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Volume 2: Herbicide Physiology

Edited by
Stephen O. Duke

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Weed Physiology

Volume II

Herbicide Physiology

Editor

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PREFACE

Weeds are plants existing at places and/or times at which they are considered undesirable by man. Thus, man's primary interest in weeds is in finding methods for eliminating their presence. Understanding the physiology of weeds and how it differs from that of crop plants is becoming increasingly important in discovering new chemical, genetic, and cultural methods of controlling weeds. The areas of highest interest and most promise in accomplishing this goal are (1) the physiology of weed reproduction, (2) the ecophysiology of weeds, (3) the mechanisms of herbicide action, and (4) the mechanisms of herbicide resistance and tolerance. Volume I deals with the first two of these areas and Volume II discusses the latter two. In these two volumes we have tried to cover each of the above areas at a level that will be useful to weed scientists, plant physiologists, agricultural chemists, and agricultural ecologists. Also, we have tried to provide enough background information for advanced weed science students to effectively utilize these reviews.

In Chapter 1 of Volume I Professor Gutterman discusses flowering, seed development, and influences during seed maturation on seed germination of annual weeds. The emphasis is on how the mother plant and its environment influences seed development and germination characteristics of weed seed. Chapter 2, by Dr. Egley and me, deals with weed seed germination and dormancy. This is an area of tremendous volumes of data, but of little actual biochemical or physiological information at the tissue or cellular level. Where possible, we have tried to discuss the ecological implications of what is known of the physiology. In Chapter 3, by Professors Kigel and Koller, asexual reproduction of weeds is reviewed. This is increasingly becoming an important area of research because annual and perennial weeds with primarily asexual means of reproduction are becoming more troublesome in many agricultural ecosystems. The next two chapters fall into the realm of weed ecophysiology. Dr. Patterson discusses the comparative ecophysiology of weeds and crops and Professor Putnam covers the area of allelopathy as it pertains to weed physiology. Improving our basic understanding of weed physiology in the context of the biotic and abiotic environments within which weeds exist is important in developing a practical knowledge of physiological factors involved in weed competition, weed population dynamics, and other important weed-related processes of agricultural ecosystems.

Volume II deals with the mechanisms of herbicide action and of resistance and tolerance to herbicides. The first five chapters of this volume cover the effects of herbicides and adjuvants on the physiology of plants. Professor Black's chapter begins by covering the effects of herbicides on photosynthesis, including photosynthetic assimilation of nitrogen, sulfur, and phosphorus. This is followed by Dr. Moreland's chapter on herbicide interactions with plant respiration. The third chapter by Professor Bartels deals with the effects of herbicides on chloroplast and cellular development with emphasis on correlating physiological information with ultrastructural effects. My chapter covers herbicidal effects on non-photosynthetic, biosynthetic processes. Nonorganelle membrane functions as affected by herbicides are discussed in Chapter 5 by Professor Balke. In field situations, herbicides are almost always applied with adjuvants. Thus, inclusion of the chapter by Dr. McWhorter on the physiological effects of adjuvants on plants compliments the coverage of herbicides. The last three chapters of the second volume concern the mechanisms of plant resistance and tolerance to herbicides. Professor Gressel's chapter summarizes resistance due to alteration at the site of action of the herbicide. His chapter deals mainly with resistance to herbicides that inhibit photosystem II, since this is the most studied and best understood mechanism of resistance. How absorption and translocation affect tolerance and susceptibility of plants to herbicides is reviewed by Professor Hess (Chapter 8). In the final chapter, Dr. Shimabukuro discusses detoxication of herbicides as a mechanism of tolerance of plants to herbicides.

The rate of increase in the number of publications in the area of weed physiology is

growing yearly — a trend seen in many other scientific disciplines. In the case of weed physiology, the increase can be related, at least partially, to the more than 20 billion dollar annual economic loss due to weeds in the U.S. alone and the growing realization that physiological information will be necessary to substantially reduce this loss. For instance, much new research effort is being expended on production of herbicide resistance in crop plants with the techniques of molecular biology. This work can not be intelligently conducted without adequate physiological knowledge of the mechanism of action of the herbicide for which resistance is being sought. Thus, mechanism of herbicide action research is being conducted at a previously unparalleled rate and many of the existing gaps in our knowledge that have been pointed out in these volumes may soon be filled. We hope these reviews will be stimulating and useful to those engaged in this effort and to those interested in plant physiology as it relates to weeds and herbicides.

Stephen O. Duke
February, 1984

THE EDITOR

Stephen O. Duke, Ph.D., is a Plant Physiologist at the USDA's Southern Weed Science Laboratory at Stoneville, Mississippi. He is a member of the Weed Biology and Mechanisms of Control research unit of this Agricultural Research Service laboratory. Dr. Duke received a B.S. in Biology in 1966 from Henderson State College and a M.S. in Botany in 1969 from the University of Arkansas. His education was then interrupted by two years of U.S. Army service, a year of which was spent in Viet Nam. He received a Ph.D. in Botany from Duke University in 1975. That same year he arrived at Stoneville as a National Research Council Associate to study phytochrome and weed seed dormancy. In 1976 he became a permanent staff member of the laboratory.

Dr. Duke is a member of numerous professional societies, including the Weed Science Society of America, the American Society of Plant Physiologists, the American Society for Photobiology, the Japanese Society of Plant Physiologists, the American Chemical Society, and the Scandinavian Society of Plant Physiologists. He is the Vice-Chairman of the Southern Section of the American Society of Plant Physiologists. As a member and chairman of several committees and as an associate editor of Weed Science, he has been an active member of the Weed Science Society of America. In 1984 he was elected the society's outstanding young weed scientist.

Dr. Duke has authored more than 50 research and review papers and has given more than 25 invited lectures at symposia and seminars. His current research interests include herbicide mechanisms of action, plastid development, seed dormancy, plant secondary metabolism, and naturally-occurring compounds as herbicides.

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The support of my family, particularly that of my wife Barbara, is gratefully acknowledged. Finally, I would like to recognize the many fine teachers that I have had, especially my parents and Professors Adelphia Basford, James Wickliff, and Aubrey Naylor.

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Chapter 1

EFFECTS OF HERBICIDES ON PHOTOSYNTHESIS

Clanton C. Black, Jr.

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I. INTRODUCTION

The autotrophic nature of plants due to the presence of photosynthesis is a unique biological feature of plants that also presents a natural site for the development of herbicides. Indeed, photosynthesis inhibitors are widely employed as herbicides today. In addition to being a specific and natural site of herbicide action, any quantitative assessment of physiological processes in green plants will quickly show that photosynthesis is the dominant metabolic process in plants based on measurements such as rates or amounts of product. Again, if one wished to influence adversely the growth of plants, one would correctly try to inhibit photosynthesis. Thus, an understanding of weed physiology and, in turn, the roles and usages of many herbicides must be buttressed by an understanding of photosynthesis.

Both crops and their associated weeds are dependent upon photosynthesis which also may be the common site of herbicide action. Solving the dilemma of how to selectively inhibit photosynthesis in the desired plant species, therefore, is a central topic in chemical weed control research. This chapter presents our understanding of plant photosynthesis primarily at the level of the chloroplast with initial emphasis upon the utilization of light to photoassimilate the essential elements carbon, nitrogen, sulfur, and phosphorus followed by a consideration of the chloroplast as it partitions assimilates with the rest of the plant. Finally, we will consider the action of herbicides and other inhibitors on the physiological and biochemical functions of photosynthesis.

II. PLANT PHOTOSYNTHESIS

For over a century plant photosynthesis has been understood in terms of involving the use of atmospheric CO_2 to form starch plus the release of O_2 in the aqueous milieu of green cells catalyzed by light and chlorophyll. This early understanding can be presented as:

$$H_2O + CO_2 \xrightarrow[\text{sunlight}]{\text{green plant}} \text{starch} + O_2 \qquad (1)$$

Even today this is a fairly adequate representation of overall photosynthesis which certainly serves to emphasize that much of photosynthesis is related to CO_2 assimilation. Though this fact is true, e.g., about 40% to 45% of a total plant is carbon, we also know that plants photoassimilate other essential elements such as N,S, and P through photosynthesis. Not only do plants utilize photosynthesis to assimilate their essential nutrients, we also know the overall pathways and mechanisms for utilizing light in chloroplasts and we know specific plants including important weeds and crops have developed several assimilatory pathways for the same essential element.

Historically, the dominant model for photosynthesis research which lead to our current understanding of photosynthesis and its diversity come from the thinking of C.B. van Niel. About five decades ago, van Niel[1] reasoned that the overall ideas about green plant photosynthesis (Equation 1) could be modified by comparisons with bacterial photosynthesis. He knew these bacteria required a reduced substance for photosynthesis, e.g., H_2S, H_2, acetone, or a variety of oxidizable substances, which he abbreviated H_2A. He also knew photosynthetic bacteria fixed CO_2, but did not evolve O_2. Thus from his comparative thinking he presented green plant and bacterial photosynthesis as:

Green Plant Photosynthesis

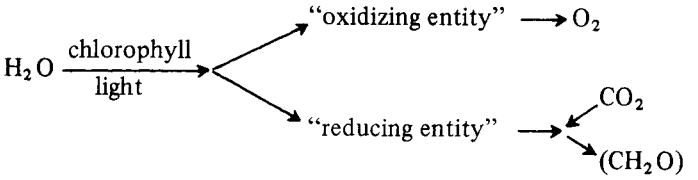
$$2H_2O + CO_2 \xrightarrow[\text{light}]{\text{chlorophyll}} (CH_2O) + H_2O + O_2 \qquad (2)$$

Bacterial Photosynthesis

$$2H_2A + CO_2 \xrightarrow[\text{light}]{\text{bacteriochlorophyll}} (CH_2O) + H_2O + 2A \qquad (3)$$

(CH_2O) represents a reduced carbon product such as starch.

Such comparisons allowed van Niel to propose that the O_2 evolved during plant photosynthesis originates from H_2O (compare Equations 2 and 3). And he formulated a model for plant photosynthesis research as:



(4)

Though we would change this model somewhat today to show a charge separation as an initial event rather than the photolysis of H_2O, this model guided photosynthesis research through the next five decades and led to the discovery and characterization of many of the "reducing" and "oxidizing" entities of Equation 4. In addition, the roles of photosynthetic pigments and learning that substances other than CO_2 also are reduced and assimilated during photosynthesis were determined.

We will soon consider these entities, pigments, and substances, but to more clearly grasp photosynthesis research in relationship to herbicide action and weed control research let us consider the times or speeds of the photosynthetic process. About two decades ago Kamen[2] presented an informative way to visualize photosynthesis based on the speed or time required for various steps in photosynthesis to occur. He divided photosynthesis into various processes and research areas based on the logarithm of time in seconds. An appropriately modified representation of his idea that has been discussed recently[3] is given in Figure 1. Think in temporial terms of orders of magnitude of time in seconds to understand Figure 1.

Photosynthesis begins with the absorption of light in about 10^{-15} sec followed by photon stabilization as excitation energy in a pigment such as chlorophyll. The excitation energy must result in photochemical conversions into oxidizing and reducing components or the photon will be re-emitted as fluorescence usually by 10^{-9} sec. The photochemical formation of oxidizing and reducing components results in photon energy conversion into components that are used in enzyme-catalyzed reactions.

The enzyme-catalyzed reactions of biochemistry require energy and transfer energy to ultimately result in the storage of energy in a variety of substances such as starch. During these biochemical conversions beginning near 10^{-7} sec, we begin to find the products of photosynthesis that most of us recognize, such as O_2, or we first observe the assimilation of CO_2.

As biochemical processes proceed, other biological processes, such as physiology of cell growth and division or plant growth, interact with photosynthetic biochemistry. Environmental constraints and genetic information also influence the products of photosynthesis. From about 10^1 to 10^9 sec photosynthesis is studied as agriculture and ecology. At these times we recognize higher plants, photosynthetic bacteria, and algae as primary products of photosynthesis; and indeed even other organisms as secondary products of photosynthesis. For the complete process of photosynthesis to occur, from the primary light absorption of (10^{-15} sec) to the production of the oldest plants (10^{11} sec), a time lapse of 26 orders of magnitude is necessary.[3]

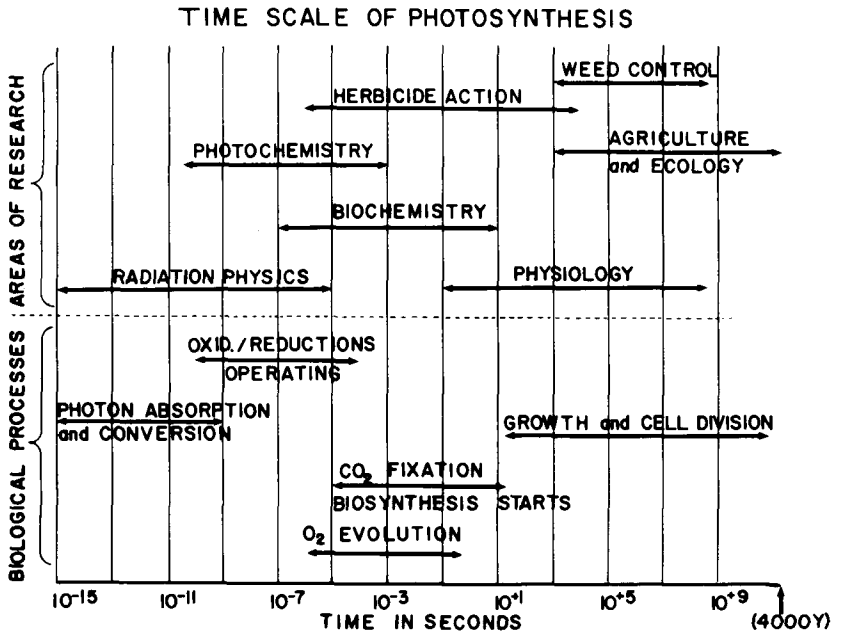


FIGURE 1. Time scale of photosynthesis in seconds. Adapted from Kamen.[2,3] Light absorption in 10^{-15} sec initiates photosynthesis and it ends in the growth of plants with an old plant being the bristle cone pine in about 10^{11} sec.

The time relationships during photosynthesis of herbicide action and weed control research also are shown on Figure 1. The initial events of herbicide action such as binding to an enzyme or membrane can begin to occur near 10^{-6} or 10^{-7} sec if present within a plant at its site of action; but from the time of field application herbicide action will not be this fast. Herbicide action continues over several time orders of magnitude and is studied in weed control research from about 10^2 to 10^8 sec. Clearly a number of early events, e.g., photon stabilization and photochemistry, have occurred in the overall process of photosynthesis before any known herbicide action occurs. Most herbicide action that we will consider occurs during biochemical reaction times and often is reflected in assimilatory activities of plants.

III. ASSIMILATORY ACTIVITIES OF PHOTOSYNTHESIS

Photosynthetic assimilation not only involves light, CO_2, and H_2O (Equation 1), but the process also provides plants with a mechanism for assimilating other essential elements. Our understanding of photosynthesis as an assimilatory process for essential elements, other than carbon, has been developing over the last half century.[3,4] Assimilation is used in this chapter to refer to the net incorporation of an element into plant organic matter. Some assimilatory processes only occur during photosynthesis, while others utilize the products of photosynthesis. For example, the net fixation and reduction of CO_2 only occurs during photosynthesis, and photosynthesis is needed for plants to reduce quantities of nitrogen and sulfur. An element such as phosphorus may be assimilated whether nonphotosynthetically, as during oxidative phosphorylation, or photosynthetically, as during photophosphorylation. However, other essential elements, such as metals in metalloenzymes, can be assimilated quite independently of photosynthesis.

If we consider C,N,S, and P assimilation in greater detail, we find that these elements generally are not directly assimilated, but rather are transformed prior to, or during, assim-

ilation into organic molecules. In the assimilation of these four major elements, photosynthetic organisms obtain them from their environments where the elements commonly exist as oxides, namely, CO_2, NO_3^-, SO_4^{2-}, PO_4^{3-}.

CO_2 is assimilated from the atmosphere via a carboxylation reaction and the carboxyl group ultimately is reduced to an aldehyde in a two electron-requiring process. Nitrate is the principal form of inorganic nitrogen available to plant roots, and it is swept up through the transpiration stream to leaves, where it is reduced in green cells to NH_3 in an eight-electron reduction process. Likewise, SO_4^{2-} is the principal form of sulfur in soils for roots, and it is brought up to leaves via transpiraton, reduced, and incorporated into cysteine, also in an eight-electron reduction process in the chloroplast. Phosphate, however, is not reduced during assimilation. In soil, roots generally take up phosphoric acid most of which also is swept up in the transpiration stream to the leaves, where it is incorporated into ATP via photophosphorylation, mitochondrial respiration, or glycolysis. Phosphate from ATP is rapidly moved into a variety of metabolites, such as sugar phosphates, phospholipids, and nucleotides. In intact plants, N,S, and P assimilation also may occur in nongreen tissue, but at much reduced rates compared to their photosynthetic tissues. Thus, C,N, and S are markedly transformed during their photosynthetic assimilation into organic molecules, whereas P is directly incorporated.

Therefore, the assimilation of either C,N,S, or P must be thought of as photosynthesis! Indeed, Equation 1 for plant photosynthesis could be modified in the following general fashions:

$$H_2O + SO_4^{2-} \xrightarrow[\text{sunlight}]{\text{green plant}} \underset{\text{(cysteine)}}{\text{organic matter}} + O_2 \qquad (5)$$

$$H_2O + NO_3^- \xrightarrow[\text{sunlight}]{\text{green plant}} \underset{\text{(amino acids)}}{\text{organic matter}} + O_2 \qquad (6)$$

$$H_2O + ADP + PO_4^{3-} \xrightarrow[\text{sunlight}]{\text{green plant}} \underset{\text{(ATP)}}{\text{organic matter}} + O_2 \qquad (7)$$

These photosynthetic assimilatory processes must occur for plants to exist.[3]

A. The Photochemical Electron-Transport Portion of Photosynthetic Nutrient Assimilation

The transformation reactions in C,N, and S assimilation, and indeed also in P assimilation, are dependent upon the rapid (Figure 1) membrane-bound reactions initiated with photon absorption by the photosynthetic pigments. These processes result in the oxidation and reduction of the "oxidizing" and "reducing" entities of Equation 4. Figure 2 diagrams the light-dependent flow of electrons through these entities into their utilization in photoassimilation reactions. The plant photosynthetic electron-transport scheme is understood today as a two-light reaction process (commonly called the "Z scheme") requiring the participation of photosystem II and photosystem I (Figure 2).

The most important leaf pigment molecules present for photon capture are the chlorophylls, *a* and *b*. Carotenoids are present that can function in photon capture and transfer, but primarily carotenoids function as photoprotectants during photosynthesis.[5] The pigments function first as antennae for the capture of photons (as shown in Figure 2), and then to funnel them to the reaction centers of either photosystem II or I. Each photosystem contains its special form

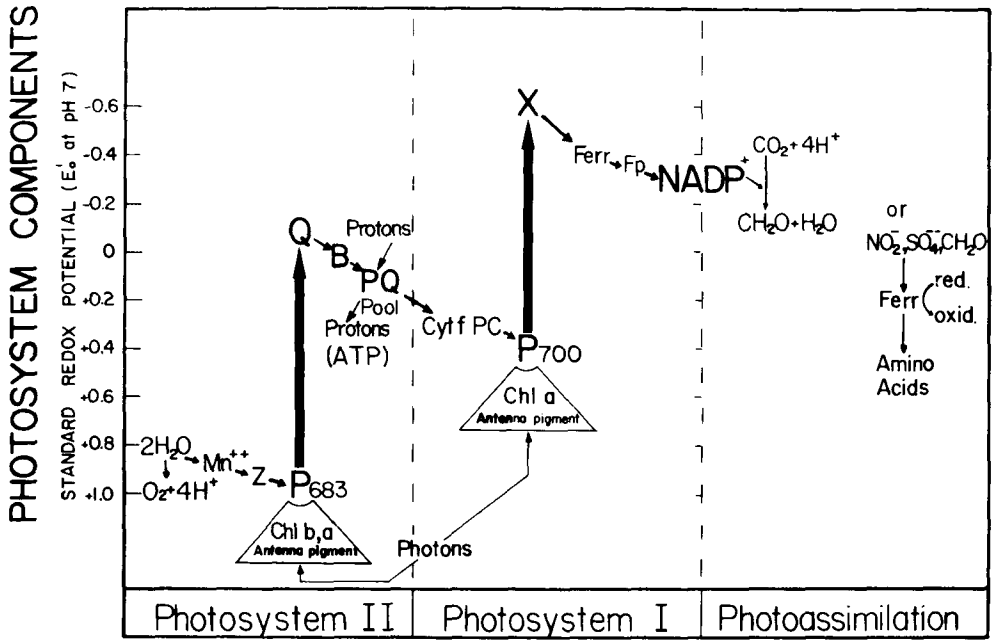


FIGURE 2. Components of photosynthesis schematically presented as a two-photosystem electron-transport pathway coupled to the photoassimilation of CO_2, NO_2^- and SO_4^{2-}. The CO_2 assimilation and electron-transport pathway are presented stoichiometrically. *No stoichiometry is implied* in the electron-transport components as drawn. For example, PQ is a pool within a leaf chloroplast that may be ten-fold larger than other electron transport components, or photosystem I may be present at two- to three-fold excess over photosystem II in a leaf chloroplast. The abbreviations used are Z an unidentified component denoting electrons to P_{683}; P_{683} the reaction center chlorophyll of photosystem II; Q is the unidentified acceptor of electrons from P_{683}; B is a protein probably bound to a quinone: PQ is plastocyanin; P_{700} is the reaction center chlorophyll of photosystem I; X is the acceptor center for electrons from P_{700} which is likely an iron-sulfur center; Ferr is ferredoxin; and Fp is the flavoprotein ferredoxin $NADP^+$ reductase.

of chlorophyll *a*, which functions as its respective reaction center, namely, P_{683} for II and P_{700} for I. Each reaction center pigment can donate an electron following photon absorption to its respective acceptor, designated Q and X in Figure 2, and the electrons then are transferred through the diagramed sequence of redox reactions. The electrons originate from H_2O, flow through the reaction centers and other redox components, and finally accumulate in reduced carbohydrate or amino acids, concurrently resulting in ATP synthesis as shown in Figure 2.

Operationally, via the Z-scheme of electron flow, green plants produce O_2 plus a strong reductant, either NADPH or reduced ferredoxin, and the ATP needed for the photoassimilation of CO_2, NO_3^-, and SO_4^{2-} to proceed. Figure 2 stoichiometrically diagrams electrons flowing from H_2O through ferredoxin or $NADP^+$ to be used in assimilatory processes along with ATP.

The mechanism of O_2 evolution during the donation of electrons to P_{683} in photosystem II is not understood though Mn is involved. The synthesis of ATP via photophorylation is only indicated in Figure 2. More details on the synthesis of ATP in the chloroplast will be given later in Figure 11, but the extraction of energy for ATP synthesis from electron flow is dependent on the production of a proton gradient from the interior to the exterior of the chloroplast membrane. Proton production and movements to the chloroplast interior are indicated during the photolysis of H_2O and the oxidation-reduction of the plastoquinone pool in Figure 2.

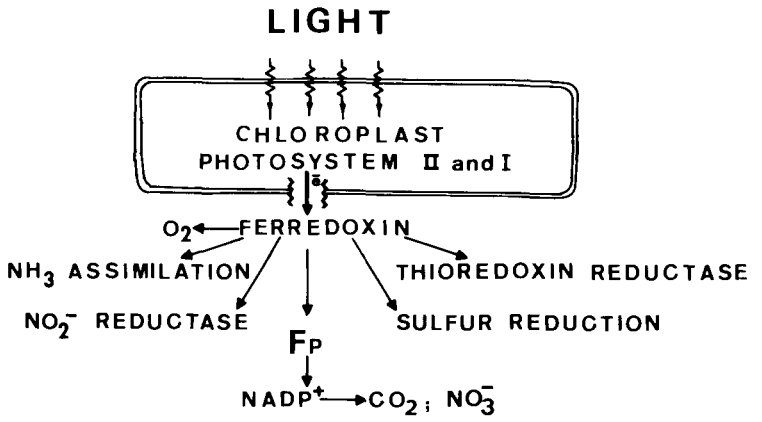


FIGURE 3. Electron-transfer reactions of ferredoxin as a branch point in chloroplast metabolism.

In addition to proton metabolism, an important branch-point in chloroplast metabolism is the oxidation-reduction protein ferredoxin. In Figure 2 ferredoxin is shown transferring electron from photosystem I to a flavoprotein enroute to NADPH. Ferredoxin is a very strong reductant with an E_o^1 near -0.41 which can be used in a variety of chloroplast redox reactions. These potential functions of chloroplast ferredoxin are outlined in Figure 3. The overall assimilation of N and S are shown in Figure 2, both of which utilize reduced ferredoxin in the chloroplast. Nitrate assimilation is initiated by nitrate reductase in the cytoplasm followed by the reduction of nitrite and ammonia assimilation into amino acids in the chloroplast. Sulfate enters the chloroplast and there is incorporated into cysteine via light-dependent reactions.

B. Pathways for the Photoassimilation of CO_2, NO_3^-, SO_4^{2-}, and PO_4^{3-}

Plants have evolved various biochemical pathways for the assimilation of their essential elements. Only a few years ago it was widely espoused that photosynthetic CO_2 fixation only occurred via the reductive pentose phosphate cycle (the C_3 cycle) and that NH_3 assimilation only occurred via glutamate dehydrogenase. Today we recognize that specific plants have diversified their biochemistry, such that plants are not dependent upon a single pathway for assimilating essential nutrients. The photoassimilation of CO_2 can proceed in specific organisms by one of seven known biochemical pathways. NO_3^- and SO_4^{2-} can be assimilated in some green cells but not in others and ammonia can be assimilated by one of two enzymes. This biochemical diversity in the photoassimilation capability of specific plants is important in weed physiology and will be illustrated in that context. The presence of specific proteins and biochemical reactions in specific plants results in many ideas that can be used in understanding herbicide selectivity and in choosing specific targets for developing new selective herbicides.

1. CO_2 Assimilation Pathways

The most widely distributed pathway of CO_2 assimilation in nature is the reductive pentose phosphate cycle (also known as the Calvin-Benson cycle or the C_3 cycle).[6,7] The C_3 cycle is well known[6] and can be considered as consisting of three phases.[8] The first phase is the generation of ribulose 1, 5-P_2, the CO_2 acceptor, by the enzyme ribulose 5-P kinase (Reaction 8)

$$\text{Ribulose 5-P} + \text{ATP} \xrightarrow[\text{kinase}]{\text{Ru 5-P}} \text{ribulose 1,5-P}_2 + \text{ADP} \qquad (8)$$

followed by its carboxylation (Reaction 9) to form 2 molecules of 3-PGA.

$$\text{Ribulose 1,5-P}_2 + \text{CO}_2 \xrightarrow[\text{carboxylase}]{\text{ribulose 1,5-P}_2} 2\ \text{3-PGA} \qquad (9)$$

The second phase is the use of ATP to form 1,3-DiPGA, the acyl phosphate of 3-PGA, which then is reduced by using NADPH to form glyceraldehyde 3-P plus PO_4^{3-}. The third phase of the C_3 cycle consists of stoichiometrically converting five molecules of the 3-carbon sugar, such as glyceraldehyde 3-P, to three molecules of the 5-carbon sugar, ribulose 5-P.

Stoichiometrically, one can balance the complete C_3 cycle by considering the fixation of three CO_2 molecules. Three molecules of ribulose 1,5-P_2 are required and six molecules of 3-PGA would be formed and then acylated and reduced to glyceraldehyde 3-P. Five molecules of glyceraldehyde 3-P would be converted to three molecules of pentose-P. One molecule of the 3-carbon sugar, glyceraldehyde 3-P, would remain as the product of the fixation, acylation, and reduction of three molecules of CO_2. This triose phosphate is a glycolytic carbon flow intermediate and a central branch point of plant metabolism since it has four other major fates, some of which we will discuss, namely, (1) the direct participation in the C_3 cycle just discussed, (2) translocation out of the chloroplast to the cytoplasm for oxidation, or condensation, or sucrose synthesis, (3) condensation with DHAP to form fructose 1,6-P_2,and on to starch storage within the chloroplast, (4) isomerization and reduction for the synthesis of glycerol-P and other lipids. In cells glyceraldehyde 3-P is in rapid equilibrium with dihydroxy acetone phosphate.

Unquestionably the C_3 cycle is widespread in nature and is a ubiquitous cycle for net sugar synthesis, though it is not present in all photosynthetic cells. The two best known green cells that lack the C_3 cycle are present within important agricultural crops and weeds. These cells are C_4 leaf mesophyll cells[9] and guard cells[10] of all leaves that have been carefully researched. Table 1 compiles some common C_3 cycle weeds and crops along with some illustrative C_4 cycle and Crassulacean acid metabolism plants.

Until about 1964 to 1969 most plant scientists thought the C_3 cycle was the only photosynthetic pathway for assimilating and reducing net quantities of CO_2. During that exciting half-decade the assimilation of CO_2 via (1) a ferredoxin-linked reductive carboxylic acid cycle was discovered in photosynthetic bacteria,[11,12] (2) a carboxylation of phosphoenolpyruvate (PEP) and the subsequent formation of sugars in sugarcane[13,14] and other plants[15] was discovered, and (3) the night CO_2 fixation in cactus and other plants known for many decades[16] was reevaluated and accepted as a unique pathway for net CO_2 assimilation in specific plants.[15] We will not discuss CO_2 fixation in photosynthetic bacteria such as *Chlorobium thiosulfatophilum*[12] further except to note this is a distinct biochemical pathway for net CO_2 fixation and reduction via photosynthesis. The sugarcane work led to our current understanding of the C_4-dicarboxylic acid cycles (C_4 cycle) of CO_2 fixation which involves many weeds and crops. The night fixation of large quantities of CO_2 is via Crassulacean acid metabolism (CAM), which also involves weeds and crops (Table 1).

a. C_4 Photosynthesis

Reports on the discovery of C_4 photosynthesis in 1965-1966[13,14] not only heralded the beginnings of new understandings about photosynthesis, but the discovery immediately was implicated as a basis for new understandings of agricultural crop productivity, weed control, and ecology[17-19] in terms of the biochemistry and physiology of C_3 vs. C_4 vs. CAM photosynthesis.[15,20] C_4 photosynthesis also attracted much research attention because its unique biochemistry was shown to be related to a distinctive leaf anatomy.[15] Since there is a large

Table 1
ILLUSTRATIVE LIST OF WEEDS AND CROPS/PASTURES THAT UTILIZE ONE OF THE SIX KNOWN BIOCHEMICAL PATHWAYS FOR THE PHOTOSYNTHETIC ASSIMILATION OF CO_2[a]

C_3 Cycle	C_4 cycle			Crassulacean acid metabolism	
	PEPCK	NADP malic enzyme	NAD malic enzyme	PEPCK	NADP/NAD malic enzyme
Crops	**Crops/pastures**	**Crops**	**Crops/pastures**	**Crops**	**Crops**
Soybeans and other legumes	*Panicum maximum*	Sugarcane, corn	*Panicum milaceum*	Pineapple	*Opuntia ficus-indica*
Small grains	*Chloris gayana*	*Sorghum bicolor*	*Eragrostis curvula*	*Aloe arborescens*	*Nopalea* sp.
Cotton	*Urochloa panicoides*				*Agave sisalina*
Weeds	**Weeds**	**Weeds**	**Weeds**	**Weeds**	**Weeds**
Cocklebur	*Panicum maximum*	Crabgrass	Bermudagrass	*Tillandsia usneoides*	*Opuntia inermis*
Jimson weed					
Sicklepod	*Panicum texanum*	*Sorghum halapense*	*Eleusine indica*		*Opuntia polyarantha*
Morningglory					
Sida sp.	*Sporobolus poiretii*	*Echinochloa crussgali*	*Amaranthus retroflexus*		

[a] More exhaustive lists of C_3, C_4, and CAM plants are available.[24]

published literature on C_4 photosynthesis, we will move now to our current understanding of the C_4 cycle[21] and simply cite key references interested readers may use to study the original literature.

The distinctive leaf anatomy initially associated with C_4 photosynthesis was called Kranz over a hundred years ago when a leaf like sugarcane or crabgrass (Figure 4) was viewed microscopically in cross section. It is true that all leaves with Kranz anatomy, namely a single layer of green bundle sheath cells surrounded by a single layer of green mesophyll cells (Figure 4), exhibit C_4 photosynthesis. However, not all C_4 plants have Kranz leaf anatomy and all plants with green bundle sheath cells are not C_4 plants. The essential or fundamental feature of C_4 anatomy which has emerged from the last 1.5 decades of research can be stated simply:[21] two green cell types are always found in a C_4 plant. These two cells do not have a fixed morphological or anatomical arrangement but share, in an interdependent manner, the tasks of photosynthesis including photon capture, electron transport through the photosystems, photoassimilation of essential elements, plus the storage and utilization of nutrients. Thus research work on the C_4 cycle has established that a definite division of metabolism of elements such as C,N, and S occurs between the two cell types and that this cooperation results in an efficient operation of the C_4 cycle such that C_4 plants often are productive crops or competitive weeds (see Chapter 4, Volume I).

Figure 5 is a current overall scheme for the photoassimilation of CO_2 in a typical C_4 plant. The cellular specialization so characteristic of C_4 photosynthesis is clearly evident, beginning with the major fixation of leaf CO_2 via the carboxylation of PEP in the mesophyll cell cytoplasm, followed by the reduction of OAA to malate or its amination to aspartate. Then these four carbon organic acids move to the bundle sheath cell where a decarboxylation occurs and CO_2 is generated to operate the chloroplast C_3 cycle. Apparently the C_3 cycle in C_4 bundle sheath cells operates similarly to its operation in green cells, including a low level of photorespiration. The C_4 mesophyll cell chloroplast lacks the C_3 cycle but carries out the critical CO_2 metabolism functions of (1) regenerating the CO_2 acceptor, PEP, from 3-carbon fragments, either pyruvate or alanine, moving into it from the bundle sheath cell, (2) the photochemcal production of the reductant to reduce the OAA produced by PEP carboxylase when CO_2 is fixed, and (3) synthesizing starch (Figure 5) from trioses. Obviously, to prove such a cellular specialization requires proof of compartmentation of enzymes, and much supporting data such as $^{14}CO_2$ metabolism studies. Indeed, such data have accumulated over the last decade.[9,15,20-23]

While Figure 5 outlines the major overall pathways of carbon flow in C_4 mesophyll cells and bundle sheath cells, it does not fully allow one to appreciate the degree of specificity in the exact biochemical pathway present in a given C_4 plant species. In fact, C_4 photosynthesis plants can be divided into three groups, based on the primary decarboxylase found in their bundle sheath cells. Table 2 summarizes some salient features of the three groups of C_4 plants and lists some representative weeds. The decarboxylation reaction for a specific organic acid in the bundle sheath cell of a C_4 plant is a key feature of C_4 carbon metabolism because the type of biochemistry of both the mesophyll cell and the bundle sheath cell plus their energetics and metabolite transport are dependent on this reaction (Table 2).

The scheme for C_4 photosynthesis in Figure 5 and the division of C_4 plants into three groups (Table 2) are generally accepted today. Before gaining wide acceptance, questions were raised concerning the functions, or roles, or results of two interconnecting cycles of CO_2 metabolism spatially separated into two cell types. The environmental physiology of C_4 plants was subsequently found to be quite different from that of C_3 or CAM plants.[15,17,20] Briefly, C_4 leaf photosynthesis is near saturation at current levels of atmospheric CO_2 and C_4 photosynthesis maintains a high rate without a detectable loss of CO_2 due to photorespiration. Apparently cellular specialization of C_4 tissues results in the mesophyll cell effectively trapping all CO_2 available either from the atmosphere or from internal leaf respiratory

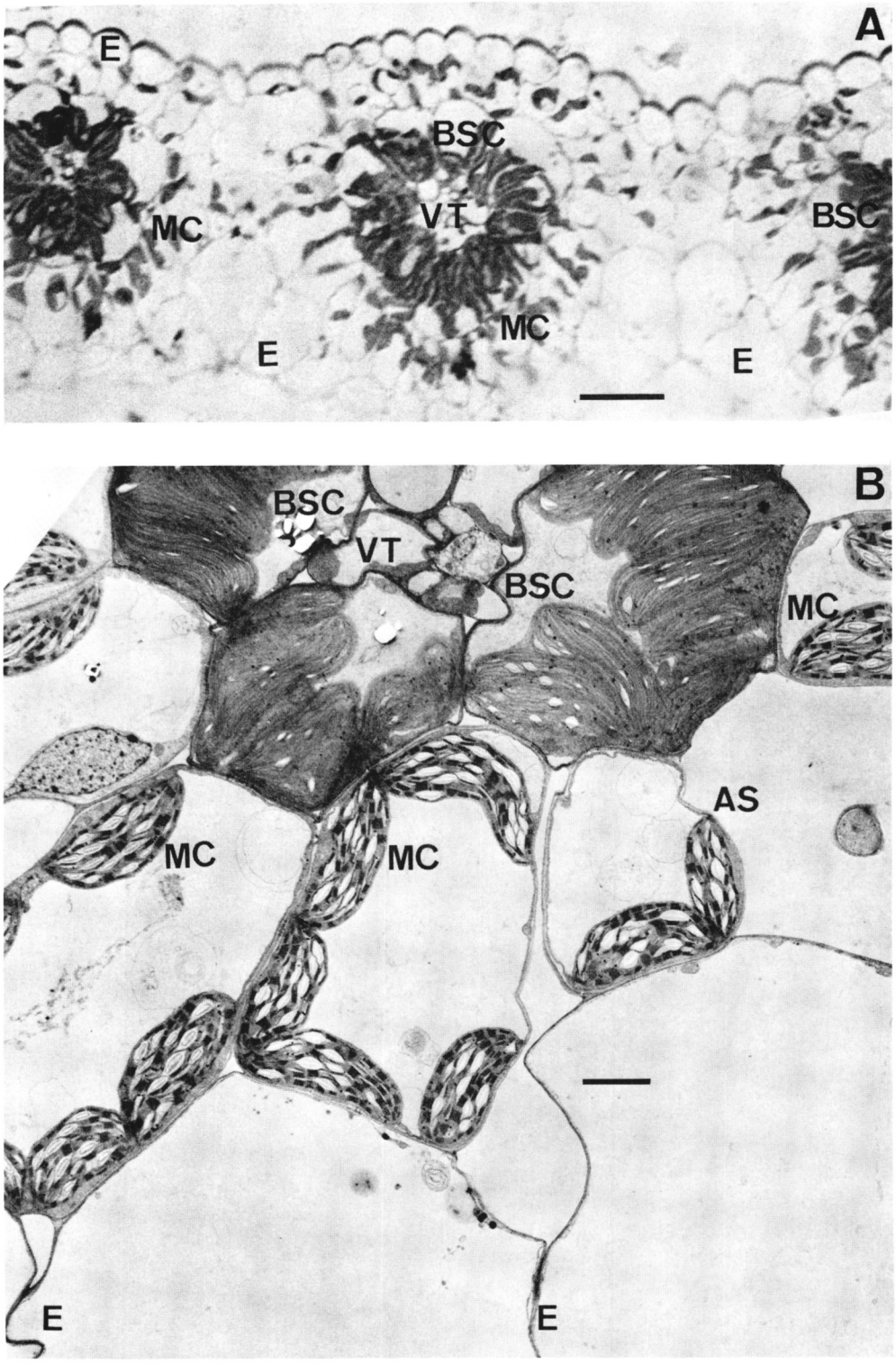


FIGURE 4. (A) Light micrograph of a typical C_4 leaf in cross section showing Kranz leaf anatomy. Leaf is *Leptochlora dubia*. Bar = 100 gμm. (B) Electron micrograph showing chloroplast ultrastructure and starch accumulation in a C_4 plant. Leaf is crabgrass, *Digitaria sanguinalis*. Bar = 1 μm. MC = mesophyll cell; BSC = bundle sheath cell; E = epidermis; VT = vascular tissue; AS = leaf air space.

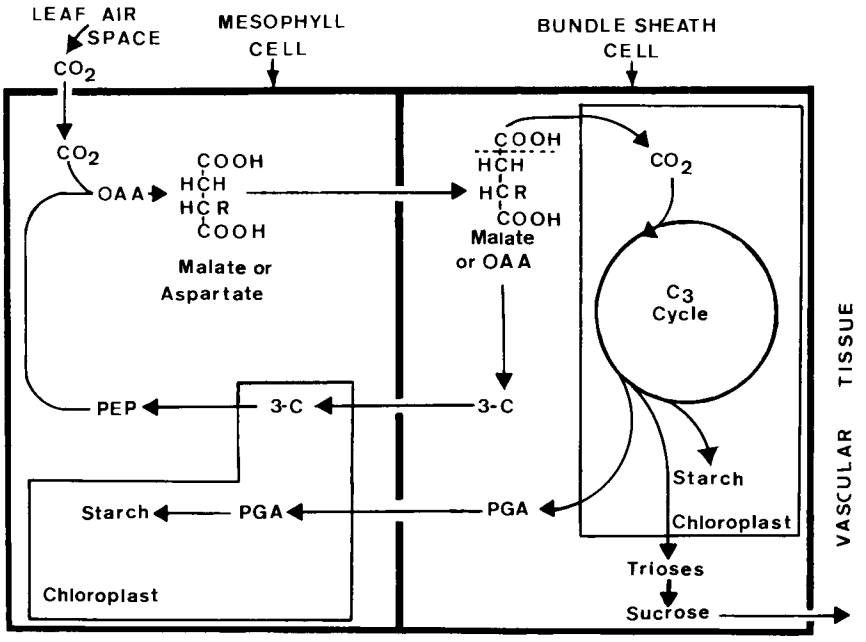


FIGURE 5. Biochemical scheme for photosynthetic CO_2 fixation and carbon flow in mesophyll cells and bundle sheath cells of C_4 plants. C-3 refers to a three-carbon fragment, either pyruvate or PEP, from the decarboxylation of malate or oxaloacetate.[9]

processes. The combination of this CO_2 trapping and the movement of C_4 organic acids to the bundle sheath cells results in release of CO_2 at the site of RuBP carboxylase in a concentrated fashion such that CO_2 in air is not rate limiting for C_4 photosynthesis. Hence, C_4 plants are an adaptation of photosynthesis that allows the efficient use of current day levels of CO_2 in the earth's atmosphere. Additional advantages accrue to the C_4 plant as a result of its highly efficient CO_2 scavenging. Since intracellular CO_2 concentrations can fall to quite low levels without decreasing the fixation rate greatly, the C_4 leaf can effectively conserve water by restricting its stomatal aperture. Thus, C_4 plants also represent an adaptation of photosynthesis for the effective utilization of water.[17] Other apparently beneficial features of C_4 photosynthesis will be presented when N and S assimilation are considered.

b. CO_2 Assimilation During Crassulacean Acid Metabolism

Though CAM has been known for 1.5 centuries[16] it was not widely appreciated by plant scientists for its unique CO_2 metabolism nor for other adaptative features, particularly the abilities to use water efficiently and to survive extreme environmental stresses, including common weed control practices such as plowing. The discovery of C_4 photosynthesis also initiated a resurgence of interest in CAM because both pathways biochemically use 4-carbon acids, e.g., malic acid is a key metabolite in both types of metabolism.

CAM plants are characterized by the features listed in Table 3.[25] The adaptative biochemical and anatomical features in Table 3 result in CAM plants being very efficient in utilizing water. Thus, CAM plants are an adaptation to arid or dry environments. For example, the cactus in deserts or spanish moss hanging in trees in the southeastern U.S. are CAM plants. This discussion will center on CO_2 fixation in CAM plants, but discussions and extensive literature citations for each feature in Table 3 are available elsewhere.[15,16,19,20,22,25-28]

A general pattern of daily CO_2 fixation measured in CAM plants is shown in Figure 6.

Table 2
THREE GROUPS OF C_4 PHOTOSYNTHESIS PLANTS WITH SEPARATE BIOCHEMICAL PATHWAYS OF CO_2 ASSIMILATION

Group	Major BSC decarboxylase (Substrate)	Energetics of:		Major substrate moving from:		Representative weeds
		Carboxylation, etc. in MC	Decarboxylation in BSC	MC to BSC	BSS to MC[a]	
1	$NADP^+$ malic enzyme (malate)	Consumption of 1 NADPH/OAA	Production of 1 $NADPH/CO_2$	Malate	Pyruvate	Crabgrass Johnsongrass
2	NAD^+ malic enzyme (malate)	Consumption of 1 NADPH/OAA	Production of 1 $NADPH/CO_2$	Aspartate	Pyruvate/ alanine[b]	Saltbush Panicgrass
3	PEP carboxy-kinase (oxaloacetate)	Consumption of 1 aminogroup/OAA	Consumption of 1 ATP/CO_2			
			Production of 1 aminogroup/OAA	Aspartate	PEP/alanine[b]	Elephantgrass Smutgrass

Note: BSC = bundle sheath cell; MC = mesophyll cell.

[a] 3-PGA also moves to the mesophyll cell (Figure 5) to support carbohydrate metabolism in all three groups of plants.

[b] Nitrogen balance between green cell types presumably is maintained because of the large amounts of substrates involved; most likely via an aminotransferase-type shuttle involving alanine. PEP also may be converted to pyruvate plus ATP in BSC.

Table 3
ADAPTATIVE FEATURES OF CAM PLANTS

1. Major quantities of CO_2 fixed at night in green cells.
2. Major utilization of a starch or glucan or freesugar (storage carbohydrate) supply at night in green cells to form the CO_2 acceptor, phosphoenolpyruvate.
3. Formation of a large vacuolar storage pool of malic acid at night in green cells which is depleted the next day to furnish CO_2 for C_3 photosynthesis in the green tissue chloroplast.
4. The features above combined in succulent green cells. (Succulence refers to green cells with large vacuoles.)
5. Ability to reduce stomatal apertures as a standard function in daylight.
6. Plants with the lowest known H_2O requirement or dry matter production ranging from 18 to 155 g of H_2O transpired per g of dry matter produced. (Therefore, CAM is an adaptation to arid or otherwise dry growth habitats.)
7. Ability to withstand loss of contact with soil and its moisture for as much as several years and then resume growth when planted and watered.
8. Ability to shift the relative amounts of atmospheric CO_2 assimilated either via PEP carboxylase or ribulose 1,5-P_2 carboxylase in response to environmental changes.

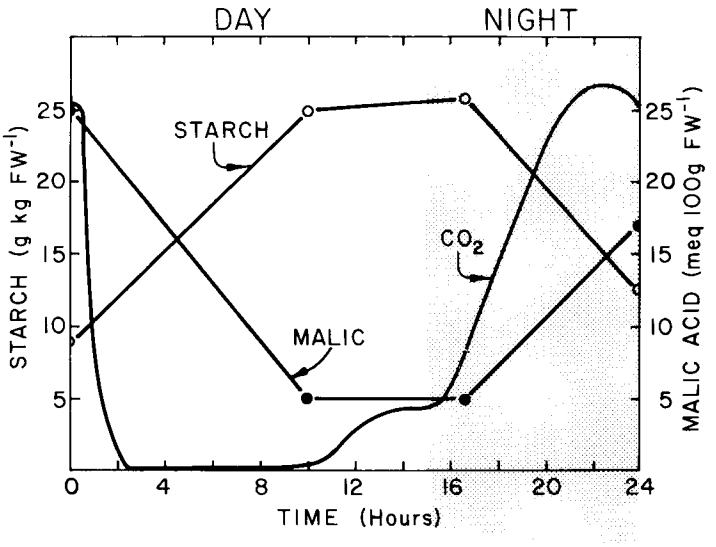


FIGURE 6. Typical diurnal patterns of CO_2 uptake, starch (or storage carbohydrate) content, and titratable acidity (or malic acid) changes in green Crassulacean acid metabolism tissues.[25]

Clearly, most net CO_2 fixation occurs at night, and concurrently the green tissue accumulates acid. The small CO_2 fixation early and late in a day may occur under well-watered growth conditions, but under dry conditions no day CO_2 fixation will occur. Acid accumulation may be measured by titration with base over 24 h and the malic acid (titratable acidity) pattern shown in Figure 6 for the whole green tissue will be obtained. Similarly, one can measure the storage carbohydrate content and a reciprocal pattern relative to titratable acid or malic acid content will be obtained.

The participation of storage carbohydrates as a major component of CAM can be calculated from Figure 6 by knowing that the tissue is near 90% H_2O.[28,29] The daily change of 17 g of starch (see Figure 6) is nearly 17% of the total composition of the plant, excluding H_2O. Thus, each day, green CAM tissues devote a major portion of their organic constituents to the synthesis of malic acid from carbohydrate and to the synthesis of storage carbohydrate

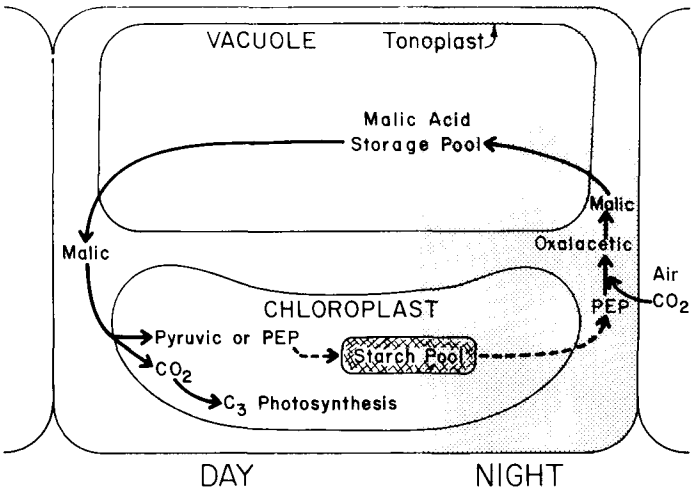


FIGURE 7. CO_2 fixation and the compartments of green cells involved in the diurnal flow of carbon during Crassulacean acid metabolism.[25]

from pyruvate acid or PEP. This diurnal cycle is a dominant feature of CAM, and it furnishes most of the CO_2 each day for photosynthesis.

On a cellular level, the biochemical details of CAM are outlined in Figure 7. Most net CO_2 fixation occurs at night by PEP carboxylase to form oxalacetate, which is reduced to malate. Malic acid is stored in the large vacuole at night, removed the next day, and decarboxylated to furnish CO_2 for C_3 photosynthesis in the chloroplast. This temporal separation of CO_2 metabolism within an individual green cell distinguishes CAM CO_2 fixation from C_4 cycle CO_2 fixation, which occurs only during the day and involves the spatial separation of PEP carboxylation in green mesophyll cells from decarboxylation followed by the carboxylation of ribulose 1,5-P_2 in green bundle sheath cells. Otherwise, as far as is known today, the C_3 cycle operates in CAM plants quite similarly to the C_3 cycle in other plants.

The depletion of malic acid via a decarboxylation each day occurs through the action of either PEP carboxykinase or $NADP^+$ malic enzyme in specific CAM plants (Table 1). An NAD^+ malic enzyme is known to be present in some CAM plants that also contain high activities of $NADP^+$ malic enzyme; however, the role of NAD^+ malic enzyme is unknown.

CAM plants exhibit one other unique feature when compared to other higher plants. CAM plants can change the amount of CO_2 fixed from the air via PEP or ribulose 1,5-P_2 carboxylase (see Table 3, Item 8). This response to environmental changes such as water status is not exhibited by other plants. The most direct measurement of this feature is the variable $\delta^{13}C$ values found with a given CAM plant grown in various environments.[30] This feature is closely related to the ability of CAM plants to survive in arid agriculture and to become severe weeds.

CAM plants are not common weeds in much of the world's cropping agriculture but they can be very severe weeds in arid agriculture, e.g., cactus in Australia[27] or *Euphorbia* sp. in North Africa. Certainly the ability of *Portulaca* sp. to exhibit CAM[31] is related to their persistence particularly when plowed for weed control. These plants withstand uprooting for several days, weeks, or even months, due to CAM, and then can root and grow again (see Item 7, Table 3). *Portulaca* also will flower and seed after being uprooted or hoed, thus insuring seeds in the next crop. The author has observed seed production by CAM

plants without roots in a variety of agricultural cropping systems ranging from the Colorado/Kansas prairie to tropical rice production.

2. Nitrogen Assimilation

Data and ideas on the photosynthetic assimilation of nitrogen (Equation 6 overall) have been accumulating for a half century which emphasize the central role of plants in biological nitrogen assimilation. We will consider green plant nitrogen assimilation from two substrates, NO_3^- and NH_3, omitting N_2 fixation and reduction by algae or bacteria. The reduction of NO_3^- to NH_3 must precede NH_3 assimilation; though plants do have additional pathways for producing sizeable amounts of NH_3, for example via photorespiration, and NH_3 can be supplied to plants to be translocated into green cells. Both NO_3^- reduction and NH_3 assimilation require photosynthesis.

3. Nitrate and Nitrite Photoreduction

The net reduction of plant NO_3^- occurs in illuminated leaves[32,33] as outlined in Figure 8. The first reaction is conducted by NO_3^- reductase in the cytoplasm according to Equation 10.

$$NO_3^- + NADH + H^+ \xrightarrow[\text{reductase}]{NO_3^-} NO_2^- + H_2O + NAD^+ \qquad (10)$$

The NADH is generated from a triose phosphate translocated out of the chloroplast followed by its oxidation (see Figure 12). NO_3^- reductase is localized in the cytoplasm of green leaf cells though sporatic unconfirmable reports have associated it with the chloroplast envelope where operationally it is appealing to have it localized so NO_2^- could be produced near or inside the chloroplast stroma.

Nitrite reduction occurs within the leaf chloroplast according to Equation 11.

$$NO_2^- + 6\bar{e} + 7H^+ \xrightarrow[\text{reductase}]{NO_2^-} NH_3 + 2H_2O \qquad (11)$$

The direct donation of electrons from the chloroplast photosystems via ferredoxin (Figures 2 and 3) clearly requires light. Indeed, one can balance the reduction of NO_2^- in the chloroplast to show that 1.5 O_2 is evolved per NO_2^- reduced to NH_3 (Equation 12):

$$NO_2^- + 3H_2O \xrightarrow[\text{photosystems, Chl}]{NO_2^- \text{ reductase}} NH_4^+ + 2OH^- + 1.5\ O_2 \qquad (12)$$

At the pH of the chloroplast the ammonium ion is the major species present rather than NH_3.

Both NO_3^- and NO_2^- reductase are substrate inducible in leaves in that a leaf supplied with NH_3 will have low levels of both activities in contrast to a leaf being supplied NO_3^- through its roots. NO_3^- reductase also is light-induced each day but NO_2^- reductase maintains a fairly level diurnal activity. NO_3^- reductase activities are compatible with NO_3^- accumulation in leaves but not with NO_2^- accumulation. Since NO_2^- is toxic as is NH_3, both of these substrates are assimilated continuously to avoid a build-up in plant tissues.[32-35]

4. Ammonia Assimilation

Prior to 1973-1974 the main pathway in plants for NH_3 assimilation was thought to be the reductive amination of α-ketoglutarate as shown in Equation 13.

$$NH_3 + \text{α-ketoglutarate} + NAD(P)H + H^+ \xrightarrow[\text{dehydrogenase}]{\text{glutamate}} \text{glutamate} + NAD(P)^+ + H_2O \quad (13)$$

Several problems with this pathway were clear, however, namely the low affinity of glutamate dehydrogenase for NH_3 and its main localization in mitochondria where it couldn't effectively remove NH_3 from the chloroplast where it is a potent uncoupler of photophosphorylation.

In 1973-1974 two enzymes were discovered in chloroplasts which revealed an additional pathway for NH_3 assimilation in which both enzymes also utilized substrates produced by the photosystems. In 1973 the convincing demonstration of glutamine synthetase in plant chloroplast[36,37] gave strong support for an active chloroplast route of NH_3 assimilation (Equation 14).

$$\text{Glutamate} + ATP + NH_3 \xrightarrow[\text{synthetase}]{\text{glutamine}} \text{glutamine} + ADP + Pi + H_2O \quad (14)$$

In 1974, the discovery of a ferredoxin-dependent glutamate synthase[38] in the chloroplast gave a light-dependent linkage between transferring the amide group of glutamine (Equation 14) to the more useful α-amino group for plant amino transferases. Equation 15 outlines this transfer.

$$\text{Glutamine} + \text{α-ketoglutarate} + \text{reduced ferredoxin} \xrightarrow[\text{synthase}]{\text{glutamate}} 2 \text{ glutamate} + \text{oxidized ferredoxin} \quad (15)$$

Glutamate is readily utilized by several plant amino transferases in forming other amino acids from α-keto acids. Glutamate synthase also has an acronym of GOGAT (glutamine amide: 2-oxoglutarate amino transferase oxido-reductase) which occurs frequently in the literature. In the overall, both NO_2^- and NH_3 assimilation occur sequentially in the chloroplast along with amino transferase action to result in the net synthesis of amino acids from NH_3 or NO_3^- (Equation 6; Figure 8).

In intact leaves these photosynthetic processes use electrons and ATP produced by the chloroplast. If we examine these pathways, however, in specific weeds and crops we do find variations. The most striking variation is found in leaves of C_4 plants where the overall process of NO_3^- assimilation shown in Figure 8 is compartmentalized in the two green cell types (Figure 9).

In 1974, using the common weed nutsedge, *Cyperus rotundus*, from which we had prepared highly purified mesophyll cells and bundle sheath strands (strands are leaf vascular tissues with their green bundle sheath cells still attached), we determined that bundle sheath cells of C_4 plants could not assimilate NO_3^- or NO_2^-. Essentially all of the leaf NO_3^- and NO_2^- reductase activity is in the green C_4 mesophyll cell. Table 4 shows these results for leaf and isolated cell preparations from two weeds, crabgrass and nutsedge.[39,40] Therefore, as NO_3^- moves through vascular tissues from the roots into a C_4 leaf, it passes the green bundle sheath cell to initiate NO_3^- assimilation in the mesophyll cell. However, NH_3 assimilation

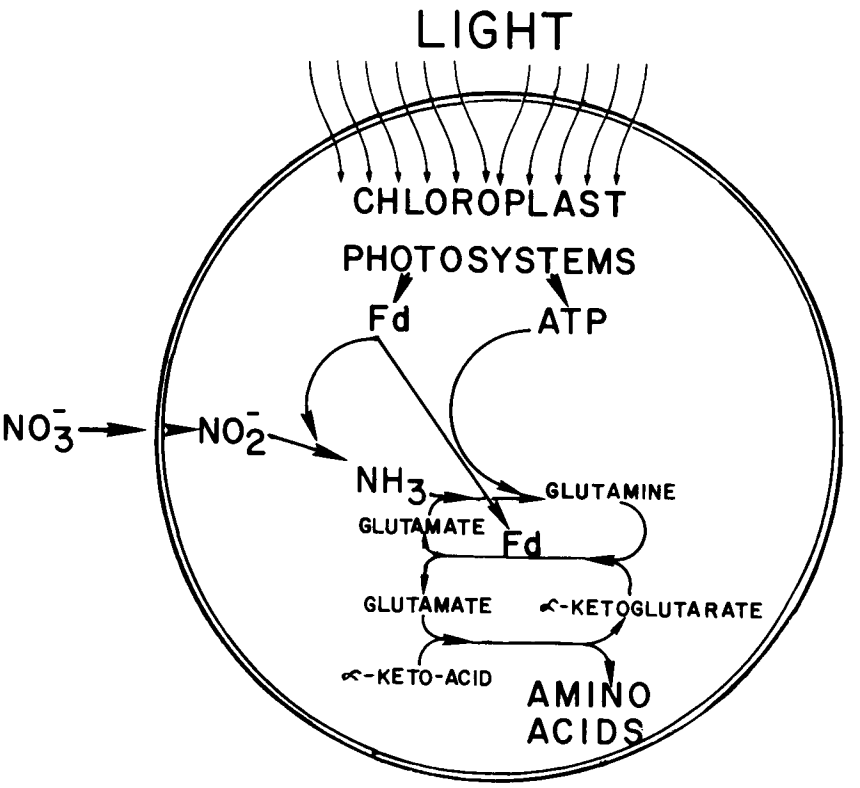


FIGURE 8. Scheme for chloroplast photoassimilation of nitrate and nitrite. Adapted from Lea and Miflin.[38]

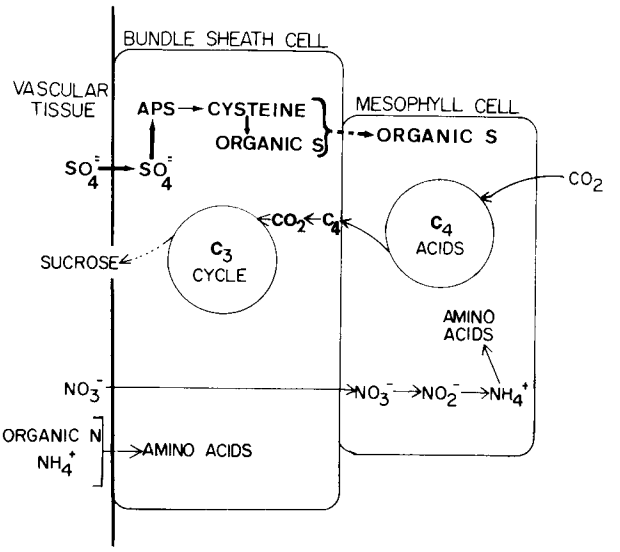


FIGURE 9. Integrated scheme during C_4 photosynthesis for the photoassimilation of CO_2, SO_4^{2-}, NO_3^-, NH_3, and organic N by intact bundle sheath and mesophyll cells.[21]

Table 4
LOCALIZATION AND ACTIVITY OF NITROGEN ASSIMILATION ENZYMES IN CRABGRASS AND NUTSEDGE LEAVES (μmol/mg chlorophyll/hr)

C_4 Plant enzyme	Whole leaf	Mesophyll cells	Bundle sheath strands
Crabgrass:			
Nitrate reductase	5.1	8.3	N.D.
Nitrite reductase	25.7	39.4	N.D.
Glutamine synthetase	44.2	33.6	45.6
Glutamate synthase	10.1	6.5	13.9
Glutamate dehydrogenase			
NADH-dependent	16.7	4.1	28.3
NADPH-dependent	6.3	1.1	11.1
Glutamate-OAA transaminase	190	202	148
Nutsedge:			
Nitrate reductase	5.6	9.8	N.D.
Nitrite reductase	15.6	33.0	N.D.
Glutamate dehydrogenase (NAD^+)	25.0	2.0	49.5
Alanine aminotransferase	120	108	195

Note: N.D. = not detectable.

occurs in each of the green C_4 leaf cell types (Table 4; Figure 9). In C_3 and CAM plants apparently all green cell types assimilate both NO_3^- and NH_3, though guard cells have not been studied yet. In brief, with C_3 and CAM weeds and crops leaf nitrogen assimilation via photosynthesis follows the pathway presented in Figure 8 for NO_3^-. Their NH_3 is primarily assimilated via glutamine synthetase and GOGAT; though glutamate dehydrogenase may have a role in mitochondria. With C_4 weeds and crops NO_3^- and NO_2^- reduction to NH_3 only occurs in leaf mesophyll cells. However, NH_3 assimilation occurs in both mesophyll and bundle sheath cells as do transaminations (Table 4, Figure 9).

There are definite practical implications and applications in weed control research and agriculture from understanding the C_4 pathway of NO_3^- photoassimilation. The fact that neither a C_4 mesophyll nor bundle sheath cell has to synthesize a full complement of photosynthetic enzymes[21] immediately implies that their nitrogen utilization is different from C_3 or CAM plants. Indeed this idea has been developed by observing that C_4 plants, particularly grasses, utilize their leaf nitrogen more efficiently than C_3 plants in producing dry matter (Table 5).[41] Table 5 shows that a C_4 plant grows more than a C_3 plant at any of these levels of nitrogen nutrition. This efficient use of nitrogen is due to the selective compartmentation of enzymes in C_4 leaf green cell types.[21,41] Thus in weed control work the growth of C_4 plants, either as crops or weeds in appropriate environments, will be greater per unit of available nitrogen than the competing plants.

5. Sulfur Assimilation

The major form of sulfur available to plants in soil is SO_4^{2-}. Sulfate is provided to leaves via transpiration and it is reduced in light-driven chloroplast reactions to the level of sulfide which then is used for cysteine synthesis. Thus Equation 5 defines photosynthesis as the photoassimilation of SO_4^{2-} to form cysteine. The valency state of sulfur changes from +6 in SO_4^{2-} to −2 in sulfide or cysteine, therefore, eight electrons supplied by the photosystems (Figure 2) are needed for SO_4^{2-} assimilation in the chloroplasts. There are a few reports on the limited use of H_2S or SO_2 for sulfur nutrition by plants, but SO_4^{2-} is the major and ubiquitous source of plant sulfur.

Table 5
NITROGEN USE EFFICIENCY, DRY MATTER YIELD, AND NITROGEN CONTENT AT VARIOUS LEVELS OF NITROGEN FERTILIZATION IN A C_3, FESCUE, AND A C_4, BERMUDAGRASS, GRASS SOD

Fertilizer nitrogen (kg/ha)	Dry matter (DM) yield (metric tons/ha)		Nitrogen content of grass (% of DM)		Nitrogen use efficiency (tons DM/% N)		Nitrogen use efficiency (kg forage/kg N applied)	
	C_3	C_4	C_3	C_4	C_3	C_4	C_3	C_4
112	3.8	8.4	2.52	2.13	1.5	3.89	33.9	74.1
224	5.8	11.4	2.77	2.26	2.09	5.04	25.9	50.9
448	7.2	16.1	3.25	2.75	2.21	5.85	16.1	35.9
896	6.9	17.5	3.5	3.0	1.97	5.83	7.7	19.5

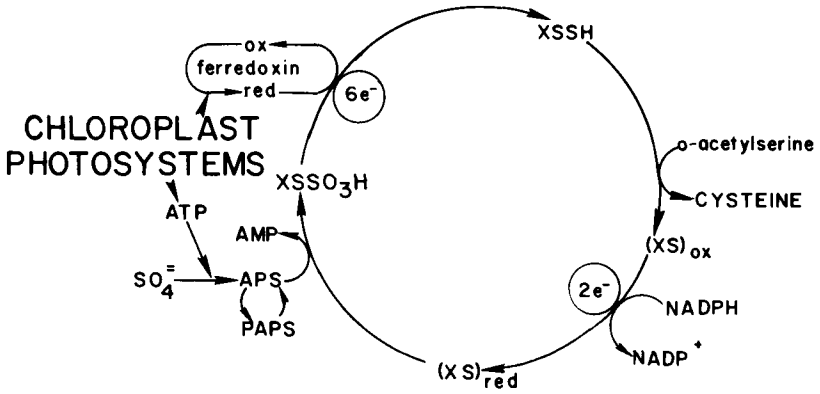


FIGURE 10. Scheme for chloroplast photoassimilation of sulfate. Adapted from Trebst and associates.[42-45]

6. Sulfate Reduction Cycle in Chloroplasts

The sulfate-reduction system through to the formation of cysteine has been partially characterized with leaves and chloroplasts fed $^{35}SO_4^{2-}$. The kinetics of the sequential labeling of intermediates after a pulse (usually 20 to 30 min) of $^{35}SO_4^{-}$ allowed the cycle in Figure 10 to be constructed.[42-45] Several unknown compounds are indicated in the cycle and these chloroplast-bound intermediates have not been identified. But the cycle clearly shows its dependency upon photosynthesis to produce ATP, reduced ferredoxin, NADPH, plus an acceptor of sulfide.

This assimilatory SO_4^{2-} reduction cycle is present in the chloroplasts of C_3 plants but it has not been studied in CAM plants. In C_4 plants the cycle is initiated in the bundle sheath cells.[46,47] Table 6 shows that the enzyme, ATP sulfurylase, which activates SO_4^{2-} with ATP[42,47] is localized in the bundle sheath cells of these C_4 weeds. The surprising localization of SO_4^{2-} activation in C_4 bundle sheath cells, considering that CO_2 and NO_3^- assimilation is initiated in mesophyll cells, was, however, not true for other sulfur metabolism enzymes or for the final incorporation of $^{35}SO_4^{2-}$ into proteins. These activities are present in both mesophyll and bundle sheath cells.[21,47] Thus as SO_4^{2-} moves up from the roots of C_4 plants via transpiration, it moves from the vascular tissue to the adjacent bundle sheath cells (Figures

Table 6
INTERCELLULAR LOCALIZATION OF ATP SULFURYLASE IN THE LEAVES OF VARIOUS C_4 WEEDS (μmol/mg chlorophyll/hr)

Plant	Whole leaf	Mesophyll protoplasts	Bundle sheath strands
$NADP^+$ malic enzyme type			
Digitaria sanguinalis	42.2	6.0	91.9
Echinochloa colonum	23.6	3.8	56.0
Echniochloa crus-galli	18.4	1.4	72.6
Euchlaena maxicana	24.1	2.4	64.4
Pennisetum americanum	79.1	0.7	183.8
NAD^+ malic enzyme type			
Chloris distichophylla	26.6	N.D.	36.8
Eleucine indica	16.2	0.8	21.5
Panicum bergii	24.4	0.8	39.7
Panicum miliaceum	10.9	N.D.	15.5
PEP-carboxykinase type			
Brachiaraia erucaeformis	20.0	3.2	64.3
Chloris gayana	28.7	1.1	53.2
Panicum maximum	35.5	2.5	37.8
Panicum molle	33.0	4.1	67.2
Urochloa mosambicensis	51.2	0.5	162.9

Note: N.D. = not detectable.

4 and 9) for activation and reduction. The form of sulfur moving between leaf cell types is unknown. Whether or not these pathways of sulfur assimilation (Figure 9) make C_4 crops or weeds more productive or more competitive than their attendant weeds or crops also is unknown.

7. Phosphorus Assimilation

Phosphoric acid in soil is the primary available form of this essential plant nutrient. Since 1954 we have known that green plants[48] and photosynthetic bacteria[49] use the energy of light through photosynthesis to assimilate PO_4^{3-} into ATP. Of course ATP is synthesized by other organelles and processes but chloroplast photophosphorylation will be emphasized here.

How is light energy used to assimilate PO_4^{3-} into ATP? A large body of data has accumulated supporting the chemiosmotic model, proposed originally by Mitchell,[50] of coupling ATP synthesis to electron flow via a membrane-associated vectorial flow of protons and electrons.

As drawn in Figure 11, the components of electron transport and ATP synthesis have a spatial orientation within the chloroplast thylakoid membrane. As a result of this spatial positioning, electrons originate from H_2O within the lumen of a thylakoid and proceed to flow through the Z scheme (Figure 2) using light energy to produce NADPH or reduced ferredoxin in the stroma. Protons are produced simultaneously within the lumen by two processes associated with electron transport. First, from the removal of electrons and the production of O_2 from H_2O. Second, from the vectorial flow of protons from the stroma across the thylakoid membrane to the lumen when the membrane pool of PQ is reduced and oxidized (Figure 11). Thus protons are moved from the stroma of an illuminated chloroplast across the membrane into the lumen.

The protons in the lumen then are channeled continuously during illumination back through

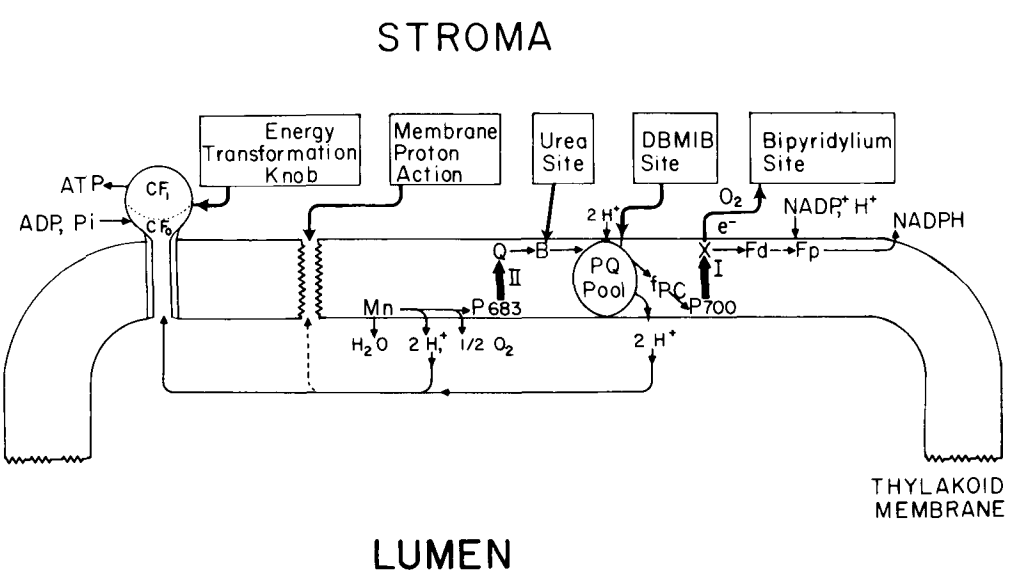


FIGURE 11. Spatial relationships of the photosynthetic electron-transport and photophosphorylation components within the chloroplast thylakoid membrane as they catalyze the vectorial flow of electrons and protons across the membrane upon illumination. Note the electron transport component abbreviations are the same as Figure 2. The sites or general location of known and potential herbicide action mechanisms are boxed in and connected by arrows to the thylakoid membrane components.

the membrane using enzymes and other proteins required for ATP synthesis with the resultant formation of ATP in the stroma (Figure 11). The exact mechanism of ATP synthesis through the CF_1-CF_0 complex is unknown but is a topic of much research.[51] The photophosphorylation model shown in Figure 11, involving a vectorial movement of protons, is strongly supported by classical photosynthesis experiments with isolated chloroplasts showing light-dependent proton uptake[52] and an acid bath to base bath transition phosphorylation.[53]

Clearly a chemical that interrupts proton movements or electron flow or changes membrane proton permeability is likely to inhibit photophosphorylation and may be a herbicide. We will consider such chemical action soon, but first, how can this ATP be utilized within a green cell? We know that within the chloroplast ATP can be used to assimilate CO_2, NO_2^-, and SO_4^{2-} plus various other synthetic uses such as starch, lipids, hormones, or nucleic acids. However, the chloroplast envelope is not freely permeable to ATP, NADPH, or to many other of its synthetic products. This raises the question of how the chloroplast communicates with the rest of the cell.

C. Chloroplast Communication with the Rest of the Plant Cell

Within the higher plant cell the chloroplast is bounded by an envelope consisting of a double membrane[54,55] that has its own enzyme complement, including synthetic activities. Gases such as CO_2 and O_2 readily cross the envelope but ions, particularly cations, either do not penetrate or cross at a characteristic rate for each ion. Ammonia crosses the envelope freely, NO_2^- crosses slowly, and SO_4^{2-} apparently is transported by a specific transport protein in the membrane.[56] Most C_3 cycle metabolites do not freely cross the envelope; indeed specific proteins, called translocators, transfer some intermediates across the envelope, often in exchange for PO_4^{3-}.

Early understanding of the envelope's selective permeability came from work with isolated intact chloroplasts which would not reduce added NADP nor utilize ADP to synthesize ATP. Breakage of the envelope resulted in the photochemical reduction of NADP and photo-

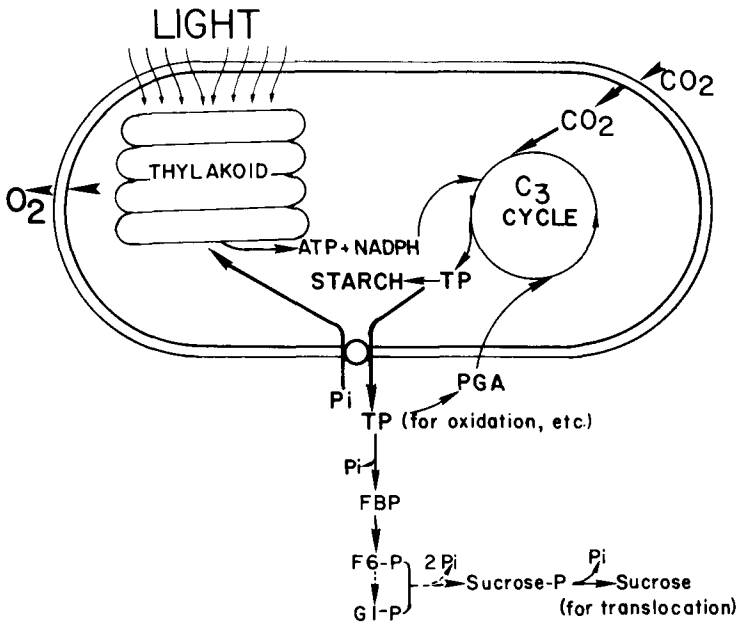


FIGURE 12. Scheme for a major pathway whereby intact chloroplasts communicate with the green cell cytoplasm via the exchange of Pi (PO_4^{3-}) for triose-Ps (primarily dihydroxyacetone phosphate and 3-phosphoglycerate, PGA).

phosphorylation. In an important study, Stocking and co-workers[57,58] realized that trioses were being shuttled across the envelope of intact chloroplasts to provide a sugar and ultimately ATP and reduced pyridine nucleotide in the green cell cytoplasm. This led to the discovery of the active exchange of PO_4^{3-} for triose-P or 3-PGA in the envelope.[55]

The phosphate translocator in the envelope catalyzes the export of fixed reduced carbon from the chloroplast to the rest of the cell. As diagramed in Figure 12, the phosphate translocator is a major site of communication between the chloroplast and the rest of the cell. The shuttling of PO_4^{3-} into the chloroplast from the cell cytoplasm results in a triose-P sugar, dihydroxyacetone phosphate primarily, addition to the cytoplasm. As illustrated (Figure 12) the triose in the cytoplasm can lead into several metabolic pathways such as: (1) its oxidation to yield reduced pyridine nucleotide and ATP, or (2) its condensation to form hexoses and then sucrose which is the major carbon transport material in most plants, or (3) since triose-Ps are central glycolytic intermediates their carbon can flow into essentially any pathway or product the plant may be operating or producing.

Other metabolites also return to the stroma across the envelope, no doubt including metabolites from the cytoplasm or other organelles such as amino acids for protein synthesis, photorespiration intermediates, or mitochondrial metabolites. And during the three types of C_4 photosynthesis each cell type must transport specific metabolites (Table 2, Figures 5 and 9) at rapid rates commensurate with their high rates of leaf CO_2 fixation. Thus green cells operate specific integrated networks of metabolism which have many controls we fail to understand in both the light and the dark. But we do know the chloroplast can selectively communicate with the cytoplasm (Figure 12) and instinctively we know this communication is finely regulated, otherwise the loss of trioses would stop photosynthesis or conversely the lack of sucrose would starve the rest of the plant or C_4 photosynthesis or other essential functions such as guard cell metabolism would not be possible.

D. Summary of Photosynthetic Assimilating Activities

Photosynthesis as an essential assimilatory process has been presented so it can be integrated into one's understanding of herbicide action and weed control usage. During photosynthesis photons are collected continuously on the antenna chlorophyll molecules (Figure 2) to steadily furnish the energy for producing low potential electrons and ATP. Simultaneously roots and leaves are collecting essential nutrients, e.g., PO_4^{3-}, NO_3^-, SO_4^{2-}, CO_2, and moving them to the chloroplast for assimilation. In the chloroplast these essential plant nutrients are united with low potential electrons and ATP to be transformed into substances such as starch, triose-P, amino acids, or lipids. These substances blend into other metabolic processes such that the autotrophic growth, maintenance, and reproduction of plants occurs. This continuous utilization of light, of course, makes plants the primarily biological producers in our biosphere. Thus we expect a chemical that inhibits some aspect of photosynthesis could be a herbicide since the very existence of plants can be dependent on photosynthesis; let us substantiate this expectation.

IV. HERBICIDE ACTION IN PHOTOSYNTHESIS

Having studied the diversity of reactions that make up the photosynthetic process, we can now critically evaluate the question of what the initial sites or primary modes of action of herbicides that affect photosynthesis are. The precision of our understanding about the molecular site or mechanism of action of a photosynthesis herbicide is dependent upon the depth of our current understanding of photosynthesis, which is a subject of intensive, continual research. Therefore, our understanding of herbicide action changes so that we can be more specific about where and how a herbicide inhibits photosynthesis. We will illustrate such changes in understanding in the next section with diuron.

In our considerations of herbicide action, we also will mention chemicals that inhibit some portion of photosynthesis but which turn out not to be herbicides. Within the intensive effort expended on photosynthesis research a variety of inhibitors are discovered, but these inhibitors often do not become herbicides. Why? It is beyond the scope of this chapter to pursue the reasons for not developing a photosynthesis inhibitor into a commercial herbicide, but generally the reasons center around cost, instability of the chemical during agricultural use, mammalian toxicity, or less activity in field usage than standard commercial herbicides. We have two major reasons for considering these photosynthesis inhibitors; first, to know they are available even though not commercialized as herbicides and second, to emphasize the fact that the known commercial herbicides are active at only a limited number of sites within the photosynthetic process. For easy location of the sites of inhibitor action, we will use the division of photosynthesis outlined by Moreland and associates[60] into processes such as electron transport, photophosphorylation, assimilation pathways, or indirect aspects such as the inhibition of chloroplast development. In evaluating herbicide and inhibitor action literature, the problem of deciding on site specificity continually arises. The approach taken in this review is the primary action occurs at the lowest inhibitor concentration. Neither herbicide action at higher concentrations nor other plant responses leading to weed control and phytotoxicity are considered primary. Even deciding on this approach does not allow the action of some inhibitors to be specifically classified since multiple sites are supported by data in the currently available literature.

A. Electron-Transport Inhibitors

By far the largest group of herbicides known (nearly 50% of the commercial herbicides) are active in the chloroplast electron-transport system at the so-called "urea site." The "urea site" is located between photosystem II and I prior to the reduction of the plastoquinone pool. Knowledge about the presence of a chloroplast "urea binding site" was initiated about

1956 from studying the inhibition of photosynthetic O_2 evolution by diuron with chloroplast fragments in the presence of the nonphysiological electron acceptor dichlorophenolindophenol;[59] this is the Hill reaction. At that time it was not known that plant photosynthesis involved two-light reactions, so only later was the site located between the photosystems. Only recently has it been demonstrated that diuron and related compounds bind to defined molecular weight proteins at the "urea site" while other herbicides, specific *s*-triazines, bind the proteins differently.[60-63]

The urea binding site in the chloroplast electron-transport chain is indicated in Figure 11. Numerous herbicides, as listed in Table 7, act at this site. This urea binding site is the most studied photosynthesis herbicide action site because diuron[59] and the triazines[60] have been standard research tools in photosynthesis for over two decades and a large number of structurally related herbicides have been synthesized and developed (Table 7). More detailed considerations and citations to the original literature of each herbicide in Table 7 are given by Moreland[61] and Fedtke.[62] The chloroplast urea binding site and the mode of *s*-triazine herbicide action is the topic of Chapter 7 so we will not consider it further; but it is clear that the urea site (Figure 11) can be subdivided into at least two classes (urea's and triazine's) of binding.

Photosynthetic electron-transport herbicides/inhibitors classically are identified with some characteristic response patterns by isolated broken chloroplasts. This pattern includes: (1) inhibition of the Hill reaction, which is reversible by washing or dilution; (2) a high affinity for chloroplast activities such as O_2 evolution, usually requiring only one herbicide/inhibitor molecule per 300 to 600 chlorophyll molecules or one molecule per electron-transport chain as in Figure 2; (3) an increase in the initial variable chlorophyll fluorescence upon illumination; (4) insensitivity of reactions by segments isolated from the electron-transport chain in Figure 2, e.g., urea binding site inhibitors do not effect isolated photosystem I reactions. A number of reactions catalyzed by isolated segments of the electron-transport chain in Figure 2 are well known[63] in which artificial electron donors and acceptors are used to study chain segments such as the donation of electrons from H_2O, photosystem II activity, photosystem I activity, or pyridine nucleotide reduction.

The high affinity of herbicides for the urea binding site is shown in the pI_{50} values for inhibition of the Hill reaction in Table 7. Generally a molar inhibitor concentration of 10^{-7} to $\sim 10^{-6}$ with isolated, broken chloroplasts will result in about one inhibitor molecule per 300 to 600 chlorophyll molecules in the chloroplast preparation. Most of the herbicides in Table 7 show this high affinity for chloroplasts. Clearly broken chloroplast preparations are not catalyzing CO_2 fixation or any of the other aspects of intact cellular chloroplast metabolism that we have studied. Most of the herbicides in Table 7 are specific with high binding affinities for inhibiting the urea binding site in chloroplasts, though some do show lower affinities and higher ranges of concentration in the pI_{50} values. Some of this variation is related to the fact that the "urea site" really is a "location" with several proteins and other components present and involved in the thylakoid membrane (see Chapter 7). At similar molar concentrations, a few herbicides (e.g., fluometuron, metflurazone, norflurazon, and ioxynil) also inhibit other plant processes such as carotenoid synthesis or respiration. Thus, their site of action is not specific, even at a low herbicide concentration. Furthermore, these additional activities are important in their agricultural usage for weed control; e.g., these carotenoid inhibitors are good commercial herbicides on germinating and developing seedlings (see Chapters 3 and 4).

A second site of inhibitor action during chloroplast electron transport is the "DBMIB site" (Figure 11). This group of compounds, which act as antagonists of the large chloroplast plastoquinone pool were introduced early in the 1970s.[64,65] They are halogenated or hydroxylated benzoquinone derivatives with dibromothymoquinone (DBMIB) being the most

Table 7
HERBICIDES THAT ACT AT THE CHLOROPLAST UREA BINDING SITE TO INHIBIT PHOTOSYNTHETIC ELECTRON TRANSPORT

Herbicide group and common name	pI_{50}[a]
UREA Herbicides	
Benzthiazuron	6.4
Buthidazole	—
Buthiuron	—
Buturon	—
Chlorbromuron	7.2
Chloroxuron	6.8 — 7.3
Chlortoluron	7.0
Cisanilide	6.8
Cycluron	5.3
Dimefuron	6.6
Diuron	6.7 — 7.5
Ethidimuron	6.8
Fenuron	4.6 — 5.5
Fluometuron	6.0
Isocarbamid	4.4
Isoproturon	6.8
Linuron	6.7 — 7.0
Methabenzthiazuron	6.5
Methazole	—
Metobromuron	6.0
Metoxuron	6.6
Monolinuron	5.6 — 6.1
Monuron	5.6 — 6.8
Neburon	6.7 — 6.9
Norea (Noruron)	5.8
Siduron	5.2
Tebuthiuron	6.9
Thiochlormethyl	7.3
ANILIDE Herbicides	
Chlorancryl	6.5 — 6.7
Cypromid	6.2
Karsil	6.5 — 7.0
Pentanochlor	—
Propanil	5.6 — 6.8
Swep	5.4 — 5.5
***s*-TRIAZINE Herbicides**	
Ametryn	6.8 — 7.3
Atraton	6.1 — 6.3
Atrazine	6.1 — 6.6
Cyanazine	—
Prometon	5.9
Prometryn	7.0
Propazine	5.4 — 6.3
Simazine	5.4 — 6.4
Simeton	5.2 — 6.3
Simetryn	6.6

Table 7 (continued)
HERBICIDES THAT ACT AT THE CHLOROPLAST UREA BINDING SITE TO INHIBIT PHOTOSYNTHETIC ELECTRON TRANSPORT

Herbicide group and common name	pI_{50}[a]
s-TRIAZINES	
Terbutryn	—
***as*-TRIAZINONE Herbicides**	
Isomethiozin	<5
Metamitron	5.4 — 6.1
Metribuzin	6.5 — 6.7
URACIL Herbicides	
Bromacil	5.8 — 6.3
Isocil	6.0
Lenacil	6.6
Terbacil	6.4
***bis*-CARBAMATE Herbicides**	
Desmedipham	6.2
Karbutilate	5.7
Phenmedipham	6.8 — 7.0
PYRIDAZINONE Herbicides	
Pyrazon	4.8 — 5.4
Metflurazone	5.4
Norflurazon	4.6
HYDROXYBENZONITRILE Herbicides	
Bromoxynil	4.8 — 6.6
Ioxynil	6.0 — 6.8

[a] pI_{50} equals -log of the molar concentration that inhibits chloroplast photosynthesis 50%. Photosynthesis was measured in various ways with these herbicides. The Hill reaction was the most common assay employed.

Data collected by Fedtke, C., *Biochemistry and Physiology of Herbicide Action*, Springer Verlag, New York, 1982.

useful inhibitor in electron-transport studies. But they also are inhibitors of mitochondrial electron transport and have not been developed into practical herbicides.

DBMIB has a high affinity for inhibiting the chloroplast Hill reaction with a pI_{50} near 7.2 with about one molecule of DBMIB needed per ~300 chlorophylls or one electron-transport chain.[64] Partial chloroplast reactions which remove electrons immediately after photosystem II are not sensitive to DBMIB; but reactions which involve the flow of electrons through PQ, cytochrome f, and plastocyanin to P_{700} are sensitive. Thus DBMIB has found wide experimental use in chloroplast electron-transport studies,[64] even though it is not a commercial herbicide.

In Figure 11, only the urea and DBMIB sites of electron-transport inhibition are indicated. Other inhibitors are known but they usually require high inhibitor concentrations for activity. For example, carboxyl cyanide phenylhydrazone (CCP) and NH_4OH inhibit near Mn in the oxygen evolution system; and $HgCl_2$ and KCN inhibit the copper protein plastocyanin. None of these chloroplast electron-transport inhibitors are commercial herbicides nor have herbicides been developed that are effective at these sites.

B. Electron-Transport Diversion

One could readily theorize electron diversion as a mode of herbicide action because the photosynthetic electron-transport chain involves essentially the entire span of biologically important oxidation reduction potentials from ~1.0 to -0.55 volts (Figure 2). Diverting electrons away from physiological functions such as NADP reduction or proton movements clearly should be lethal to plants. But in fact synthetic electron acceptors that can divert electrons from the chloroplast electron-transport chain are rare. Still, in the no-till agriculture of today, an important group of herbicides, the bipyridyliums, diquat and paraquat, can very effectively divert electrons from photosynthesis in the intact leaf. As shown in Figure 11, electrons are intercepted in photosystem I, thus stopping the reduction of ferredoxin and subsequent reactions as outlined in Figure 3.

A variety of synthetic electron acceptors are known which can divert electrons in isolated chloroplasts. These compounds include ferricyanide, dichlorophenolindophenol, phenazine methosulfate, FMN, phenylenediamines, benzoquinones, molybdates, and tungstates. These compounds often are used to study isolated segments of the electron-transport chain, but they are not highly phytotoxic nor have any been developed into agricultural herbicides. Why? One ready answer is possible failure to penetrate the intact leaf and to move to the chloroplast. Another answer lies in their chemistry, in that most of these electron acceptors are stable in air as reduced compounds. If we study the mode of action of the bipyridyliums we will understand why these other compounds are not phytotoxic.

How the bipyridyliums work as contact herbicides can be concisely understood by knowing their chemistry. The chemical properties of bipyridyliums which allow their successful use as contact herbicides are: (1) readily soluble in water with the capability of dissociation into a positive and negative ion; (2) the positive ion (the herbicide) can be tightly adsorbed by plants or other organic matter, or by soil clay minerals (this property results in an inactivation essential for use in no-till agriculture); (3) a negative chemical oxidation-reduction potential, ~ -0.3 to -0.4 volts which is near that of important biological electron-transport systems (Figure 2) and which is independent of pH; (4) a positive ion that readily accepts a single electron; and (5) a reduced form that is stable, but which is readily oxidized by O_2. With the constant regeneration by O_2 of the oxidized bipyridylium the herbicide is essentially always available to shunt electrons to O_2 and, therefore, is effective at catalytic amounts inside of leaves.[66]

The ability to intercept electrons in biological electron-transport systems and shunt them off to O_2 is the basic mode of action of the bipyridyliums. When these herbicides penetrate plant cells they can immediately intercept electrons from photosystem I (Figure 11) and/or oxidize $NADH_2$ and $NADPH_2$. Thus, two major energy-yielding oxidation/reduction processes in plants, namely photosynthesis and the oxidation of $NADH_2$ and $NADPH_2$ are stopped. The bipyridyliums short-circuit these normal routes of electron flow in plants, thereby removing reduced metabolites from plants.

Even though we now have determined how the bipyridylium herbicides work in short-circuiting electron flow, death results from a number of other processes which also occur because the plant has lost its natural biochemical equilibrium state through the loss of reduced materials. Thus a cascading of oxidation reactions, excess free radical production in the oxidizing environment of air, membrane breakdown, chlorophyll oxidation, and a variety

of other secondary responses can be observed[67,68] as phytotoxicity occurs (see Chapter 9 for further details).

Indeed, with such a fundamental type of action in biological systems through the acceptance of electrons and shunting them off to O_2 instead of to their natural electron acceptors, the bipyridyliums will kill plants in the light or in the dark (at a much slower rate) and they are toxic in mammals. Therefore, intelligent, careful use of the bipyridylium compounds is a necessity in handling them for agricultural purposes.

Most of the other diverters cited above, such as ferricyanide, do not regenerate their oxidized forms readily in air. Thus they must be present in substrate quantities to greatly influence the massive flow of electrons passing through photosynthesis in a weed or crop in the field. Since they are not active in catalytic amounts, even if they were phytotoxic, the cost of large chemical applications would not be economically feasible.

C. Uncouplers and Inhibitors of ATP Synthesis

The transformation by chloroplasts of light energy ultimately into ATP makes this energy conservation process another likely site for photosynthetic herbicide development. We know (Figure 11) ATP synthesis involves the vectorial movements of protons across the thylakoid membrane toward the stroma plus the action of the chloroplast coupling factor complex[69] (we have called this complex CF_1-CF_0). These sites or modes of inhibitor action are shown on Figure 11 as "membrane proton action" and as the "energy transformation knob."

Classically, compounds which dissociate ATP synthesis from electron transport are classified as uncouplers and normally a stimulation of electron transport follows the dissociation. Since the exact mode of uncoupler action still is uncertain, we will group them as compounds causing "membrane proton action." Moreland[61] has catagorized a number of herbicides as "inhibitory uncouplers;" these usually are active uncouplers both in chloroplasts and mitochondria. The photosynthetic "inhibitory uncoupler" herbicides include: acylanilides, benzimidazoles, bromofenoxim, dinitroanilines, dinitrophenols, halogenated benzonitriles, imidazoles, *N*-phenylcarbamates, pyridinols, and thiadiazoles. These herbicides also block electron transport at the urea site and uncouple ATP synthesis.[61] In addition, as already mentioned, when considering other herbicides acting at the "urea site" in Table 7, e.g., ioxynil,[70] some of these herbicides also act simultaneously as both electron-transport inhibitors and as uncouplers of photophosphorylation. The exact mode of action of herbicide uncouplers in perturbing the thylakoid membrane is not certain, but whatever this membrane action is, be it a change in fluidity or proton action (Figure 11) or otherwise, it results in a dissipation of proton and other ion gradients across the membrane, stopping ATP synthesis.

Perfluidone appears to function solely as an uncoupler of photophosphorylation.[61,70] It is active near physiological chloroplast pH values, $\sim$ 7.8 to 8.0, and inhibits the chloroplast Mg-ATPase with a pI_{50} of 5×10^{-5} *M* without inhibiting electron transport.[70] However, its primary mode of herbicidal action still is obscure since effects on hormone-type plant growth responses and on lipid metabolism also can be observed at similar molar concentrations of perfluidone.[62]

Other uncouplers are well known in photosynthesis research including: NH_4^+, amines, atebrin, FCCP, CCP, gramicidin, and nigericin. A thorough listing of photophosphorylation uncouplers is available.[71] However, these uncouplers do not block chloroplast electron transport. It is notable that NH_4^+ and amines do not uncouple mitochondrial oxidative phosphorylation so their action is specific for the chloroplast membrane. None of these uncouplers are highly phytotoxic in amounts which could be economically applied in agriculture for herbicidal purposes.

Chemicals that inhibit ATP synthesis act within the energy transformation knob site shown in Figure 11. This site is called a knob because it appears as a knob protruding from the thylakoid membrane in electron microscopy studies. CF_1-CF_0 acts as a proton-translocating

ATP-synthesizing complex; but it also may act in reverse as an ATPase and this is the way it often is measured in in vitro work.[69] Inhibitors acting at the energy transformation knob do not directly effect electron transport. One herbicide, nitrofen, acts on the CF_1-CF_0 complex to inhibit ATP synthesis at near 10 μM concentrations.[72] Since, as just mentioned, perfluidone also inhibits the Mg-ATPase,[70] perhaps it also is active within the energy transformation knob (Figure 11).

Several other chemicals, known in photophosphorylation research with isolated chloroplast fragments or with purified proteins,[69,71] also act in the energy transformation knob. DCCD and triphenyltin chloride bind to a membrane protein-lipid, while phlorizin inhibits CF_1 at similar concentrations that inhibit photophosphorylation without affecting electron transport. However, none of these chemicals are phytotoxic enough to be herbicides; likely because they don't move to the knob inside intact leaves in sufficient amounts to be toxic.

D. Carbon Metabolism Inhibitors

In this consideration of the inhibitors known to be acting within carbon metabolism, we will find that few commercial herbicides are recognized as having a site of specific action in chloroplast carbon metabolism. Usually these inhibitors act on specific enzymes but one herbicide, benzadox, seems to have a unique mode of action and potential usefulness in altering carbon metabolism. Each of the herbicides just considered that inhibit photosynthetic electron transport or ATP synthesis also would inhibit CO_2 fixation because all chloroplast carbon metabolism cycles and pathways require ATP and NADPH.

About a decade ago a mixture of DL-glyceraldehyde was recognized as an inhibitor of the C_3 cycle, apparently by blocking the conversion of triose-P into ribulose 1,5-P_2.[73] Phosphoribulokinase is inhibited by millimolar amounts of DL-glyceraldehyde,[73] but a more sensitive site is present within the conversion of fructose 1,6-P_2 to pentose-P in the chloroplast reductive pentose phosphate pathway.[74] Unfortunately, millimolar concentrations of DL-glyceraldehyde are required for these inhibitions so no commercial use has been made of this inhibitor.

Within the C_3 cycle, the inhibition of any enzyme would probably result in phytotoxicity. For example, a lethal mutant of *Arabidopsis* has been discovered with a lesion in the carbon oxidation cycle.[75] The mutant lacks the enzyme phosphoglycolate phosphatase, therefore, phosphoglycolate accumulates. Phosphoglycolate is a potent, pI_{50} = 15 μM, inhibitor of triose-P isomerase[76] and apparently is the cause of death.[75] In other work on triose-P isomerase, analogues of its substrate, dihydroxyacetone-P, were synthesized and shown to be good inhibitors.[77,78] Recently one of these, iodoacetol-P, was shown to inhibit chloroplast CO_2 fixation at micromolar concentrations and to inhibit both the NADPH and NADH-dependent glyceraldehyde 3-P dehydrogenase at nanomolar concentrations.[79] Thus, the reductive phase of the C_3 cycle is blocked by iodoacetol phosphate. Other glyceraldehyde 3-P dehydrogenase inhibitors such as iodoacetate and iodoacetamide are well known in biochemistry research but require millimolar concentrations to be effective in inhibiting the enzyme or photosynthesis.

Of course within the C_3 cycle ribulose 1,5-P_2 carboxylase is an obvious site one might inhibit and thus produce a herbicide. This carboxylase is perhaps the most studied plant enzyme. Consequently, a number of substrate analogues and other inhibitors are known. A carboxyribitol 1,5-P_2 preparation was shown to be a competitive inhibitor with ribulose 1,5-P_2 using purified ribulose 1,5-P_2 carboxylase about a decade ago.[80,81] It is likely carboxyarabinitol 1,5-P_2 also was a contaminant in that chemical preparation and it also is a potent ribulose 1,5-P_2 carboxylase inhibitor.[82] Other carboxylase inhibitors are known such as *p*-chloromercuribenzoate,[83] xyulose 1,5-P_2,[84] pyridoxal 5′-P,[85] 2,3-butanedione, phenyl-glyoxal, and other inhibitors, many of which bind at the active site of the enzyme.[86] None of these inhibitors are known to be herbicides. Ribulose 1,5-P_2 carboxylase is a regulatory enzyme.

Seemingly its in vitro activity is being understood,[87] but as a herbicidal target one in vitro problem is the large amount of this protein in the chloroplast. In vitro values of ribulose 1,5-P_2 carboxylase comprising 25 to 35% of the total soluble chloroplast protein are common in weeds and crops. Clearly to inhibit such a large amount of leaf protein would require logistically difficult and, probably, prohibitively expensive herbicide levels.

Also related to the C_3 cycle, the potential for chemical inhibition of the photosynthetic carbon oxidation cycle (PCO), or photorespiration, has been an active research area. Initially it was thought that inhibition of the PCO cycle would be beneficial for plant growth by stopping this carbon loss cycle.[15,20,22] Inhibitors within the PCO cycle are known including α-hydroxypyridinemethanesulfonic acid,[88] isonicotinic acid hydrazide,[89] and α-hydroxy-3-butynoate.[90] However, these inhibitors are needed at millimolar concentrations to affect intact leaves, thus limiting their potential agriculture use. And by extrapolation from the lethal PCO cycle *Arabodopsis* mutant work,[75] it is likely they would be phytotoxic rather than beneficial. Indeed the PCO cycle is a likely target for herbicide development.

C_4 photosynthesis has a number of unique biochemical steps (several are given in Table 2 or shown in Figure 9), in comparison with C_3 photosynthesis, where herbicides could be used selectively. The C_4 decarboxylases, Table 2, are unique sites and several decarboxylase inhibitors are known. 3-Mercaptopicolinic acid (3-MPA) is an effective inhibitor of PEP carboxykinase with an in vitro pI_{50} of near 10^{-5} *M* in C_4 plants such as *Panicum maximum*.[91] Oxalate is an inhibitor of $NADP^+$ malic enzyme.[92] However, neither compound has been effective in blocking net CO_2 fixation. Another possible specific decarboxylase inhibitor is the recent discovery of the herbicidal effects of methanearsonate on johnsongrass.[93] Shoot necrosis of johnsongrass (but not of corn) is observed and it is speculated that johnsongrass malic enzyme is sensitive to this sulfhydryl group reagent.[93]

Another potential site for inhibiting C_4 photosynthesis was recently found which also has ramifications in altering C_3 photosynthesis. Aminooxyacetate, a herbicide and a known inhibitor of transaminases,[94,95] was shown to inhibit an aspartate-dependent photosynthetic O_2 evolution in crabgrass mesophyll cells.[96] Benzadox is a herbicide that controls C_4 weeds such as *Kochia scoparia*, *Setaria viridis*, and *Salsola kali*.[97] Benzadox was shown to inhibit *Panicum miliaceum* leaf photosynthesis at millimolar concentrations, and also to be an inhibitor of leaf transaminase activity.[97] Benzadox in vivo is a poor inhibitor of transaminase activity but aminooxyacetate is a good inhibitor at 10^{-6} to 10^{-5} *M*. It is speculated that benzadox is metabolized to aminooxyacetate in these C_4 leaves; thereby inhibiting transaminases and exerting a herbicidal effect.[97] In work with C_3 cycle algae[98,99] aminooxyacetate stimulates the light-dependent formation of glycolate. Thus, it may also inhibit the transaminase involved in the PCO cycle. Collectively this research indicates that plant transaminases are a potential site for altering carbon flux and exerting herbicidal action.

Finally, a negative aspect of many known carbon metabolism inhibitors such as some of those just presented, is that they often are phosphate esters. Many highly active phosphate ester phosphatases are present in nature. Thus it is unlikely that a phosphate ester would be sufficiently stable in nature to be agriculturally useful.

E. Nitrogen Assimilation Inhibitors

The use of benzadox in controlling some C_4 plants was just presented.[96] Benzadox's possible involvement through inhibiting amino transferase activities is a key aspect of plant nitrogen utilization. There are no known herbicidal inhibitors of NO_3^-, or NO_2^- reduction; though inhibitors of these enzymes, such as cyanide, are known.[100] However, a putative mode of herbicide action is the accumulation of NO_2^- (discussed further in Chapter 4).[101] Nitrite accumulation in the presence of herbicides is not limited to herbicides acting on chloroplasts and high herbicide concentrations are necessary. This possible mode of herbicide action is not well understood.[101] Within ammonia assimilation and transfer two inhibitors

are widely employed in plant research. Methionine sulfoximine is an inhibitor of glutamine synthetase[102] and azaserine is an inhibitor of glutamine-amide transfer reactions including GOGAT.[103] Neither inhibitor, however, inhibits leaf photosynthesis[104] and both are used in millimolar concentration in in vitro studies, which indicates impractical usage in agriculture.

F. Photosynthetic Pigment Synthesis Inhibitors

Inhibiting synthesis of the major leaf pigments, either chlorophylls or carotenoids, surely will be phytotoxic since plants must cope with light daily. Surprisingly, no known herbicide action initially is directed at these pigments once they are in place within an intact mature chloroplast. But carotenoid synthesis is a favorite herbicide target and at least one herbicide inhibits chlorophyll synthesis (see Chapter 4).

The chemical DTP (1,3-dimethyl-4-(2,4-dichlorobenzoyl)-5-hydroxypyrazole) has herbicidal properties which are consistent with it blocking chlorophyll synthesis.[105] With new leaves chlorosis occurs; but developed leaves do not bleach at micromolar DTP treatment levels. With mature leaves and millimolar DTP treatment, chlorosis will develop slowly. It is suggested that DTP blocks the synthesis of protochlorophyllide in leaves and consequently induces chlorosis.[105]

Carotenoid synthesis inhibitors comprise a major class of herbicides that generally cause leaf chlorosis as a result of chlorophyll degradation in the light when carotenoid synthesis is blocked. These herbicides include amitrole, pyrichlor, difunone, dichlomate, haloxydine, norflurazon, metflurazon, and fluridone.[106] Most of these herbicides act to inhibit α- and β-carotene formation and result in the accumulation of intermediates of their biosynthesis such as phytoene, phytofluene, and sometimes δ-carotene. A more detailed view of the action of carotenoid inhibitors is in Chapter 4. But it is pertinent to our understanding of the intact chloroplast that carotenoids are in both the chloroplast envelope and the thylakoid membrane plus carotenoid synthesis apparently occurs in the chloroplast envelope.[54]

V. SUMMARY AND CONCLUSIONS

We have examined the physiological and biochemical functions of the intact photosynthesizing chloroplast. Powered by the energy of sunlight, the chloroplast allows plants to photoassimilate essential elements such as C,N,S, and P, thereby living autotrophically. This natural site has been exploited for herbicide development in that the majority of the available commercial herbicides act on the chloroplast. Indeed over 50% of the known herbicides act at the urea site (Figure 11). The urea site should today be considered as a location in the photosynthetic electron-transport chain (Figure 2) which has several proteins and binding sites involved in addition to an electron-transport capability.

A more detailed study of photosynthetic herbicide action, as in Figure 11 or in pigment synthesis, reveals that surprisingly few herbicides act with other electron-transport components, in carbon metabolism, in nitrogen metabolism, in phosphorus metabolism, in sulfur metabolism, or in chloroplast communication with the cytoplasm. Indeed, there are many likely targets of herbicide action unique to photosynthesis that remain to be exploited. The limited number of herbicide sites in photosynthesis (Figure 11) is apparent when one considers that the major sites of action of currently used herbicides are limited to the stroma (or outside) surface of the thylakoid membrane. The lumen surface has not been susceptible to herbicide action. Furthermore, the soluble enzymes in the stroma have not been susceptible to herbicide development. So the spatial positioning of various photosynthesis components has somehow rendered the outer thylakoid surface more susceptible to herbicide development than any other position in the chloroplast. Collectively, if one also carefully considers the differences in anatomy (Figures 4 and 9) and physiology of specific weeds and crops, plus the known existence of multiple pathways of biochemistry (Tables 1 and 2), a variety of other targets exist for developing specific herbicides for specific plant groups.

REFERENCES

1. **van Niel, C. B.,** The bacterial photosynthesis and their importance for the general problem of photosynthesis, *Adv. Enzymol.*, 1, 263, 1941.
2. **Kamen, M.,** *Primary Processes in Photosynthesis*, Academic Press, New York, 1963.
3. **Black, C. C.,** Biosolar resources; Fundamental biological processes, in *CRC Handbook of Biosolar Resources*, Vol. 1, Part 1, Mitsui, A. and Black, C. C., Eds., CRC Press, Boca Raton, Fla., 1982, 3.
4. **Myers, J. and Cramer, M. L.,** Reconsideration of the photosynthetic mechanism in Chlorella, *Science*, 105, 552, 1947.
5. **Krinsky, N. L.,** Function, in *Carotenoids*, Isler, O., Ed., Birkhäuser Verlag, Basel, 1971, 669.
6. **Bassham, J. A. and Calvin, M.,** *The Path of Carbon in Photosynthesis*, Prentice-Hall, Englewood Cliffs, N.J., 1957.
7. **Norris, L., Norris, R. E., and Calvin, M.,** A survey of the rates and products of short-term photosynthesis in plants of nine phyla, *J. Exp. Bot.*, 6, 64, 1955.
8. **Bassham, J. A.,** Photosynthetic carbon dioxide assimilation via the reductive pentose phosphate cycle (C_3 cycle), in *CRC Handbook of Biosolar Resources*, Vol. 1, Part 1, Mitsui, A. and Black, C. C., Eds., CRC Press, Boca Raton, Fla., 1982, 181.
9. **Ray, T. B. and Black, C. C.,** The C_4 pathway and its regulation, in *Photosynthesis II. Photosynthetic Carbon Metabolism and Related Processes*, Vol. 6, Gibbs, M. and Latzko, E., Eds., Encyclopedia of Plant Physiology, New Series, Springer-Verlag, New York, 1979, 77.
10. **Outlaw, W. H., Jr., Manchester, J., DiCamelli, C. A., Randall, D. D., Rapp, B., and Veith, G. M.,** Photosynthetic carbon reduction pathway is absent in chloroplast of *Vicia faba* guard cells, *Proc. Nat. Acad. Sci. U.S.A.*, 76, 6371, 1979.
11. **Bachofen, R., Buchanan, B. B., and Arnon, D. I.,** Ferredoxin as a reductant in pyruvate synthesis by a bacterial extract, *Proc. Natl. Acad. Sci. U.S.A.*, 51, 690, 1964.
12. **Buchanan, B. B.,** Carbon dioxide assimilation in photosynthetic bacteria, in *CRC Handbook of Biosolar Resources*, Vol. 1, Part 1, Mitsui, A. and Black, C. C., Eds., CRC Press, Boca Raton, Fla., 1982, 175.
13. **Kortschak, H. P., Hartt, C. E., and Burr, G. O.,** Carbon dioxide fixation in sugarcane leaves, *Plant Physiol.*, 40, 209, 1965.
14. **Hatch, M. D. and Slack, C. R.,** Photosynthesis by sugar-cane leaves, *Biochem. J.*, 101, 103, 1966.
15. **Hatch, M. D., Osmond, C. B., and Slatyer, R. D., Eds.,** *Photosynthesis and Photorespiration*, John Wiley & Sons, New York, 1971.
16. **Ranson, S. L. and Thomas, M.,** Crassulacean acid metabolism, *Annu. Rev. Plant Physiol.*, 11, 81, 1960.
17. **Black, C. C., Chen, T. M., and Brown, R. H.,** Biochemical basis for plant competition, *Weed Sci.*, 17, 338, 1969.
18. **Chen, T. M., Brown, R. H., and Black, C. C.,** CO_2 compensation concentration, rate of photosynthesis, and carbonic anhydrase activity of plants, *Weed Sci.*, 18, 399, 1970.
19. **Black, C. C.,** Ecological implications of dividing plants into groups with distinct photosynthetic production capacities, *Adv. Ecol. Res.*, 1, 87, 1971.
20. **Black, C. C.,** Photosynthetic carbon fixation in relation to net CO_2 uptake, *Annu. Rev. Plant Physiol.*, 24, 253, 1973.
21. **Campbell, W. H. and Black, C. C.,** Cellular aspects of C_4 leaf metabolism, *Recent Adv. Phytochem.*, 16, 223, 1982.
22. **Burris, R. H. and Black, C. C.,** *Carbon Dioxide Metabolism and Plant Productivity*, University Park Press, Baltimore, 1976.
23. **Hatch, M. D. and Osmond, C. B.,** Compartmentation and transport in C_4 photosynthesis, in *Encyclopedia of Plant Physiology, New Series*, Vol. 3, Heber, U. and Stocking, C. R., Eds., Springer-Verlag, Heidelberg, 1976, 144.
24. **Smith, B. N.,** General characteristics of terrestrial plants (agronomic and forests) — C_3, C_4, and Crassulacean acid metabolism plants, in *CRC Handbook of Biosolar Resources*, Vol. 1, Part 1, Mitsui, A. and Black, C. C., Eds., CRC Press, Boca Raton, Fla., 1982, 99.
25. **Black, C. C.,** Crassulacean acid metabolism, in *CRC Handbook of Biosolar Resources*, Vol. 1, Part 1, Mitsui, A. and Black, C. C., Eds., CRC Press, Boca Raton, Fla., 1982, 191.
26. **Osmond, C. B.,** Crassulacean acid metabolism: a curiosity in context, *Annu. Rev. Plant Physiol.*, 29, 379, 1978.
27. **Kluge, M. and Ting, I. P.,** *Crassulacean Acid Metabolism* (Analysis of an Ecological Adaptation), Springer-Verlag, Berlin, 1978.
28. **Ting, I. P. and Gibbs, M., Eds.,** *Crassulacean Acid Metabolism*, American Society of Plant Physiologists, Rockville, Md., 1982, 316.

29. **Pucher, G. W., Vickery, H. B., Abrahams, M. D., and Leavenworth, C. S.,** Studies in the metabolism of crassulacean plants; diurnal variation in organic acids and starch in excised plants *Bryophyllum calycinum*, *Plant Physiol.*, 24, 610, 1949.
30. **Bender, M. M., Rouhani, I., Vines, H. M., and Black, C. C.,** $^{13}C/^{12}C$ ratio changes in Crassulacean acid metabolism plants, *Plant Physiol.*, 52, 427, 1973.
31. **Ku, S. B., Shieh, Y. J., Reger, B. J., and Black, C. C.,** Photosynthetic characteristics of *Portulaca grandiflora*, a succulent C_4 dicot, *Plant Physiol.*, 68, 1073, 1981.
32. **Beevers, L. and Hageman, R. H.,** The role of light in nitrate metabolism of higher plants, *Photophysiology*, 7, 85, 1972.
33. **Hewitt, E. J.,** Assimilatory nitrate nitrite reduction, *Annu. Rev. Plant Physiol.*, 26, 73, 1975.
34. **Miflin, B. J. and Lea, P. J.,** The pathway of nitrogen assimilation in plants, *Phytochemistry*, 15, 873, 1976.
35. **Leech, R. M. and Murphy, D. J.,** The cooperative function of chloroplasts in the biosysnthesis of small molecules, in *The Intact Chloroplast*, Barber, J., Ed., Elsevier, Amsterdam, 1976.
36. **O'Neal, D. and Joy, K. W.,** Localization of glutamine synthetase in chloroplasts, *Nature (London) New Biol.*, 246, 61, 1973.
37. **Haystead, A.,** Glutamine synthetase in the chloroplasts of *Vicia faba*, *Planta*, 111, 271, 1973.
38. **Lea, P. J. and Miflin, B. J.,** Alternative route for nitrogen assimilation in plants, *Nature (London)*, 251, 614, 1974.
39. **Chen, T. M., Dittrich, P., Campbell, W. H., and Black, C. C.,** Metabolism of epidermal tissues, mesophyll cells, and bundle sheath strands resolved from mature nutsedge leaves, *Arch. Biochem. Biophys.*, 163, 246, 1974.
40. **Moore, R. and Black, C. C.,** Nitrogen assimilation pathways in leaf mesophyll and bundle sheath cells of C_4 photosynthesis plants formulated from comparative studies with *Digitaria sanguinalis* (L.) Scop., *Plant Physiol.*, 64, 309, 1979.
41. **Brown, R. H.,** A difference in N use efficiency in C_3 and C_4 crop plants and its implications in adaptation and evolution, *Crop Sci.*, 18, 93, 1978.
42. **Trebst, A. and Schmidt, A.,** Photosynthetic sulfate and sulfite reduction by chloroplasts, in *Progress in Photosynthesis Research*, Vol. 3, Metzner, H., Ed., W. Junk, The Hague, The Netherlands, 1969.
43. **Schmidt, A. and Trebst, A.,** The mechanism of photosynthetic sulfate reduction by isolated chloroplasts, *Biochim. Biophys. Acta*, 180, 529, 1969.
44. **Schmidt, A.,** Photosynthetic assimilation of sulfur compounds, in *Encyclopedia of Plant Physiology*, New Series, Vol. 6, Gibbs, M. and Latzko, E., Eds., Springer-Verlag, Heidelberg, 1979.
45. **Trebst, A.,** Sulfur assimilation in higher plants and algae, in *CRC Handbook of Biosolar Resources*, Vol. 1, Part 1, Mitsui, A. and Black, C. C., Eds., CRC Press, Boca Raton, Fla., 1982, 289.
46. **Gerwick, B. C. and Black, C. C.,** Sulfur assimilation in C_4 plants, *Plant Physiol.*, 64, 590, 1979.
47. **Gerwick, B. C., Ku, S. B., and Black, C. C.,** Initiation of sulfate activation: a variation in C_4 photosynthesis plants, *Science*, 209, 513, 1980.
48. **Arnon, D. I., Whatley, F. R., and Allen, M. B.,** Photosynthesis by isolated chloroplasts. II. Photosynthetic phosphorylation, the conversion of light into phosphate bond energy, *J. Am. Chem. Soc.*, 76, 6324, 1954.
49. **Frenkel, A. W.,** Light induced phosphorylation by cell-free preparations of photosynthetic bacteria, *J. Am. Chem. Soc.*, 76, 5568, 1954.
50. **Mitchell, P.,** Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism, *Nature (London)*, 191, 144, 1961.
51. **Selman, B. R. and Selman-Reimer, S., Eds.,** *Energy Coupling In Photosynthesis*, Elsevier/North Holland, Amsterdam, 1981.
52. **Neumann, J. and Jagendorf, A. T.,** Light-induced pH changes related to phosphorylation by chloroplasts, *Arch. Biochem. Biophys.*, 107, 109, 1964.
53. **Jagendorf, A. T. and Uribe, E.,** ATP formation caused by acid-base transition of spinach chloroplasts, *Proc. Natl. Acad. Sci. U.S.A.*, 55, 170, 1966.
54. **Douce, R. and Joyard, J.,** Structure and function of the plastid envelope, *Adv. Bot. Res.*, 7, 1, 1979.
55. **Heber, U. and Heldt, H. W.,** The chloroplast envelope: Structure, function, and role in leaf metabolism, *Annu. Rev. Plant Physiol.*, 32, 139, 1981.
56. **Hampp, R. and Ziegler, I.,** Sulfate and sulfite translocation via the phosphate translocator of the inner envelope membrane of chloroplasts, *Planta*, 137, 309, 1977.
57. **Robinson, M. J. and Stocking, C. R.,** Oxygen evolution and the permeability of the outer envelope of isolated whole chloroplasts, *Plant Physiol.*, 43, 1597, 1968.
58. **Stocking, C. R. and Larson, S.,** A chloroplast-cytoplasmic shuttle and the reduction of extraplastid NAD, *Biochem. Biophys. Res. Commun.*, 37, 278, 1969.
59. **Wessels, J. S. C. and van der Veen, R.,** The action of some derivatives of phenylurethan and 3-phenyl-1, 1-dimethylurea on the Hill reaction, *Biochim. Biophys. Acta*, 19, 548, 1956.

60. **Moreland, D. E., Gentner, W. A., Hilton, J. L., and Hill, K. K.,** Studies on the mechanism of herbicidal action of 2-chloro-4,6-bis(ethylamino)-s-triazine, *Plant Physiol.*, 34, 432, 1959.
61. **Moreland, D. E.,** Mechanism of action of herbicides, *Annu. Rev. Plant Physiol.*, 31, 597, 1980.
62. **Fedtke, C.,** *Biochemistry and Physiology of Herbicide Action*, Springer-Verlag, New York, 1982.
63. **Izawa, S.,** Acceptors and donors for chloroplast electron transport, *Methods Enzymol.*, 69, 413, 1980.
64. **Trebst, A.,** Inhibitors in electron flow: tools for the functional and structural localization of carriers and energy conservation sites, *Methods Enzymol.*, 69, 675, 1980.
65. **Trebst, A., Harth, E., and Draber, W.,** On a new inhibitor of photosynthetic electron-transport in isolated chloroplasts, *Z. Naturforsch.*, 25b, 1157, 1970.
66. **Black, C. C.,** How herbicides work—the bipyridylium herbicides, *Weeds Today*, Fall, 12, 1977.
67. **Dodge, A. D.,** Some mechanisms of herbicide action, *Sci. Prog. (London)*, 62, 447, 1975.
68. **Summers, L. A.,** *The Bipyridylium Herbicides*, Academic Press, New York, 1980.
69. **McCarty, R. E.,** Delineation of the mechanism of ATP synthesis in chloroplasts: use of uncouplers, energy transfer inhibitors, and modifiers of coupling factor 1, *Methods Enzymol.*, 69, 719, 1980.
70. **Alsop, W. R. and Moreland, D. E.,** Effects of herbicides on the light-activated, magnesium-dependent ATPase in isolated spinach (*Spinacia oleracea* L.) chloroplasts, *Pestic. Biochem. Physiol.*, 5, 163, 1975.
71. **Izawa, S. and Good, N. E.,** Inhibition of photosynthetic electron transport and photophosphorylation, *Methods Enzymol.*, 24, 355, 1972.
72. **Lambert, R., Kunert, K.-J., and Böger, P.,** On the phytotoxic mode of action of nitrofen, *Pestic. Biochem. Physiol.*, 11, 267, 1979.
73. **Stokes, D. M. and Walker, D. A.,** Photosynthesis by isolated chloroplasts. Inhibition by DL-glyceraldehyde of carbon dioxide assimilation, *Biochem. J.*, 128, 1147, 1972.
74. **Bamberger, E. S. and Avron, M.,** Site of action of inhibitors of carbon dioxide assimilation by whole lettuce chloroplasts, *Plant Physiol.*, 56, 481, 1975.
75. **Somerville, C. R. and Ogren, W. L.,** A phosphoglycolate phosphatase-deficient mutant of *Arabidopsis*, *Nature (London)*, 280, 833, 1979.
76. **Anderson, L. E.,** Chloroplast and cytoplasmic enzymes. II. Pea leaf triose phosphate isomerases, *Biochim. Biophys. Acta*, 235, 237, 1971.
77. **Hartman, F. C.,** Haloacetal phosphates. Potential active-site reagents for aldolase, triose phosphate isomerase, and glycerophosphate dehydrogenase. I. Preparation ond properties, *Biochemistry*, 9, 1776, 1970.
78. **Hartman, F. C.,** Haloacetol phosphates. Characterization of the active site of rabbit muscle triose phosphate isomerase, *Biochemistry*, 10, 146, 1971.
79. **Usada, H. and Edwards, G. E.,** Inhibition of photosynthetic carbon metabolism in isolated chloroplasts by iodoacetal phosphate, *Plant Physiol.*, 67, 854, 1981.
80. **Wishnick, M., Lane, M. D., and Scrutton, M. C.,** The interaction of metal ions with ribulose 1,5-diphosphate carboxylase from spinach, *J. Biol. Chem.*, 245, 4939, 1970.
81. **Siegel, M. I. and Lane, M. D.,** Chemical and enzymatic participation of a 2-carboxy-3-ketoribitol-1,5-diphosphate intermediate in the carboxylation of ribulose-1,5-diphosphate, *J. Biol. Chem.*, 248, 5486, 1973.
82. **Pierce, J., Tolbert, N. E., and Barker, R.,** Interaction of ribulose bisphosphate carboxylase/oxygenase with transition-state analogues, *Biochemistry*, 19, 934, 1980.
83. **Takabe, T. and Akazawa, T.,** The role of sulfhydryl groups in the ribulose-1,5-bisphosphate carboxylase and oxygenase reactions, *Arch. Biochem. Biophys.*, 169, 686, 1975.
84. **Paech, C., Pierce, J., McCurry, S. D., and Tolbert, N. E.,** Inhibition of ribulose-1,5-bisphosphate carboxylase/oxygenase by ribulose-1,5-bisphosphate epimerization and degradation products, *Biochem. Biophys. Res. Commun.*, 83, 1084, 1978.
85. **Paech, C., Ryan, F. J., and Tolbert, N. E.,** Essential primary amino groups of ribulose bisphosphate carboxylase/oxygenase indicated by reaction with pyridoxal 5′-phosphate, *Arch. Biochem. Biophys.*, 179, 279, 1977.
86. **Hartman, F. C., Norton, I. L., Stringer, C. D., and Schloss, J. V.,** Attempts to apply affinity labeling techniques to ribulose bisphosphate carboxylase/oxygenase, in *Photosynthetic Carbon Assimilation*, Siegelman, H. and Hind, G., Eds., Plenum Press, New York, 1978, 245.
87. **Lorimer, G. H., Badger, M. R., and Heldt, H. W.,** The activation of ribulose 1,5-bisphosphate carboxylase/oxygenase, in *Photosynthetic Carbon Assimilation*, Siegelman, H. W. and Hind, G., Eds., Plenum Press, New York, 1978, 283.
88. **Zelitch, I.,** The relationship of glycolic acid to respiration and photosynthesis in tobacco leaves, *J. Biol. Chem.*, 234, 3077, 1959.
89. **Pritchard, G., Griffin, W., and Whittingham, C. P.,** The effects of CO_2 concentration, light intensity, and isonicotinyl hydrozide on the photosynthetic production of glycollic acid by *Chlorella*, *J. Exp. Bot.*, 13, 176, 1962.
90. **Jewess, P. J., Kerr, M. W., and Whitaker, D. P.,** Inhibition of glycolate oxidase from pea leaves, *FEBS Lett.*, 53, 292, 1975.

91. **Ray, T. B. and Black, C. C.,** Inhibition of oxalacetate decarboxylation during C_4 photosynthesis by 3-mercaptopicolinic acid, *J. Biol. Chem.*, 251, 5824, 1976.
92. **Sarawek, S. and Davies, D. D.,** The identification and properties of a naturally occurring inhibitor of malic enzyme, *Phytochemistry*, 15, 479, 1976.
93. **Knowles, F. C. and Benson, A. A.,** The mode of action of a herbicide. Johnsongrass and methanearsonic acid, *Plant Physiol.*, 71, 235, 1983.
94. **John, R. A., Charteris, A., and Fowler, L. J.,** The reaction of amino-oxyacetate with pyridoxal phosphate-dependent enzymes, *Biochem. J.*, 171, 771, 1978.
95. **Hoagland, R. E. and Duke, S. O.,** Effects of glyphosate on metabolism of phenolic compounds VIII. Comparison of the effects of aminooxyacetate and glyphosate, *Plant Cell Physiol.*, 23, 1081, 1982.
96. **Shieh, Y-J., Ku, M. S. B., and Black, C. C.,** Photosynthetic metabolism of asparate in mesophyll and bundle sheath cells isolated from *Digitaria sanguinalis* (L.) Scop., a $NADP^+$-malic enzyme C_4 plant, *Plant Physiol.*, 69, 776, 1982.
97. **Nakamoto, H., Ku, M. S. B., and Edwards, G. E.,** Inhibition of C_4 photosynthesis by (benzamidooxy) acetic acid, *Photosynthesis Res.*, 3, 293, 1982.
98. **Krampitz, L. O. and Yarris, C. E.,** Glycolate formation and excretion by *Chlorella pyrenoidosa* and *Netrium digitus*, *Plant Physiol.*, 72, 1084, 1983.
99. **Tolbert, N. E., Harrison, M., and Selph, N.,** Aminooxyacetate stimulation of glycolate formation and excretion by *Chlamydomonas*, *Plant Physiol.*, 72, 1084, 1983.
100. **Guerrero, M. G., Vega, J. M., and Losada, M.,** The assimilatory nitrate-reducing system and its regulation, *Annu. Rev. Plant Physiol.*, 32, 169, 1981.
101. **Klepper, L. A.,** Effects of certain herbicides and their combination on nitrate and nitrite reduction, *Plant Physiol.*, 64, 273, 1979.
102. **O'Neal, D. and Joy, K. W.,** Glutamine synthetase of pea leaves. Divalent cation effects, substrate specificity, and other properties, *Plant Physiol.*, 54, 773, 1974.
103. **Wallsgrove, R. M., Harel, E., Lea, P. J., and Miflin, B. J.,** Studies on glutamate synthase from the leaves of higher plants, *J. Exp. Bot.*, 28, 588, 1977.
104. **Woo, K. C. and Canvin, D. T.,** Effect of ammonia, nitrite, glutamate, and inhibitors of N metabolism on photosynthetic carbon fixation in isolated spinach leaf cells, *Can. J. Bot.*, 58, 511, 1980.
105. **Kawakubo, K., Shindo, M., and Konotsune, T.,** A mechanism of chlorosis caused by 1,3-dimethy-4-(2,4-dichlorobenzoyl)-5-hydroxypyrazole, a herbicidal compound, *Plant Physiol.*, 64, 774, 1979.
106. **Sandmann, G. and Böger, P.,** Mode of herbicidal bleaching, in *Biochemical Responses Induced By Herbicides*, Moreland, D. E., St. John, J. B., and Hess, F. D., Eds., American Chemical Society, Washington, D.C., 111, 1982.

Chapter 2

EFFECTS OF HERBICIDES ON RESPIRATION

Donald E. Moreland

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I. INTRODUCTION

Respiration is the process through which energy obtained from the oxidation of proteins, polysaccharides, and lipids is conserved in the form of the phosphate-bond energy of adenosine triphosphate (ATP) (Figure 1). In the respiratory process, macromolecules are metabolically converted to acetyl-CoA predominantly by cytoplasmic enzymes. The acetyl-CoA is metabolized oxidatively, by enzymes located in the matrix of mitochondria, to CO_2 through the Krebs cycle (citric acid cycle or tricarboxylic acid cycle). Electrons and protons that are removed from the organic acids by Krebs cycle enzymes are transferred through a series of oxidation/reduction reactions by components located in the mitochondrial inner membrane with the ultimate reduction of oxygen to form water. This latter pathway is referred to as the electron-transport pathway, the cytochrome system, or the respiratory chain. The sequential transfer of electrons involves carriers that possess increasing redox potentials. Associated with the electron-transport chain is the transduction of electron energy to bond energy in the form of ATP. The latter reaction in which inorganic phosphate (Pi) is esterified with adenosine diphosphate (ADP) is referred to as oxidative phosphorylation. ATP is an extremely pivotal compound in metabolism because it provides, directly or indirectly, the energy that drives all of the biosynthetic, mechanical, and transport activities of the cell (Figure 1). Interference with the production or hydrolysis of ATP could be one mechanism through which herbicides express chronic toxicity.

Attention in this chapter will focus on the effects expressed by herbicides on electron transport, oxidative phosphorylation (ATP generation), and properties of mitochondria.

II. MITOCHONDRIA

A. Occurrence, Structure, and Composition

Space limitations will permit only a superficial summary of the properties of mitochondria. For more detailed information, the reader is referred to texts and chapters written by Lehninger,[1] Tzagoloff,[2] Hanson and Day,[3] and Storey.[4]

Mitochondria are found in all aerobic eukaryotic cells. The structure, basic composition, enzymatic complement, and reactions catalyzed are very similar in plant, mammalian, and insect mitochondria. Plant cells typically contain from 500 to 1,000 mitochondria. The numbers correlate with the cell's need for ATP. The more metabolically active cells (secretory cells, phloem companion cells, and transfer cells) possess the greater number. In vivo, mitochondria are typically about 3 μm long and 1 μm in diameter, and tend to be spherical in shape, but size may vary and transient changes in shape are exhibited. Mitochondria move freely in streaming cytoplasm, appear to divide by fission, and coalesce with transient adherence to chloroplasts and other organelles. Mitochondria persist throughout the life of the cell and are resistant to degradative changes associated with senescence. The organelle consists of a sophisticated membrane system within which are incorporated the enzymes that mediate cellular respiration.

A diagrammatic representation of the membrane systems and spaces of mitochondria is shown in Figure 2A. The two double-membrane systems are referred to as the outer membrane and the inner membrane, respectively. Invaginations occur only in the inner membrane and are referred to as cristae. The space between the two membrane systems is called the intracristal or intermembrane space, whereas the space in the interior of the mitochondrion is referred to as the matrix space. The membranes are approximately 5 to 7 nm thick. The outer membrane is freely permeable to most solutes that have molecular weights up to 4,000. The inner membrane constitutes an osmotic barrier and is relatively impermeable to protons, many ions, and hydrophilic organic solvents. However, it is readily permeable to water, carbon dioxide, and oxygen.

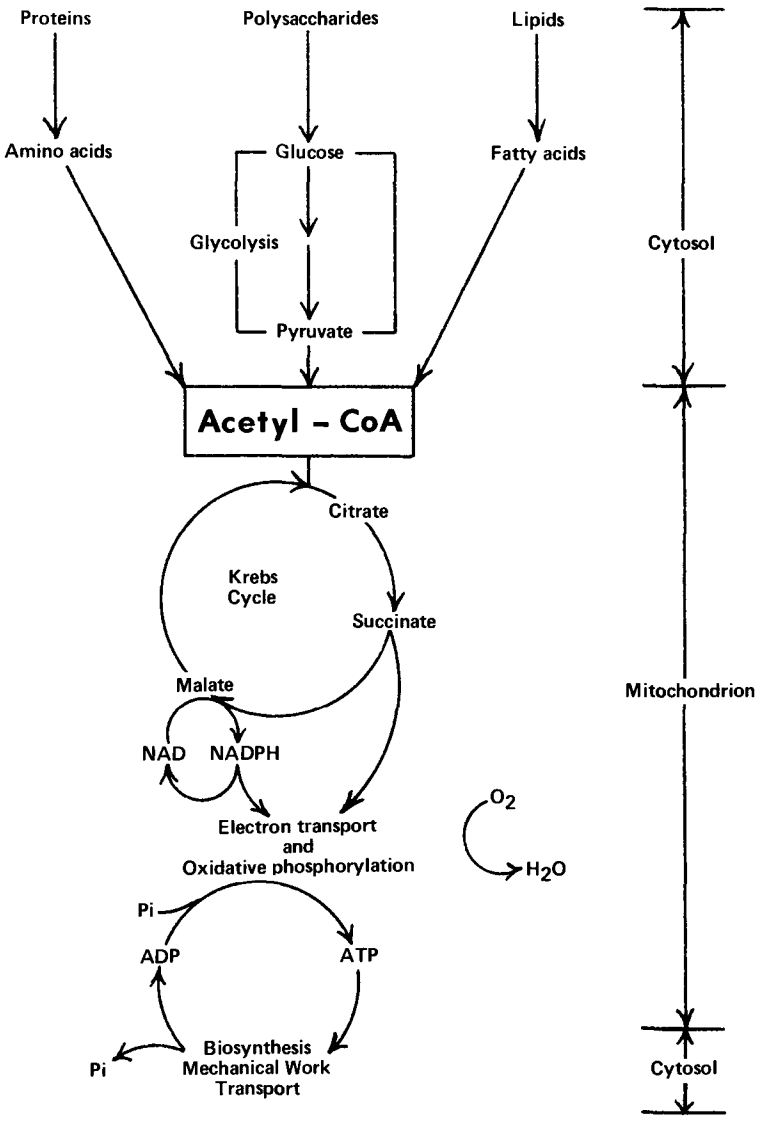

FIGURE 1. Diagrammatic representation of the respiratory process.

The inner mitochondrial membrane is composed of about 30% lipid and 70% protein, by weight. Phospholipids comprise about 98% of the lipid with the remaining 2% being galactolipids. The outer membrane contains about 40% lipid by weight and, in addition to phospholipids, some sterols are present. The phospholipids of the inner membrane primarily contain unsaturated 18-carbon fatty acyl chains (approximately 90%) with 18:3 predominating and some 16:0, whereas the phospholipids of the outer membrane are about 50% 16:0 and 50% 18-carbon fatty acyl chains.[5]

Shown in Table 1 are enzymes or enzymatic systems associated with the different components of mammalian and plant mitochondria. The inner membrane contains the enzymes and factors responsible for electron transport and synthesis of ATP. Located in the matrix are all of the Krebs cycle enzymes and NAD-linked dehydrogenases. The matrix also contains DNA, which is circular (30 nm long, 60×10^6 daltons, 90×10^3 base pairs), and ribosomes

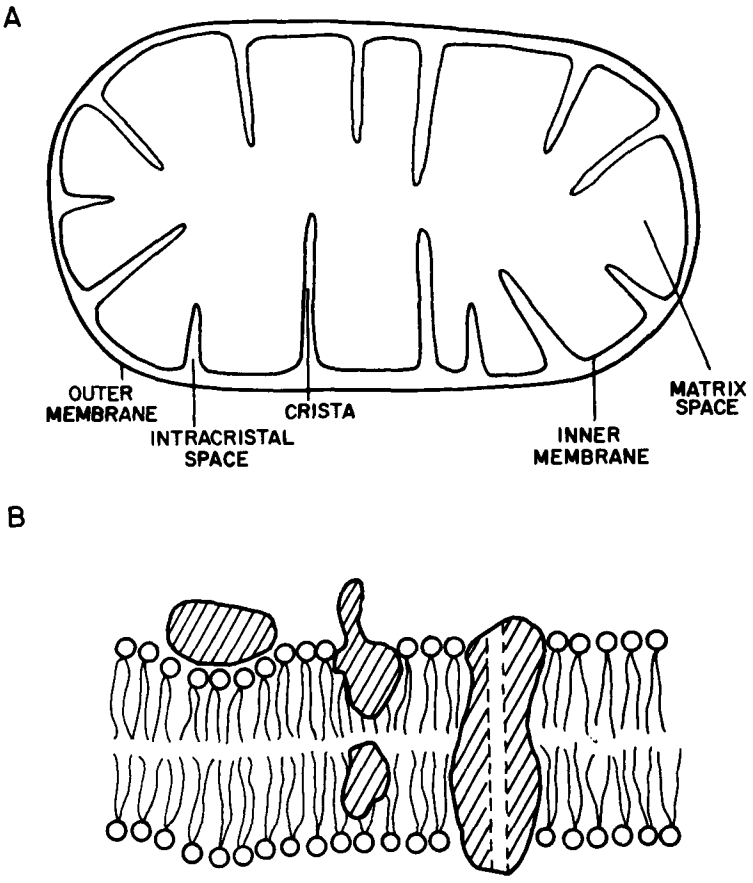

FIGURE 2. Diagrammatic representations of (A) cross-section of a mitochondrion, and (B) structure of a biological membrane (Legend: , polar phosphatidyl head group and fatty acid tail groups; slashed areas, proteins; vertical dotted zone, transport pore within a protein).

(RNA). Hence, mitochondria have a capacity for protein synthesis. Constitutive differences between animal and plant mitochondria have been identified. For example, enzymes involved in the elongation of fatty acids by the addition of acetyl-CoA and enzymes for β-oxidation of fatty acids are located in the matrix of mammalian mitochondria, whereas these enzymes are found not in the mitochondria, but in the glyoxysomes of plants. Glycine decarboxylase and isocitrate lyase are present in plant foliar mitochondria, but not in mitochondria isolated from hypocotyls.

The enzymes involved in electron transport and ATP generation are embedded in, or associated with, the lipid bilayer that forms the inner membrane. Shown diagrammatically in Figure 2B is a membrane model composed of polar phosphatidyl head groups and fatty acid tail groups that depicts association of lipoidal and proteinaceous components. Proteins may (a) be sorbed or attached to the membrane surface; (b) be embedded entirely within the lipid; (c) be partially embedded, but with portions projecting into the matrix or inner membrane space; or (d) contain transport pores. When lipophilic herbicides partition into the lipid phases of membranes, they can perturb lipid-lipid, lipid-protein, and protein-protein interactions that are required for membrane functions such as electron transport, ATP formation, and active transport. Hence, interference by herbicides with membrane functions

Table 1
DISTRIBUTION OF ENZYMES IN PLANT AND MAMMALIAN MITOCHONDRIAL COMPARTMENTS

Outer membrane	Intermembrane space	Inner membrane	Matrix
NADH dehydrogenase	*Superoxide dismutase (Cu-Zn)	Respiratory complexes	Krebs cycle enzymes
Cytochrome *b*-555	*NADH dehydrogenase	Coupling ATPase	NAD-linked dehydrogenases
*Acid phosphatase	*NADPH dehydrogenase	Pyridine nucleotide transhydrogenase	Glutamate dehydrogenase
**Monoamine oxidase	*Malic enzyme	NADH dehydrogenase (external)	Amino transferase
**Kynurine hydroxylase	**Adenylate kinase (myokinase)	Metabolite and ion transporters	Circular DNA
**Glycerolphosphate acyl transferase	**Nucleoside diphosphokinase	*Adenylate kinase (external)	Ribosomes
**Phosphatidate phosphatase	**Nucleoside monophosphokinase	*Alternate cyanide insensitive oxidase	Superoxide dismutase (Mn)
**Phospholipase A		*CDP-diglyceride synthetase	*Malic enzyme (NAD-linked)
**Nucleoside diphosphokinase		**β-Hydroxybutyrate dehydrogenase	*Glycine decarboxylase (leaf)
		**Carnitine palmityl transferase	*Isocitrate lyase (leaf)
			*PEP carboxykinase
			*α-Ketoglutarate-glyoxylate carboxyligase
			**Ornithine carbamyl transferase
			**Carbamyl phosphate synthetase
			**Fatty acyl-CoA synthetase
			**Fatty acyl-CoA dehydrogenase
			**Enoyl hydrase
			**β-Hydroxyacyl-CoA dehydrogenase
			**β-Ketoacyl-CoA thiolase

Note: No symbol = common to both plant and mammalian mitochondria. * = reported in plant mitochondria. ** = reported in mammalian mitochondria.

Adapted from Tzagoloff[2] and Hanson and Day.[3]

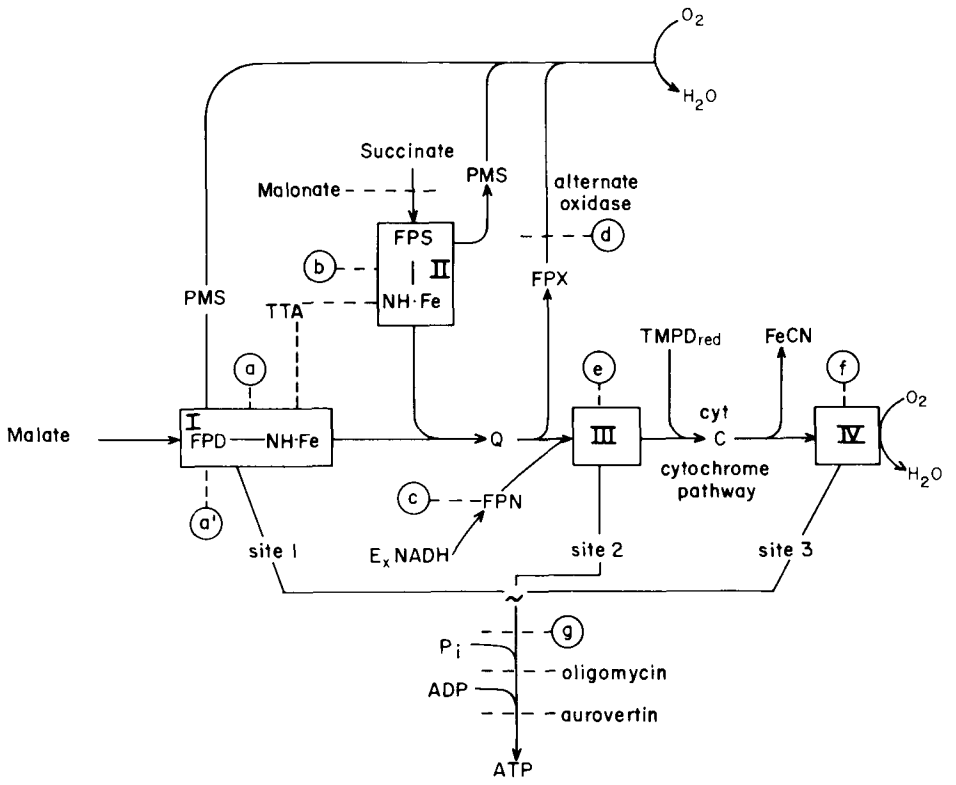

FIGURE 3. Schematic representation of electron transport and phosphorylation in plant mitochondria showing postulated sites of action of inhibitors. Abbreviations used: I, II, III, and IV, complex I, II, III, and IV, respectively; site 1, 2, and 3, phosphorylation site 1, 2, and 3; ExNADH, exogenous NADH; FPD, flavoprotein associated with complex I; FPS, flavoprotein associated with complex II; FPX, flavoprotein postulated to be associated with the alternate pathway; FPN, flavoprotein postulated to be associated with the oxidation of exogenous NADH; NH · Fe, nonheme (iron-sulfur) protein; Q, ubiquinone; FeCN, ferricyanide; and a, a', b, c, d, e, f, and g, postulated sites of action of inhibitors (see text for details). (Reproduced, with permission, from the *Annual Review of Plant Physiology*, Vol. 31. © 1980 by Annual Reviews, Inc.)

such as electron transport and phosphorylation may result from interactions with lipoidal as well as proteinaceous components.

B. Electron Transport and Phosphorylation

A generalized scheme that shows components of the electron transport and coupled phosphorylation pathways as they are considered to occur in the inner membrane of mitochondria isolated from nonchlorophyllous plant tissue is presented in Figure 3. The scheme shows four complexes (customarily designated by Roman numerals). Each complex contains several of the components of the electron-transport chain. Each complex is the smallest unit in which a sector of the electron-transfer chain can be isolated without a loss of native characteristics, such as the ability to react with natural electron acceptors, to show susceptibility to specific inhibitors, and to maintain the appropriate redox values of the component proteins. Additional details on the composition of the several complexes are given in Table 2. Components of the complexes undergo oxidation and reduction, and participate in the transfer of electrons down the chain. Electron transport drives proton efflux. Many redox components have been isolated and characterized, but the exact location, role, and function of some have

Table 2
IDENTIFICATION AND COMPOSITION OF PLANT MITOCHONDRIAL COMPLEXES

Complex number	Name	Composition	Inhibitors
I	NADH-CoQ reductase	FMN 4 Fe-S proteins (N-1, N-2, N-3, N-4)	Piericidin A, amytal, rotenone, TTA
II	Succinate-CoQ reductase	FAD (covalently bound) 3 Fe-S proteins (S-1, S-2, S-3)	Malonate, TTA
III	$CoQH_2$-cytochrome *c* reductase	Fe-S protein 2 *b* Cytochromes (*b*-556, *b*-560) Cytochrome c_1 (*c*-552)	Antimycin A, HOQNO
IV	Cytochrome *c* oxidase	Cytochrome *a* and a_3 2 mol Copper	Cyanide, azide, carbon monoxide
V	Coupling ATPase	Coupling factors F_0, F_1	Oligomycin

not been identified. The redox components can be titrated with appropriate techniques so that their mid-point potentials can be determined. Some can be assayed spectrophotometrically. Differences in the composition of the complexes with respect to iron-sulfur proteins have been identified between mammalian and plant mitochondria.

Components of the complexes are asymmetrically distributed across the inner membrane. Complexes I and II involved in the oxidation of malate and succinate, respectively, are considered to be associated with the matrix side of the inner membrane, whereas complexes III and IV can be accessed from the cytosol side of the inner membrane. ATP is also generated on the matrix side of the inner membrane. Coenzyme Q and cytochrome c are considered to be mobile within the membrane and to provide communication between the different complexes.

The classical concept of electron transport considered that there was a continuous flow of electrons from one redox component to the next as presented diagrammatically (Figure 3).[6] However, there are suggestions that a discontinuous flow of electrons may be involved, i.e., the redox components may not be in electronic communication at all times. When electrons are delivered to a complex (reduction), a conformational rearrangement occurs. Only then is electronic communication established between the respective complexes. The conformational changes result from the shift of a component from its oxidized to its reduced state, or vice versa. Shifts in conformation then lead to the making and breaking of electronic interactions. The mobile carriers (Coenzyme Q and cytochrome *c*) transfer electrons between the complexes.

In contrast to isolated animal mitochondria, intact plant mitochondria readily oxidize malate, in the absence of pyruvate. Only plant mitochondria oxidize exogenously supplied NADH, because of the presence of a dehydrogenase located on the cytosol side of the inner membrane. A cyanide- and antimycin A-insensitive pathway (the alternate pathway) is present in plant mitochondria isolated from various types of tissue, but is absent in mammalian mitochondria. For the most part, plant and mammalian mitochondria are affected similarly by inhibitors and uncouplers; however, different sensitivities to the same compound are observed. For example, plant mitochondria are generally less sensitive to rotenone and antimycin A, but more sensitive to oligomycin than are mammalian mitochondria.

1. ATP Generation

The machinery for coupled synthesis of ATP by the union of ADP and Pi is located on the matrix side of the inner membrane. This coupling ATPase is sometimes referred to as complex V (Table 2). Coupling involves the interdigitation of the electron-transport chain and the ATP generating system. Electron transport drives a proton flux. The inner membrane is impermeable to a back flow of extruded protons. Proton extrusion is electrogenic and produces an electrochemical gradient of protons, or pmf (Δp). The coupling ATPase consists of a hydrophilic knob (F_1) joined by a stalk-piece (OSCP, or oligomycin sensitivity-conferring factor) to a hydrophobic base-piece (F_0).

There are three "sites" of energy conservation (Figure 3). Each "site" represents a point of generation of a transmembrane proton gradient. The proton gradient is dissipated through the inner membrane-bound protein complex (F_1-F_0) that couples the breakdown of the gradient to the synthesis of ATP in the matrix. In an uncoupled system, substrate is oxidized, electron transport occurs, but no ATP is generated.

C. Isolation and Characterization of Plant Mitochondria

The art of isolating plant mitochondria that are intact and in which electron transport is coupled tightly to phosphorylation, and will remain coupled for several hours, has advanced significantly in recent years. As opposed to mammalian tissue, plant tissues have tough cell walls that have to be broken to release the cytoplasmic contents. Rough and/or prolonged

Table 3
CHARACTERISTICS OF RESPIRATORY STATES 3 AND 4 OF MITOCHONDRIA ACCORDING TO CHANCE AND WILLIAMS[11]

	Respiratory state	
Characteristics	**3**	**4**
Respiration rate	Fast	Slow
$[O_2]$	>0	>0
[Substrate]	High	High
[ADP]	High	Low
Rate limitation imposed by	Electron transport chain	ADP

homogenization and isolation procedures damage the mitochondria. In addition, plant cells have vacuoles that contain acidic constituents. Hence, when plant cells are homogenized, vacuolar contents are released and the pH of the homogenization medium is lowered. Consequently, the extracting medium must be buffered to maintain a pH from 7.1 to 7.4. If the pH should drop much below 7.0, irreversible changes occur and the mitochondria are unsuitable for use in critical studies.

Successful isolation of intact mitochondria has been accomplished from a variety of plant tissue, such as dark-grown leguminous hypocotyls, cauliflower buds, white potato tubers, sweet potato roots, corn roots, and corn mesocotyls, by gentle homogenization of the tissue in an isotonically buffered osmoticum, followed by differential centrifugation.[7] Further purification may be accomplished by gradient centrifugation in sucrose or Percoll.[8,9] The latter method has been utilized to isolate foliar mitochondria that are essentially free of chloroplast fragments.[10]

Traditionally, Warburg manometry was used to monitor oxygen uptake. However, in recent years, manometry has been replaced with Clark-type (oxygen) electrodes and measurements can be made more precisely and rapidly. The Clark is a Ag^+/Pt^- electrode with a saturated KCl bridge. A potential difference, usually of 800 mV, is imposed across the junction. Dissolved oxygen reacts with the Pt^- and a flow of current results that is proportional to the concentration of oxygen. Changes in potential are monitored with a strip-chart recorder. Measurements of mitochondrial activity are usually made in a small-volume glass reaction vessel, in which the electrode is inserted, that is accurately thermostated. Various substrates, ADP, inhibitors, and other reagents can be added to the mitochondria as desired. The contents of the reaction vessel are kept stirred magnetically. With this arrangement, Chance and Williams[11] described five respiratory states. Of these five states, states 3 and 4 (Table 3) are of most interest in studies concerned with the action of inhibitors. In state 3, all required components are present and the respiratory chain is the rate-limiting factor. State 4 is the condition in which ADP availability limits the respiratory rate. In state 1, both ADP and substrate are lacking. State 2 is the condition in which only substrate is rate-limiting, whereas in state 5, oxygen is lacking.

A polarographic trace of oxygen utilization obtained with potato tuber mitochondria as measured with a Clark-type electrode is shown in Figure 4, Trace A, for the oxidation of succinate. The trace reflects the stimulation of oxygen uptake (state 3 respiration) obtained

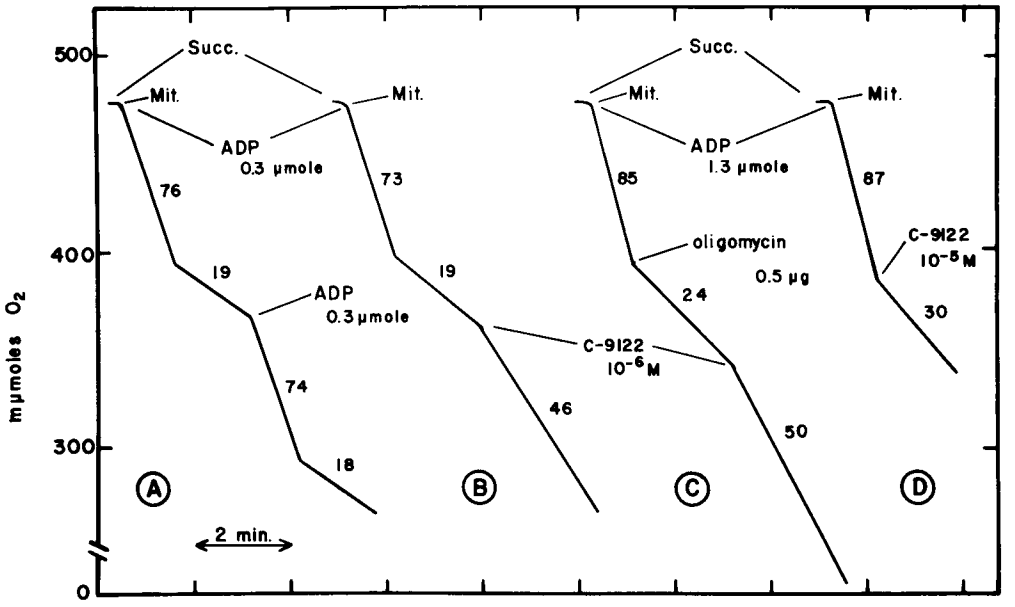

FIGURE 4. Representative polarographic traces that depict oxygen utilization obtained with potato mitochondria for succinate oxidation. Trace A, respiratory control obtained in the absence of an inhibitor; Trace B, stimulation of state 4 respiration by C-9122 (bromophenoxim) (1 μ*M*); Trace C, circumvention of oligomycin-inhibited respiration by C-9122 (1 μ*M*); and Trace D, inhibition of state 3 respiration by C-9122 (10 μ*M*). Rates of oxygen utilization (nmol/min/mg protein) are indicated above the traces. Mitochondria (Mit) containing 0.4 mg protein, succinate (8.5 μmol), ADP, oligomycin, and C-9122 were added at the points indicated. Concentrations of components are shown as μmol (succinate and ADP), or μg (oligomycin) supplied to, or as the final molarity (C-9122) in, the 2-mℓ reaction medium. (From Moreland, D.E. and Blackmon, W. J., *Weed Sci.*, 18, 419, 1970. With permission.)

by the addition of a small amount of ADP, followed by a decrease in respiration upon exhaustion of the added ADP (state 4 respiration). This sequence can be repeated by further addition of small amounts of ADP until anaerobiosis occurs (state 5). The cycling between states 3 and 4 demonstrates the presence of respiratory control that is required for the evaluation of the action of inhibitors on the energy transfer sequence. The control manifested by ADP will only be evident when mitochondria are tightly coupled.

The quality of isolated mitochondria can be evaluated by calculation of respiratory control (RC) and ADP/O ratios. The RC ratio is obtained by dividing the fast state 3 rate by the slow state 4 rate. It provides an indication of the relative efficiency of energy transduction of a mitochondrial preparation. RC values obtained with mung bean mitochondria for the oxidation of malate, NADH, and succinate are approximately 4.0 ± 0.5, 3.8 ± 0.3, and 2.2 ± 0.2, respectively. The ADP/O ratio is defined as the number of ATP molecules synthesized during the oxidation of a substrate via the respiratory chain by molecular oxygen. The ADP/O ratio can be calculated directly from polarographic traces because the concentration of the added ADP is known and the moles of atomic oxygen consumed can be measured. The ADP/O ratio for the oxidation of NAD-linked substrates such as malate is 3, whereas the ratio for oxidation of succinate is 2. These values are theoretical and are seldom achieved. Actual ADP/O values frequently obtained for the oxidation of malate, NADH, and succinate by mung bean mitochondria are approximately 2.3, 1.3, and 1.5, respectively.

In addition to studying whole-chain electron transport, it is possible to monitor partial reactions with the use of appropriate electron mediators (Figure 3). Phenazine methosulfate

(PMS) can be used to mediate electron flow from the oxidation of malate and succinate through complexes I and II, respectively. Ferricyanide can accept electrons in the region of cytochrome *c*, and reduced *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) can be oxidized through cytochrome *c* and complex IV.

D. Effects of Herbicides on Mitochondrial Activities

The combination of having tightly coupled and time-stable plant mitochondria, and the ability to measure oxygen uptake rapidly and with considerable sensitivity, has provided a clearer insight into the action of herbicides on mitochondrial reactions. Responses induced by herbicides reported in the early literature with the Warburg technique have not always been duplicated when the Clark electrode was used. Hence, only observations measured with a Clark-type electrode and with plant mitochondria that have been shown to be of acceptable quality will be considered herein. Unless mitochondria are tightly coupled, remain coupled during the course of the experiments, and possess low rates of endogenous ATPase activity, they are probably damaged and should not be used in critical studies. Some investigators are not always able to reproduce responses reported by other researchers for herbicides. Many factors can be expected to influence the responses obtained with plant mitochondria: source (species, tissue, and age of tissue), isolation method, composition of the reaction mixture, substrates oxidized, solvent in which water-insoluble inhibitors are dissolved, final concentration of the solvent in the reaction mixture, and the extent to which the mitochondria remain coupled.

1. Classification of Inhibitors that Affect Electron Transport and ATP Generation

Inhibitors have played, and will continue to play, important roles in the formulation of postulates that explain oxidative phosphorylation, and in the elucidation of components of the electron transport and ATP generation pathways. Classically, chemicals that interfere with the mitochondrial system have been classified as (a) electron-transport inhibitors, (b) uncoupling agents, (c) energy transfer inhibitors, and (d) inhibitory uncouplers. Classes (a), (b), and (c) are identified and defined in the mammalian mitochondrial literature.[13,14] Class (d), inhibitory uncouplers, has been added and is used here to identify the multiple types of responses that are observed with many of the nonclassical inhibitors of oxidative phosphorylation, including most of the inhibitory herbicides.[6]

Effects produced by herbicides on the respiratory activity of isolated plant mitochondria are summarized in Table 4. In many of the studies, the investigators examined the effects of several members of a given chemical family, however, to simplify the presentation, responses for only representative herbicides are listed.

a. Electron-Transport Inhibitors

Experimentally, electron-transport inhibitors are characterized by their ability to interrupt electron flow at some point in the respiratory chain by interacting with one of the complexes. The chemicals are thought to combine with one of the proteinaceous electron carriers of the complex and to prevent the formation of a redox couple. This action is measured most readily as inhibition of state 3 respiration. When electron flow is interrupted, the coupled phosphorylating reactions are also inhibited. Nonherbicidal inhibitors that interfere specifically with each of the complexes are identified in Table 2. Amytal, rotenone, and piericidin A are considered to interact with complex I (Figure 3, site a), and prevent the oxidation of malate and other NAD-linked substrates. Thenoyltrifluoroacetone (TTA) inhibits by binding to the nonheme iron proteins of complex I. Malonate competitively interferes with the oxidation of succinate by succinic dehydrogenase and TTA interacts with the nonheme iron proteins of complex II (Figure 3, site b). Antimycin A and 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide (HOQNO) affect complex III (Figure 3, site e). Cyanide, azide, and carbon monoxide

Table 4
EFFECTS OF REPRESENTATIVE HERBICIDES FROM DIFFERENT CHEMICAL FAMILIES ON RESPIRATORY ACTIVITY OF ISOLATED PLANT MITOCHONDRIA, AS MEASURED WITH A CLARK-TYPE ELECTRODE

FAMILY/ representative compound(s)	Tissue source[a]	Substrate[b] inhibition of state 3: S	M	N	Stimulation[c] of state 4 (succinate)	Induction of passive swelling[d]	Inhibition of other responses	Ref.
ACYLANILIDES								
Chlorfenprop-methyl	WP	+ + +	ND	ND	+ + +	ND	Membrane integrity altered	28
Karsil	MB, CB	+ + +	+ +	+ + +	+ +	+ +	Val.-induced swelling	15—17
Propanil	MB, CB	*	+ + +	+ + +	+ +	+ + +	Val.-induced swelling	15,16,18,19
BENZONITRILES								
Ioxynil	MB, WP	+ +	+ + +	+ + +	+	+ +	Val.-induced swelling	16,19,20
DINITROANILINES								
Oryzalin	MB	+ +	+ +	+ + +	NE	+ +	Val.-induced swelling	16,21,22
							Ca^{2+} transport	23
Trifluralin	MB	+ +	+ +	+ +	NE	+ +	Val.-induced swelling	16,21,22
							Ca^{2+} transport	23
DINITROPHENOLS								
Dinoseb	MB	+ +	+ +	+ + +	+	+ +	Val.-induced swelling	16,19
Dinoterb	WP	+ + +	+ + +	+ + +	+ +	ND		24
DIPHENYLETHERS								
Nitrofen	MB, WP	+ +	+ +	+ +	ENE	ND		25
OXIMES								
Bromofenoxim (C-9122)	MB, WP	+	+ +	+ +	+	+ +	Val.-induced swelling	12,16
PHENYLCARBAMATES								
Chlorpropham	MB, WP	+ + +	+ +	+ + +	+ + +	+ + +	Val.-induced swelling	15,16,19,26
							Ca^{2+} transport	23
PHENOXIES								
Diclofop-methyl	W, O	*	*	*	ENE	*		27
2,4-D	MB	*	*	*	ENE	ND		16
2,4-DB	BB,WP	*	*	ND	+ +	*		29,30
2,4,5-T	BB	*	*	ND	*	*		29

PHENYLUREAS								
D_5[e]	MB	ND	ND	ND	+ +	ND	Ca^{2+} transport	31
DCPTU[e]	WP	ND	ND	ND	+	ND		32
Diuron	MB, WP	*	+ + +	+ + +	NE	NE		15,16,19,33,34
MBPU[e]	MB	+ +	+ +	+ +	+ +	ND		16,35
Neburon	MB, WP	*	+ +	+ +	+ +	NE		16,34,36
Siduron	MB, WP	*	*	+ + +	+ +	NE		16,34,36
MISCELLANEOUS								
Perfluidone	MB	+ + +	+ + +	ENE	+ +	+ + +	Val.-induced swelling	37
Pyriclor	MB, SB	+ + +	*	*	ENE	ND		16,38
UKJ72J[e]	WP	+	ENE	ENE				39

[a] Source code: MB, mung bean hypocotyls (*Phaseolus aureus* Roxb. = *Vigna radiata* L.); WP, white potato tubers (*Solanum tuberosum* L.); CB, castor bean endosperm (*Ricinus communis* L.); W, etiolated wheat seedlings (*Triticum aestivum* L.); O, etiolated oat seedlings (*Avena sativa* L.); BB, broad bean hypocotyls (*Vicia faba* L.); SB, soybean hypocotyls (*Glycine max* [L.] Merr.).

[b] Substrate code: S, succinate; M, malate; N, NADH. I_{50} concentration range code: *, >500 μM; + + +, 100 to 500 μM; + +, 10 to 100 μM; +, <10 μM. ENE = limited (<25%) inhibition. ND = not determined. NE = no effect.

[c] Maximum stimulation for the oxidation of succinate observed within the following concentration range: + + +, 100 to 500 μM; + +, 10 to 100 μM; +, <10 μM. ENE = limited (<20%) stimulation. NE = no stimulation reported.

[d] Passive swelling reported to occur within the following concentration range: *, >500 μM; + + +, 100 to 500 μM; + +, 10 to 100 μM; +, <10 μM. ND = not determined, NE = swelling not induced.

[e] MBPU = 1-(α-methylbenzyl)-3-(3,4-dichlorophenyl)urea; DCPTU = 1-(methyl)-1-(1,2,3-thiadiazolyl)-3-(3,4-dichlorophenyl) urea; D_5 = 1-(*n*-pentyl)-1-(methyl)-3-(3,4-dichlorophenyl)urea; UKJ72J = 2-ethylamino,4-amino,5-thiomethyl,6-chloropyrimidine.

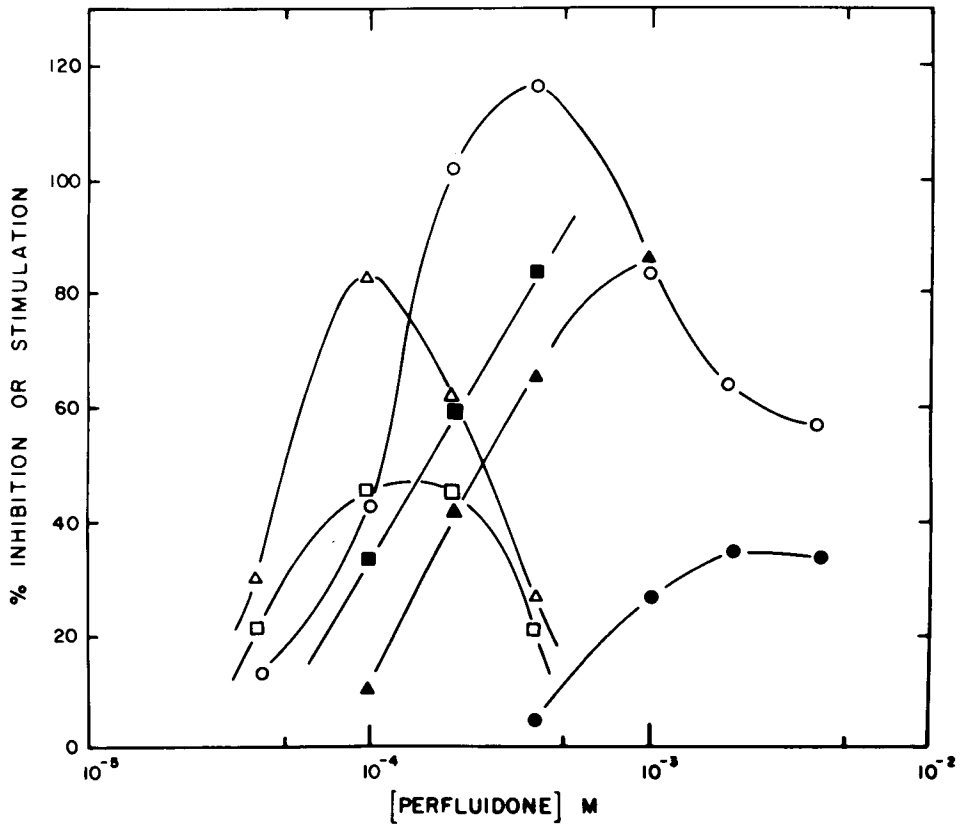

FIGURE 5. Effects of perfluidone on the oxidation of malate, exogenous NADH, and succinate by mung bean mitochondria. Legend: stimulation of state 4 respiration during the oxidation of malate (□), NADH (○), and succinate (△); inhibition of state 3 respiration during the oxidation of malate (■), NADH (●), and succinate (▲). (From Moreland, D.E., *Pestic. Biochem. Physiol.*, 15, 21, 1981. With permission.)

interfere with cytochrome oxidase located in complex IV (Figure 3, site f) by forming a complex with the copper. Cyclic hydroxamates, such as salicylhydroxamic acid (SHAM), inhibit the cyanide-insensitive pathway (Figure 3, site d).

Inhibition of state 3 respiration by C-9122 [bromofenoxim;3,5-dibromo-4-hydroxybenzaldehyde *O*-(2,4-dinitrophenyl)oxime] at a concentration of 10 μM is shown in Figure 4, trace D. Percent inhibition can be calculated from polarographic traces by comparing the inhibited state 3 rate with the no-inhibitor control rate. I_{50} values can be obtained by titration of state 3 respiration with increasing concentrations of an inhibitor. Plots of percent inhibition vs. logarithm of the molar concentration are usually linear except at the extreme concentrations as shown in Figure 5 for perfluidone.

A number of herbicides (for example, chlorpropham, propanil, dinoseb, and ioxynil) inhibit state 3 respiration, but they also uncouple state 4 respiration, usually at slightly lower molar concentrations. Hence, these herbicides will not be considered as pure electron-transport inhibitors, but instead will be classified as inhibitory uncouplers. Some of the dinitroanilines[21] and diphenylethers,[25] however, do act as multisite electron-transport inhibitors of mitochondrial respiration without having a marked uncoupling effect. Pyriclor interferes only with electron transport in soybean hypocotyl mitochondria.[38] UKJ72J (2-ethylamino,4-amino,5-thiomethyl, 6-chloropyrimidine) strongly inhibits only the oxidation of succinate by plant mitochondria.[39] The activity of plant mitochondria is much more sensitive to UKJ72J than either yeast or rat liver mitochondria.

Herbicides that are pure inhibitors of light-induced electron transport in chloroplasts, such as diuron, *s*-triazines, and the substituted uracils do not interfere strongly with state 3 or 4 respiration of plant mitochondria, but some induce marginal disturbances at concentrations >0.5 m*M*. Stimulation of state 4 respiration by these herbicides has been observed sometimes with loosely coupled, but not with tightly coupled mitochondria.

b. Uncouplers

At appropriate concentrations, classical uncouplers such as carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) prevent the phosphorylation of ADP without interfering with electron transport (Figure 3, site g). In general, any compound that promotes the dissipation of the protonmotive force generated by electron transport, other than for the production of ATP, may be regarded as an uncoupler. Uncouplers, when added in vitro to tightly coupled mitochondria, stimulate oxygen uptake by eliminating the regulatory influence exerted by Pi and ADP. Stimulation of state 4 respiration by C-9122 at a concentration of 1 μ*M* is evident in Figure 4, Trace B. As shown, all control exerted by ADP is lost. Electron transport and oxygen utilization continue at accelerated rates, but no ATP is formed. Percent stimulation can be calculated by comparing the stimulation induced by a test compound to the stimulation obtained with ADP, i.e., 100% stimulation equals that obtained with ADP. The titration of state 4 respiration with increasing concentrations of an uncoupler, when plotted as percent stimulation vs. the logarithm of the molar concentration, gives a characteristic parabolic or bell-shaped curve as shown in Figure 5 for perfluidone.

Stimulation of state 4 respiration in media deficient in ADP or Pi is one of several criteria that uncouplers should meet. Other criteria include (a) induction of ATPase activity, with the induction process being sensitive to oligomycin; (b) circumvention of oligomycin-imposed inhibition of state 3 respiration (as shown in Figure 4, Trace C for C-9122); and (c) inhibition of the various exchange reactions catalyzed by mitochondria in the absence of substrates (Pi-ATP, ADP-ATP, and oxygen of water with Pi and ATP).[13,14,40]

Several hypotheses have been proposed to explain uncoupling of oxidative phosphorylation. These include the classical uncoupler-induced hydrolysis of a high energy intermediate; conformational changes and altered functions of coupling proteins upon interaction with uncouplers; energy-linked transport of the uncoupler anion into, and passive diffusion of the protonated species out of the mitochondrion; acid- or base-catalyzed hydrolysis by uncouplers of an intermediate reaction of oxidative phosphorylation in a lipophilic membrane region; increase of membrane conductance; collapse of the transmembrane proton gradient by uncouplers acting as protonophores and ionophores in transporting cations across the mitochondrial membrane; and variations of the above.[41-44] Covalent labeling of specific mitochondrial proteins by reactive radio- and photoaffinity labeled uncouplers (2-azido-4-nitrophenol and 2-nitro-4-azidocarbonylcyanidephenylhydrazone) have identified specific uncoupler-binding sites associated with the energy conservation pathway.[45-47]

Representatives of several herbicidal families are identified in Table 4 that uncouple oxidative phosphorylation. However, the compounds also inhibit state 3 respiration usually at slightly higher molar concentrations. Consequently, these herbicides are classified as inhibitory uncouplers and their action will be discussed separately. The isopropyl ester of glyphosate, however, has shown marginal uncoupling action.[48]

c. Energy Transfer Inhibitors

Compou..ds in this group inhibit phosphorylating electron transport (state 3 respiration) when the energy conserving apparatus of mitochondria is intact. Their inhibition is circumvented by uncouplers. Energy transfer inhibitors are considered to combine with an intermediate in the energy coupling pathway and, hence, block the phosphorylation sequence that leads to the production of ATP (Figure 3). Oligomycin is the prototype. Aurovertin

also affects ATP generation by acting at a locus closer to ATP formation than that affected by oligomycin.

In Figure 4, Trace C, inhibition of state 3 respiration by oligomycin is shown with ADP present in nonlimiting concentrations. C-9122, when added at an uncoupling concentration of 1 μM, partially circumvented the oligomycin-inhibited respiration. No herbicide has been shown to act as an energy transfer inhibitor of oxidative phosphorylation in higher plants to date.

d. Inhibitory Uncouplers

Most herbicides (Table 4) that interfere with oxidative phosphorylation manifest a complex spectrum of responses and have been classified as inhibitory uncouplers.[6] At low molar concentrations, the herbicides satisfy most, if not all, of the criteria established for uncouplers, but at higher molar concentrations, they act like electron-transport inhibitors.

The herbicides classified as inhibitory uncouplers, in so far as they have been tested, stimulate state 4 respiration during the oxidation of malate, succinate, and exogenous NADH much as shown for perfluidone in Figure 5. All produce the characteristic bell-shaped curve when percent stimulation is plotted as a function of the logarithm of the concentration of the herbicide. Within the stimulatory range, inhibition of state 3 respiration becomes evident. Hence, the higher concentrations of inhibitory uncouplers both stimulate state 4 and inhibit state 3 respiration. As explained earlier herein, inhibition of state 3 respiration is generally considered to reflect interference with electron transport, whereas the state 4 response is considered to measure an effect imposed on a component of the ATP-generating sequence. The occurrence of "overlapping" effects, i.e., inhibition of state 3 and stimulation of state 4 respiration at the same molar concentration, can be expected to reduce the actual or apparent uncoupling capabilities of a compound because the capacity for utilizing oxygen has been reduced by a competing reaction.

For many herbicides, as well as other pesticides, biotransformation products may be more inhibitory to oxidative phosphorylation than the unaltered parent compound. Ring hydroxylation followed by conjugation of the phenols is a primary metabolic detoxication pathway (see Chapter 9). Most monophenols act as inhibitory uncouplers in plant mitochondria. If there should be a delay between the formation of the hydroxylated compounds and their conjugation, or if they should accumulate in their free form, interference with ATP production could result. The conjugation reaction also requires ATP energy. Hence, the accumulation of free phenols would intensify the uncoupling action and reduce the availability of ATP for detoxication (the conjugation reaction). A response of this type has been shown for dichlobenil which has essentially no effect on the activity of tightly coupled mung bean mitochondria. However, the 3- and 4-hydroxylated degradation derivatives are strong inhibitory uncouplers and have been associated with the expression of toxicity in both plants and animals.[49]

e. Summary of Responses

The mechanisms that are involved in the actions of herbicides that interfere with mitochondrial electron transport and phosphorylation are complex and remain to be identified. In addition to meeting all of the requirements for uncouplers, the herbicides classified as inhibitory uncouplers affect several sites on the electron-transport pathway.[15,22,50,51] That inhibition of state 3 respiration associated with the oxidation of malate, succinate, and NADH (Figure 3, site c) does not occur at a single site common to the three substrates is suggested by the widely differing I_{50} values that are obtained. The herbicides also inhibit malate-PMS oxidoreductase (Figure 3, site a'). Malate-PMS oxidoreductase is not inhibited by rotenone, hence, action prior to the rotenone-sensitive site in complex I is suggested. Additional evidence for interference at or near complex II (Figure 3, site b) is provided by inhibition

of succinate-PMS oxidoreductase by the inhibitory uncouplers. Succinate-PMS oxidoreductase is not inhibited by TTA, SHAM, diphenylethers, or the dinitroanilines. Inhibitory uncouplers also inhibit malate and succinate-mediated cyanide-resistant respiration (Figure 3, site d). No evidence has been published to date for interference at or near complex III and IV by any of the herbicides.

2. Perturbation of the Inner Mitochondrial Membrane

The multisite inhibitory action on electron transport and phosphorylation by herbicides is considerably more complex than that of the site-specific inhibition of standard respiratory inhibitors such as rotenone and antimycin A.[15,22,51,52] All of the inhibitory herbicides are lipophilic and can be expected to partition into the nonpolar regions of the mitochondrial inner membrane. Partitioning could produce alterations to the fluidity and permeability properties of the membrane that result in perturbational and conformational shifts to the constituent redox components. These alterations, then, could be responsible for the multiple effects imposed on state 3 and possibly state 4 respiration. Chlorpropham, propanil, dinitroanilines, ioxynil, dinoseb, and perfluidone do perturb the inner mitochondrial membrane as evidenced by changes in the cation permeability of the membrane as measured by osmotic swelling techniques (Table 4).[15,22,37,51,52] At relatively low molar concentrations, the herbicides inhibit the permeability of potassium induced by the ionophores valinomycin and gramicidin. At higher molar concentrations, membrane permeability to potassium is increased in the absence of an ionophore. In general, interference with valinomycin-induced swelling was initiated at concentrations lower than those required to stimulate state 4 respiration and inhibition of state 3 respiration occurred within the molar concentration range in which induction of passive swelling was evident.[15,22,37,51,52]

Interference with calcium uptake of plant mitochondria by oryzalin, trifluralin, chloropropham, and D_5[1-(*n*-pentyl)-1-(methyl)-3-(3,4-dichlorophenyl)urea] has also been reported (Table 4).[23,31] Conceivably, alteration of the semipermeability of the inner mitochondrial membrane to cations and anions may be a manifestation common to all inhibitory uncouplers. However, investigators have not devoted much attention to this type of study in the past. Interestingly, only herbicides classified as inhibitory uncouplers of mitochondrial reactions interfere with membrane permeability. Herbicides such as diuron, *s*-triazines, and uracils that are not very inhibitory to state 3 respiration, also do not interfere with ionophore-induced swelling.

3. Comparisons with Inhibition of Chloroplast Reactions

Consideration of herbicides that interfere with mitochondrial electron transport and phosphorylation warrants a comparison with effects produced on the light-induced companion reactions of thylakoids. The latter have, in fact, received even more attention in recent years than the mitochondrial reactions (see Chapters 1 and 7). Interfering herbicides of chloroplast reactions have also been classified into the same four groups used herein for interferences with mitochondrial reactions.[6] However, a fifth classification, namely electron acceptors was added to accommodate compounds, such as diquat and paraquat, that compete for electrons with some component of the thylakoid electron-transport pathway and subsequently undergo reduction.

The herbicides classified as electron-transport inhibitors of chloroplastic electron transport, such as dimethylphenylureas, *s*-triazines, *bis*-carbamates, diphenylethers, pyridazinones, triazinones, urea-carbanilates, and uracils do not interfere strongly with state 3 or state 4 respiration of plant mitochondria.[6] However, some may induce marginal responses at concentrations >0.5 m*M*. Diquat and paraquat, classified as electron acceptors, also do not affect responses of plant mitochondria, at least in so far as studies conducted to date have reported.[16] However, those herbicides classified herein as inhibitory uncouplers of mito-

chondrial reactions act both as uncouplers and electron-transport inhibitors of chloroplast-mediated responses. Perfluidone is an exception in that it acts only as an uncoupler and has no effect on electron transport in thylakoids.[37]

4. Structure-Activity Correlations

Structure-activity studies have not received the extensive attention in mitochondrial responses that they have in investigations with chloroplasts. However, the limited studies that have been reported suggest that ring substituents and side chain lengths associated with increased inhibitory activity in chloroplast (both electron transport and uncoupling) reactions[53] also show increased inhibitory activity against both plant and animal mitochondrial responses. In chlorinated 2-methylvaleranilides and propionanilides, the 3,4- and 3,5-dichlorinated derivatives were considerably more inhibitory to state 3 respiration and more stimulatory to state 4 respiration than 3-or 4-monochlorinated isomers.[17,18,54] Chlorination in an ortho ring position is associated with decreased inhibitory activity in all systems. Stimulation of state 4 respiration and inhibition of state 3 respiration also increased with the length of the alkyl substituent, from C_3 to C_7, in *N*-(3,4-dichlorophenyl)alkylamides.[17,18] In the substituted benzimidazoles, uncoupling activity was proportional to the acidity of the imidazole-NH group.[55,56]

As indicated previously, those herbicides that have been classified as electron-transport inhibitors in chloroplast assays affect state 3 respiration of mitochondria only at relatively high molar concentrations. For example, diuron at a concentration of 1 m*M*, was reported to only weakly affect either state 3 or state 4 respiration when tested with white potato tuber mitochondria and succinate as substrate. However, substitution of one of the terminal *N*-methyl groups with an *n*-butyl (neburon) or *n*-pentyl (D_5) group resulted in strong uncoupling action at 100 μ*M*.[31,34] Replacement of one of the *N*-methyl groups of diuron with a methylbenzyl group also resulted in the compound acting as an inhibitory uncoupler against both chloroplasts and mitochondria.[25] However, the compound was not very phytotoxic.

Failure to obtain interference with mitochondrial responses by inhibitors of chloroplast electron transport may be related to the lipophilic environment around the reactive entities. An increase in lipophilicity, achieved by the introduction of appropriate substituents, has converted the action of phenylurea herbicides to that of mitochondrial inhibitory uncouplers, at least in the examples cited above.[25,31,34]

Purity of the test chemical used by investigators in mitochondrial studies can sometimes be responsible for misleading conclusions. For example, technical grade samples of diuron and other phenylurea herbicides were observed to interfere with oxidative phosphorylation of potato tuber mitochondria. However, studies with purified samples of the herbicides failed to confirm the inhibition. The impurity responsible for the uncoupling action subsequently was identified as *N*, *N'*-bis(3,4-dichlorophenyl)urea.[33,57]

III. INTERFERENCE BY HERBICIDES WITH RESPIRATION OF INTACT SYSTEMS

Early investigations on the mechanisms of action of herbicides were concerned with effects imposed on respiration. These observations were frequently made with Warburg manometry. Measurements were made with isolated tissue to which herbicides were added or with tissue removed from treated plants. Much of this early work has been reviewed by Kirkwood.[58] For the most part, the observations have not advanced our insight into the mechanisms involved in the action of herbicides. Manometric measurements, as pointed out earlier, lack sensitivity. In addition, the investigator cannot control such factors as absorption, translocation, and metabolic alterations including the formation of complexes.

Use of tissue sections or cell suspensions eliminates some of the problems and uncertainties

associated with absorption, translocation, and metabolism of the herbicide by the tissue of interest. Oxygen uptake of treated tissue frequently is not very sensitive to most herbicides known to affect oxygen utilization by isolated mitochondria. Excised tissue frequently does not readily absorb externally applied herbicides, and in most situations, the investigators have not measured the extent of uptake of the applied treatment. In addition, most studies have not included standard respiratory uncouplers or inhibitors, such as FCCP, azide, cyanide, or HOQNO, for comparative purposes. In tissues and even cells, oxygen can be utilized or liberated by metabolic reactions other than those involving mitochondrial functions. Consequently, oxygen measurements may not be very meaningful.

There are other problems associated with measuring oxygen even with the Clark-type electrode. Air trapped within tissue, which would serve as a reservoir for oxygen, is a problem. However, this problem might not be as serious in studies that involve isolated cells.

Summarized in Table 5 are some observations made with herbicides on oxygen utilization of tissue sections or isolated cells. In some of the studies, the investigators examined the effects of several members of a given chemical family, however, to simplify the presentation, responses for only representative herbicides are listed. For the most part, results do not always agree with what would be expected based on studies conducted with isolated mitochondria. Instead of measuring effects on oxygen utilization by excised tissue or cells, some investigators have examined effects imposed on the ATP pools present in the cells (Table 5). It must be remembered, that in nonphotosynthetic tissue both glycolysis and oxidative phosphorylation contribute to the pool. In so far as they have been tested, herbicides have not been shown to inhibit glycolysis. Hence, even if herbicides effectively interfered with the oxidative production of ATP, some production of ATP by glycolysis can be expected. Competing reactions and turnover rates also play roles here. The herbicide could be interfering with reactions that utilize ATP. Hence, if these reactions were inhibited, the ATP pool concentration would increase. Conversely, the herbicide could stimulate an ATP-requiring reaction that would utilize available ATP faster than it could be synthesized by the glycolytic or mitochondrial pathways. Hence, at best, the ATP pool size may be only an indirect measure of effects expressed on mitochondrial functions. ATP can be measured in very low concentrations with the luciferin-luciferase system. Good correlations between the effects expressed by several herbicides on isolated mitochondria and tissue ATP levels can be developed from a comparison of the data in Table 5 with those in Table 4.

IV. IMPLICATIONS

A. Correlations Between In Vitro and In Vivo Responses

Extrapolation from in vitro studies with isolated mitochondria to the expression of phytotoxicity remains to be achieved. It is not known what concentration of any herbicide is inside a cell when lethality is triggered. Hence, it is not known what concentrations of herbicides a mitochondrion is exposed to in vivo. Mitochondria are isolated, stabilized, and maintained in vitro in buffered 0.3 *M* mannitol or sucrose. Yet, *in situ*, mitochondria are not surrounded with mannitol and this concentration of sucrose has not been shown to exist at least in the cytoplasm of hypocotyl cells, i.e., the tissue from which many investigators isolate mitochondria. In vivo, the major cytoplasmic cation is probably potassium and the major species of anion probably consists of macromolecules (proteins). It is not known if the mitochondria would be more or less sensitive to herbicides in this physiological environment than in the artificial one used by investigators.

B. ATP Availability and Metabolism

ATP has a ubiquitous and dominant role in cellular metabolism. ATP provides the energy,

Table 5
EFFECTS OF REPRESENTATIVE HERBICIDES FROM DIFFERENT CHEMICAL FAMILIES ON RESPIRATORY OXYGEN UTILIZATION, ON CELL GROWTH, OR, ATP CONCENTRATIONS IN, EXCISED TISSUE AND ISOLATED CELLS

FAMILY/ representative compound(s)	Tissue[a]	Assay system[b]	Responses[c] (concentration)		Ref.
ACYLANILIDES					
Propanil	AP	O_2/CG	S/L	(100 μM)	59
	SB	ATP	I	(600 μM)	60
BENZONITRILES					
Dichlobenil	AB	O_2/CG	S/L	(100 μM)	59
	SB	ATP	S	(200 μM)	49,60
	PV	CO_2	I	(200 μM)	61
Ioxynil	AP	O_2/CG	S/L	(100 μM)	59
	SB	ATP	I	(600 μM)	60
DINITROANILINES					
Oryzalin	MBrt.MBh	O_2	I	(1 mM)	62
Trifluralin	SB	ATP	NE	(200 μM)	60
	CR	O_2	I	(25 ppm)	62
	PV	CO_2	NE	(50 μM)	61
	CC	O_2/ATP	I/S	(100 μM)	63
DINITROPHENOLS					
Dinoseb	AP	O_2/CG	S/L	(100 μM)	59
	SB	ATP	I	(600 μM)	60
	PV	CO_2	I	(10 μM)	61
	CC	O_2	S	(10 μM)	63
Dinoterb	PL	O_2	S	(3 μM)	64
DNOC	AP	O_2/CG	S/L	(100 μM)	59
DIPHENYLETHERS					
Fluorodifen	AP	O_2/CG	E/L	(100 μM)	59
Nitrofen	AP	O_2/CG	E/L	(100 μM)	59
PHENYLCARBAMATES					
Chlorpropham	AP	O_2	I	(600 μM)	26
	SB	ATP	I	(600 μM)	60
	PV	CO_2	I	(100 μM)	61
PHENYLUREAS					
Diuron	AP	O_2/CG	E/I	(100 μM)	65
	SB	ATP	NE	(600 μM)	60
	PV	CO_2	I	(100 μM)	66
Monuron	AP	O_2/CG	E/E	(100 μM)	65
	PV	CO_2	E	(100 μM)	61
Neburon	AP	O_2/CG	S/L	(100 μM)	65
PHENOXIES					
2,4-D	PV	CO_2	E	(100 μM)	61
2,4,5-T	SB	ATP	I	(600 μM)	60
TRIAZINES					
Atrazine	AP	O_2/CG	E/NE	(100 μM)	59
	PV	CO_2	NE	(100 μM)	61
	SB	ATP	NE	(200 μM)	60
Prometryn	PV	CO_2	I	(100 μM)	66
URACILS					
Bromacil	AP	O_2/CG	NE/NE	(100 μM)	59
	PV	CO_2	NE	(100 μM)	61
Isocil	SB	ATP	NE	(600 μM)	60

Table 5 (continued)
EFFECTS OF REPRESENTATIVE HERBICIDES FROM DIFFERENT CHEMICAL FAMILIES ON RESPIRATORY OXYGEN UTILIZATION, ON CELL GROWTH, OR, ATP CONCENTRATIONS IN, EXCISED TISSUE AND ISOLATED CELLS

FAMILY/ representative compound(s)	Tissue[a]	Assay system[b]	Responses[c] (concentration)		Ref.
MISCELLANEOUS					
CDAA	SB	ATP	S	(600 μM)	60
	PV	CO_2	I	(5 mM)	61
Chloramben	SB	ATP	NE	(600 μM)	60
	PV	CO_2	E	(1 mM)	61
Dalapon	PV	CO_2	NE	(1 mM)	61
EPTC	SB	ATP	NE	(600 μM)	60
	PV	CO_2	E	(1 mM)	61
Paraquat	PV	CO_2	S	(1 mM)	61

[a] Tissue code: MBrt, mung bean (*Phaseolus aureus* Roxb. = *Vigna radiata* L.) root tips; MBh, mung bean hypocotyls; AP, nonphotosynthetic *Acer pseudoplatanus* suspension culture; CR, corn (*Zea mays* L.) roots; PL, pea (*Pisum sativum* L.) leaf sections; SB, soybean (*Glycine max* L. Merr.) hypocotyls; PV, *Phaseolus vulgaris* leaf cells; CC, carrot (*Daucus carota* L.) callus culture.

[b] Assay system code: O_2, oxygen evolution monitored with Clark-type (platinum) electrode; CG, cell growth; ATP, measured with luciferin-luciferase system; CO_2, $^{14}CO_2$ released from ^{14}C-glucose, trapped, and counted.

[c] Response code: I, inhibition; S, stimulation; L, lethal; E, essentially no effect (control ± 10%); NE, no effect.

directly or indirectly, to drive most biosynthetic reactions. The several nucleotide and deoxynucleotide triphosphates (CTP, GTP, UTP, and dATP, dCTP, dGTP, and dTTP) are formed by transfer of the terminal phosphate group of ATP to the 5′-diphosphates of the respective nucleotides.[67] Through these transfers, catalyzed by specific diphosphokinases, ATP energy is channeled into the major biosynthetic pathways involved in the synthesis of polysaccharides, porphyrins, cellulose, proteins, lipids, and the nucleic acids. None of the triphosphates can substitute for ATP in processes energized by ATP.

Membrane functions including active transport and the effects induced by membrane-bound hormones and phytochrome are energy dependent. The structural organization, contraction, and orientation of chromosomes and microtubules of the spindle apparatus during mitosis depend on ATP energy. The intracellular concentrations and stoichiometric relations of ATP, ADP, and AMP modulate cellular metabolism.[68]

In evaluating the role of ATP in the cellular metabolism of higher plants, all processes that contribute to the ATP pool (glycolysis, oxidative phosphorylation, and photophosphorylation) have to be considered. Even though the photosystem II inhibitors block noncyclic photophosphorylation, ATP can still be produced in vivo, under some conditions, by cyclic photophosphorylation, by glycolysis, and through oxidative phosphorylation. Apparently, sufficient energy can be provided through the last two processes, if respirable carbohydrates are supplied exogenously, to satisfy the light-induced demands and prevent phytotoxic symptoms in *s*-triazine-treated plants.[35] The uncouplers and inhibitory uncouplers interfere with the mitochondrial production of ATP, and carbohydrates do not protect against their action.[35]

All herbicides that inhibit or uncouple mitochondrial electron transport also interfere with

chloroplast electron transport and/or phosphorylation. However, the converse is not true, i.e., as indicated previously, there are a large number of herbicides such as diuron and the *s*-triazines that have only a marginal effect on mitochondrial reactions at relatively high molar concentrations.

V. CONCLUSIONS

Inhibition of chloroplast electron transport has been correlated with binding to a protein(s), however, the sites and mechanisms through which herbicides interfere with mitochondrial and chloroplast-mediated phosphorylations remain to be identified. When lipophilic herbicides partition into the lipid phases of membranes, they could perturb lipid-lipid, lipid-protein, and protein-protein interactions that are required for membrane functions such as electron transport, ATP formation, and active transport. Interference with photophosphorylation and oxidative phosphorylation may involve an interaction with the lipoidal components of the membrane.

Membrane perturbations can be induced very rapidly when lipophilic herbicides partition into membranes. However, techniques to measure directly rapid alterations in membrane fluidity and permeability induced in most cellular and organelle membranes are not yet available. If a herbicide perturbs a particular cellular membrane, it is likely that all membranes could be affected. The consequences of perturbations can be measured readily in those membranes that involve electron transport and phosphorylation. Perturbations and subsequent alterations in the tonoplast, plasmalemma, and nuclear membranes and the endoplasmic reticulum may be just as dramatic as in mitochondria and chloroplasts, but the changes may be more subtle. However, techniques are not yet available to detect the changes, or investigators have yet to identify marker systems that can be readily monitored that reflect the alterations.

Many biologically active chemicals, such as anesthetics and drugs, induce expansion when they partition into membranes. The expansion is postulated to produce multiple effects on membrane-related processes.[69,70] According to this postulate, anesthetics and other neuroactive agents adsorb to hydrophobic regions (lipid and protein) of the excitable membrane, expand the hydrophobic regions of proteins, and subsequently block the ionic conductance channels that underlie nerve cell action potentials.[69] Most of the herbicides that interfere with mitochondrial activity induce expansion of the inner membrane. Some of the inhibitory responses may be a consequence of membrane expansion.

Conceivably, for herbicides that interfere with mitochondrial activity, acute phytotoxicity could be expressed by a combination of alterations to the properties of various cellular membranes and the limited availability of ATP. Small changes in the properties of cellular membranes could have a significant and drastic effect over a time span of several hours or days.

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REFERENCES

1. **Lehninger, A. L.,** *The Mitochondrion*, W. A. Benjamin, New York, 1964, 263.
2. **Tzagoloff, A.,** *Mitochondria*, Plenum Press, New York, 1982, 342.
3. **Hanson, J. B. and Day, D. A.,** Plant mitochondria, in *The Biochemistry of Plants*, Vol. 1, Tolbert, N. E., Ed., Academic Press, New York, 1980, chap. 8.
4. **Storey, B. T.,** Electron transport and energy coupling in plant mitochondria, in *The Biochemistry of Plants*, Vol. 2, Davies, D. D., Ed., Academic Press, New York, 1980, chap. 4.
5. **Mazliak, P.,** Glyco- and phospholipids of biomembranes in higher plants, in, *Lipids and Lipid Polymers in Higher Plants*, Tevini, M. and Lichtenthaler, H. K., Eds., Springer-Verlag, Berlin, 1977, 48.
6. **Moreland, D. E.,** Mechanisms of action of herbicides, *Annu. Rev. Plant Physiol.*, 31, 597, 1980.
7. **Bonner, W. D., Jr.,** A general method for the preparation of plant mitochondria, *Methods Enzymol.*, 10, 136, 1967.
8. **Douce, R., Christensen, E. L., and Bonner, W. D., Jr.,** Preparation of intact plant mitochondria, *Biochim. Biophys. Acta*, 275, 148, 1972.
9. **Jackson, C., Dench, J. E., and Hall, D. O.,** Separation of mitochondria from contaminating subcellular structures utilizing silica sol gradient centrifugation, *Plant Physiol.*, 64, 150, 1979.
10. **Douce, R., Moore, A. L., and Neuburger, M.,** Isolation and oxidative properties of intact mitochondria isolated from spinach leaves, *Plant Physiol.*, 60, 625, 1977.
11. **Chance, B. and Williams, G. R.,** Respiratory enzymes in oxidative phosphorylation. III. The steady state, *J. Biol. Chem.*, 217, 409, 1955.
12. **Moreland, D. E. and Blackmon, W. J.,** Effects of 3,5-dibromo-4-hydroxybenzaldehyde-*O*-(2,4-dinitrophenyl)oxime on reactions of mitochondria and chloroplasts, *Weed Sci.*, 18, 419, 1970.
13. **Slater, E. C.,** Uncouplers and inhibitors of oxidative phosphorylation, in *Metabolic Inhibitors*, Vol. 2, Hochster, R. M. and Quastel, J. H., Eds., Academic Press, New York, 1963, 503.
14. **Slater, E. C.,** Application of inhibitors and uncouplers for a study of oxidative phosphorylation, *Methods Enzymol.*, 10, 48, 1967.
15. **Moreland, D. E. and Huber, S. C.,** Fluidity and permeability changes induced in the inner mitochondrial membrane by herbicides, in *Plant Mitochondria*, Ducet, G. and Lance, C., Eds., Elsevier/North-Holland, Amsterdam, 1979, 191.
16. **Moreland, D. E.,** unpublished results.
17. **Blackmon, W. J.,** The Effects of 3′,4′-Dichloro-2-methylvaleranilide and Related Acylanilides on Reactions Mediated by Mung Bean Mitochondria, Ph.D. thesis, North Carolina State University, Raleigh, 1970.
18. **Inoue, Y., Ishizuka, K., and Mitsui, S.,** Responses of isolated plant mitochondria to photosynthesis inhibiting acylanilides, *Agric. Biol. Chem.*, 34, 1029, 1970.
19. **Moreland, D. E., Huber, S. C., and Novitzky, W. P.,** Interaction of herbicides with cellular and liposome membranes, in *Biochemical Responses Induced by Herbicides*, Moreland, D. E., St. John, J. B., and Hess, F. D., Eds., American Chemical Society, Washington, D. C., 1982, chap. 5.
20. **Ferrari, T. E. and Moreland, D. E.,** Effects of 3,5-dihalogenated-4-hydroxybenzonitriles on the activity of mitochondria from white potato tubers, *Plant Physiol.*, 44, 429, 1969.
21. **Moreland, D. E., Farmer, F. S., and Hussey, G. G.,** Inhibition of photosynthesis and respiration by substituted 2,6-dinitroaniline herbicides. I. Effects on chloroplast and mitochondrial activities, *Pestic. Biochem. Physiol.*, 2, 342, 1972.
22. **Moreland, D. E. and Huber, S. C.,** Inhibition of photosynthesis and respiration by substituted 2,6-dinitroaniline herbicides. III. Effects on electron transport and membrane properties of isolated mung bean mitochondria, *Pestic. Biochem. Physiol.*, 11, 247, 1979.
23. **Hertel, C., Quader, H., Robinson, D. G., and Marme, D.,** Antimicrotubular herbicides and fungicides affect Ca^{2+} transport in plant mitochondria, *Planta*, 149, 335, 1980.
24. **Belbachir, O., Matringe, M., Chevallier, D., and Tissut, M.,** Physiological actions of dinoterb, a phenol derivative. 2. Effects on isolated plant mitochondria and chloroplasts, *Pestic. Biochem. Physiol.*, 14, 309, 1980.
25. **Moreland, D. E., Blackmon, W. J., Todd, H. G., and Farmer, F. S.,** Effects of diphenylether herbicides on reactions of mitochondria and chloroplasts, *Weed Sci.*, 18, 636, 1970.
26. **Macherel, D., Ravanel, P., and Tissut, M.,** Effects of herbicidal carbamates on mitochondria and chloroplast, *Pestic. Biochem. Physiol.*, 18, 280, 1982.
27. **Cohen, A. S. and Morrison, I. N.,** *In vitro* sensitivity of wheat and oat mitochondria to the selective herbicide, diclofop-methyl, *Pestic. Biochem. Physiol.*, 16, 110, 1981.
28. **Gauvrit, C.,** Action of chlorfenprop-methyl on membrane integrity in plant mitochondria, *Weed Res.*, 23, 85, 1983.

29. **Matlib, M. A., Kirkwood, R. C., and Smith, J. E.,** Differential effect of selected phenoxy-acid compounds on oxidative phosphorylation of mitochondria from *Vicia faba* L., *J. Exp. Bot.*, 23, 886, 1972.
30. **Budimir, M., Plesnicar, M., and Kljajic, R.,** Effects of the herbicide 2,4-DB and fungicide captan on reactions of mitochondria and chloroplasts, *Arch. Environ. Contam. Toxicol.*, 4, 166, 1976.
31. **Gauvrit, C. and Wilson, S. B.,** Uncoupling properties of the herbicide *N-n*-pentyl-*N*-methyl-*N'*-(3,4-dichlorophenyl)-urea, *J. Exp. Bot.*, 34, 367, 1983.
32. **Hauska, G., Trebst, A., Kotter, C., and Schulz, H.,** 1,2,3-Thiadiazolyl-phenyl-ureas, new inhibitors of photosynthetic and respiratory energy conservation, *Z. Naturforsch.*, 30c, 505, 1975.
33. **Blein, J. B., Ducruet, J. M., and Gauvrit, C.,** Identification et activite biologique d'une impurete du diuron technique, *Weed Res.*, 19, 117, 1979.
34. **Ducruet, J. M. and Gauvrit, C.,** Effects des urées substitutées sur les oxydations phosphorylantes des mitochondries de tubercules de pomme de terre (*Solanum tuberosum* L.): comparaison avec l'action sur liposomes, *Weed Res.*, 18, 327, 1978.
35. **Moreland, D. E. and Boots, M. R.,** Effects of optically active 1-(α-methylbenzyl)-3-(3,4-dichlorophenyl)urea on reactions of mitochondria and chloroplasts, *Plant Physiol.*, 47, 53, 1971.
36. **Gauvrit, C.,** Action of the herbicides neburon and siduron on membrane permeabilities in potato tuber mitochondria, *Physiol. Plant.*, 48, 448, 1980.
37. **Moreland, D. E.,** Interaction of perfluidone with mitochondrial, thylakoid, and liposome membranes, *Pestic. Biochem. Physiol.*, 15, 21, 1981.
38. **Killion, D. D. and Frans, R. E.,** Effect of pyriclor on mitochondrial oxidation, *Weed Sci.*, 17, 468, 1969.
39. **Gauvrit, C. and Sealla, R.,** The experimental herbicide UKJ72J is an inhibitor of succinate oxidation in plant mitochondria, *FEBS Lett.*, 158, 222, 1983.
40. **Heytler, P. G.,** Uncouplers of oxidative phosphorylation, *Methods Enzymol.*, 55, 462, 1979.
41. **Green, D. E. and Blondin, G. A.,** Molecular mechanism of mitochondrial energy coupling, *Bioscience*, 28, 18, 1978.
42. **Hanstein, W. G.,** Uncoupling of oxidative phosphorylation, *Biochim. Biophys. Acta*, 456, 129, 1976.
43. **Hatefi, Y.,** Energy conservation and uncoupling in mitochondria, *J. Supramol. Struct.*, 3, 201, 1975.
44. **Kessler, R. J., Vande Zande, H., Tyson, C. A., Blondin, G. A., Fairfield, J., Glasser, P., and Green, D. E.,** Uncouplers and the molecular mechanism of uncoupling in mitochondria, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 2241, 1977.
45. **Hatefi, Y. and Galante, Y. M.,** Organization of the mitochondrial respiratory chain, in *Energy Conservation in Biological Membranes*, Schäfer, G. and Klingenberg, M., Eds., Springer Verlag, Berlin, 1978, 19.
46. **Katre, N. V. and Wilson, D. F.,** Interaction of uncouplers with the mitochondrial membrane: A high affinity binding site, *Arch. Biochem. Biophys.*, 185, 578, 1977.
47. **Kurup, C. K. and Sanadi, D. R.,** Photoaffinity labeling of uncoupler binding sites on mitochondrial membrane, *J. Bioenerg. Biomembr.*, 9, 1, 1977.
48. **Olorunsogo, O. O., Bababunmi, E. A., and Bassir, O.,** Uncoupling of corn shoot mitochondria by *N*-(phosphonomethyl) glycine, *FEBS Lett.*, 97, 279, 1979.
49. **Moreland, D. E., Hussey, G. G., and Farmer, F. S.,** Comparative effects of dichlobenil and its phenolic alteration products on photo- and oxidative phosphorlation, *Pestic. Biochem. Physiol.*, 4, 356, 1974.
50. **Moreland, D. E. and Hilton, J. L.,** Actions on photosynthetic systems, in *Herbicides: Physiology, Biochemistry, Ecology*, Vol. 1, Audus, L. J., Ed., Academic Press, London, 1976, chap. 16.
51. **Moreland, D. E. and Huber, S. C.,** Alterations to properties of the inner mitochondrial membrane by chlorpropham and other phenylamide herbicides, *Abstr. Weed Sci. Soc. Am. Meet. San Francisco* Weed Science Society of America, Champaign, Ill., 1979, 103.
52. **Moreland, D. E. and Huber, S. C.,** Alterations to properties of mitochondrial and chloroplast membranes induced by herbicides, *Abstr. 9th Int. Congr. Plant. Prot. Washington*, No. 935, American Phytopathology Society, St. Paul, 1979.
53. **Moreland, D. E.,** Inhibitors of chloroplast electron transport: Structure-activity relations, in *Progress in Photosynthesis Research*, Metzner, H., Ed., International Union of Biological Science, Tubingen, Federal Republic of Germany, 1969, 1693.
54. **Inoue, Y., Ishizuka, K., and Mitsui, S.,** Effects of photosynthesis inhibiting acylanilides on the activity of mammal mitochondria, *Agric. Biol. Chem.*, 33, 1798, 1969.
55. **Büchel, K. H., Korte, F., and Beechey, R. B.,** Uncoupling of the oxidative phosphorylation in mitochondria by NH-acidic benzimidazoles, *Angew. Chem. Int. Ed.*, 4, 788, 1965.
56. **Jones, O. T. G. and Watson, W. A.,** Properties of substituted 2-trifluoromethylbenzimidazoles as uncouplers of oxidative phosphorylation, *Biochem. J.*, 102, 5641, 1967.
57. **Gauvrit, C.,** Effects of some substituted urea herbicides on oxidative phosphorylation in potato tuber mitochondria (*Solanum tuberosum* L.), in *Plant Mitochondria*, Ducet, G. and Lance, C., Eds., Elsevier/North-Holland, Amsterdam, 1978, 199.

58. **Kirkwood, R. C.,** Action on respiration and intermediary metabolism, in, *Herbicides: Physiology, Biochemistry, Ecology*, Vol. 1, Audus, L. J., Ed., Academic Press, London, 1976, chap. 15.
59. **Blein, J. P.,** Action of some herbicides on growth, respiration, plasmalemma integrity, and proton extrusion of *Acer pseudoplatanus* cells. II. Amides, diphenyl ethers, nitriles, phenols, triazines, and uracils, *Pestic. Biochem. Physiol.*, 17, 156, 1982.
60. **Gruenhagen, R. D. and Moreland, D. E.,** Effects of herbicides on ATP levels in excised soybean hypocotyls, *Weed Sci.*, 19, 319, 1971.
61. **Ashton, F. M., DeVilliers, O. T., Glenn, R. K., and Duke, W. B.,** Localization of metabolic sites of action of herbicides, *Pestic. Biochem. Physiol.*, 7, 122, 1977.
62. **Moreland, D. E., Farmer, F. S., and Hussey, G. G.,** Inhibition of photosynthesis and respiration by substituted 2,6-dinitroaniline herbicides. II. Effects on responses in excised plant tissues and treated seedlings, *Pestic. Biochem. Physiol.*, 2, 354, 1972.
63. **Sloan, M. E. and Camper, N. D.,** Trifluralin effects on carrot culture tissue, *Pestic. Biochem. Physiol.*, 15, 201, 1981.
64. **Belbachir, O., Matringe, M., Tissut, M., and Chevallier, D.,** Physiological actions of dinoterb, a phenol derivative. 1. Physiological effects on the whole plant and on tissue fragments of pea, *Pestic. Biochem. Physiol.*, 14, 303, 1980.
65. **Blein, J. P.,** Action of some herbicides on growth, respiration, plasmalemma integrity, and proton extrusion of *Acer pseudoplatanus* cells. 1. Substituted ureas, *Pestic. Biochem. Physiol.*, 16, 179, 1981.
66. **DeVilliers, O. T. and Marais, H. J.,** Comparative effects of diuron and prometryne on biochemical processes in isolated leaf cells, *Proc. Natl. Weeds Conf. S. Afr.*, Vol. 3, A. A. Balkema, Rotterdam, 1979, 199.
67. **Lehninger, A. L.,** *Bioenergetics*, W. A. Benjamin, Menlo Park, Calif., 1971, 245.
68. **Atkinson, D. E.,** Regulation of enzyme function, *Annu. Rev. Microbiol.*, 23, 47, 1969.
69. **Seeman, P.,** The membrane actions of anesthetics and tranquilizers, *Pharmacol. Rev.*, 24, 583, 1972.
70. **Ritchie, J. M.,** Mechanisms of action of local anaesthetic agents and biotoxins, *Br. J. Anaesth.*, 47, 191, 1975.

Chapter 3

EFFECTS OF HERBICIDES ON CHLOROPLAST AND CELLULAR DEVELOPMENT

Paul G. Bartels

TABLE OF CONTENTS

I. INTRODUCTION

Since two excellent comprehensive reviews describing the action of herbicides on the ultrastructure of plant cells were published in 1973[1] and 1976,[2] many new articles dealing with this subject have appeared. Recent books[3-5] have included the results from some of these published articles, but this subject has not been recently reviewed as a single topic. In this chapter, the literature since 1972 will be surveyed, covering the ultrastructural effects caused by herbicides on chloroplasts, microtubules, endoplasmic reticulum, and cell walls. Effects on chloroplasts will be emphasized, because chloroplasts appear to be more sensitive to the majority of the available herbicides than other organelles. The ultrastructural effects of herbicides on nuclei and mitochondria are rarely found, except during late stages of cellular deterioration.

A long sought-after goal of weed scientists is to elucidate how the primary action of a herbicide alters the cellular morphology and growth of a plant. Most electron microscopic studies describing the action of herbicides on the ultrastructure of plant cells do not provide clues or data as to the possible primary site(s) of action of a specific herbicide. The ultrastructural organization of plant organelles represents a complex system that includes several metabolic pathways and chemical constituents. If one step of a pathway is inhibited by a herbicide (primary site), the other steps in the pathway or in other pathways of the complex system may not be able to operate or to remain stable, because the pathways are integrated. Ultrastructural studies are typically limited to observing or detecting structural changes caused by several biochemical alterations originating from one primary biochemical site of action. Another complication of ultrastructural research is that the inhibition of a single primary biochemical action may produce two independent structural effects, depending on the concentration of herbicides reaching the biochemical site. Consequently, it is the detective work of the electron microscopist to guess or theorize how these structural changes may have been produced in the cell by the herbicides. Obviously, the best approach to this work is to correlate the ultrastructural observations with biochemical changes that occur during the early stages of herbicidal activity. Research that examines herbicide-treated tissue, both biochemically and ultrastructurally, during and immediately following treatment, may provide an insight into the events that lead to structural alteration. The detection of these ultrastructural changes may occur as early as 24 h with some herbicides, whereas with other herbicides the changes do not occur until later. Thus, it is the purpose of this chapter to compare and evaluate the literature describing the effects of herbicides on the ultrastructure of plants and attempt to correlate, when possible, published ultrastructural observations with reported biochemical changes.

II. ULTRASTRUCTURE OF PLASTIDS

A. The Chloroplast

The structure of mature, green chloroplasts of angiosperm plants, as revealed by the electron microscope, has been discussed in several reviews.[6-7] The chloroplast is surrounded by a limiting double membrane or envelope. Its internal structure is a complex membrane system embedded in a granular matrix, the stroma. The pigments involved in photosynthesis are found in the membranes, whereas the enzymes involved in carbon fixation, 70S ribosomes, strands of naked DNA fibrils, and often starch grains and lipid globules, are located in the stroma. The specific location and orientation of the proteins, lipids, and pigments within the membrane are still a matter of controversy and various molecular models have been proposed. Most of the recent models are based on the fluid mosaic model.[8] The membranes form flattened discs or sacs that may be layered into stacks called grana (Figure 1). The number of discs per granum is influenced by genetic and environmental factors. In

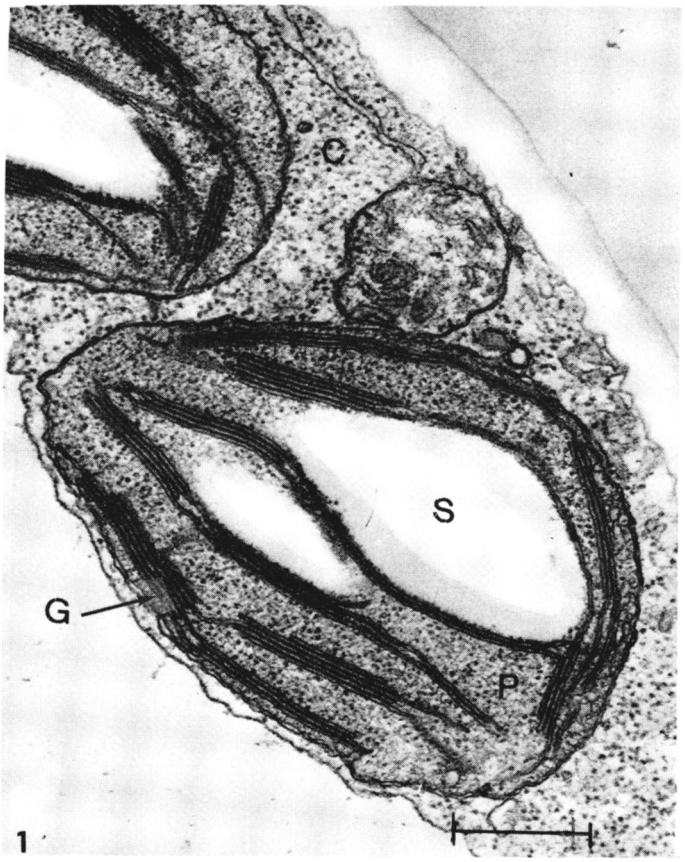

FIGURE 1. Chloroplast from wheat seedling contains grana (G), stoma thylakoids (T), plastid ribosomes (P), cytoplasmic ribosomes (C), and starch (S). Scale marker equals 1 μm.

this chapter, the discs or sacs will be referred to as lamellae or thylakoids. The thylakoid is a single membrane completely bounding a narrow space. Views of freeze-etched preparations of chloroplast membranes show that the internal faces of the chloroplast thylakoids are composed of different-sized particles that may perform photosynthetic activities. The area where two thylakoids touch each other in a granum will be called a partition. The thylakoids of a chloroplast are not completely separated discs, but are interconnected to form a unified membrane system continuous with the inner membrane of the plastid envelope.

The organization of the thylakoids and the starch levels in the chloroplast of some C_4 plants differ from that of the C_3 plants. C_4 plants are those which form C_4 dicarboxylic acids as the primary products of photosynthesis, whereas C_3 plants produce phosphoglyceric acid (3 carbon acid) as a primary product (Calvin cycle, see Chapter 1 for further discussion). The mesophyll cells of C_3 plants have chloroplasts with grana and starch grains, whereas C_4 plant mesophyll cells have chloroplasts with grana, but no starch grains. The bundle sheath chloroplasts of C_4 plants have an abundance of starch and certain species (NADP-ME types) are agranal (see Chapter 1).

B. Nongreen Plastids

Angiosperm plants also contain various nongreen plastids and they are generally classified on the basis of pigment and internal membrane structures they contain. In meristematic and

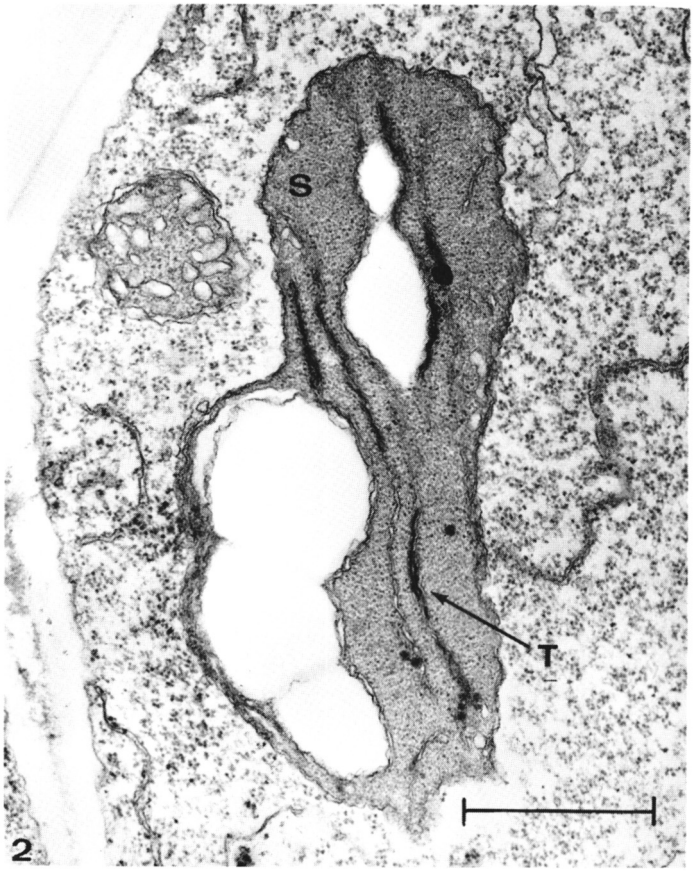

FIGURE 2. Protoplastid from intercalary meristem of wheat seedling contain few thylakoids (T) and stroma (S). Scale marker equals 1 μm.

reproductive cells of higher plants, mature green chloroplasts are absent, but there are numerous proplastids present. These plastids are precursors to other plastids and can develop into plastids with specialized biochemical attributes (Figure 2). They consist of a double membrane envelope and stroma containing few or no thylakoid membranes, 70S ribosomes, and often starch grains. Connections of thylakoids to inner membranes of the proplastid envelope are common.

If plants are grown in the absence of light, the proplastids of the leaf cells undergo development into plastids called etioplasts, which lack chlorophyll but contain small amounts of protochlorophyllide and carotenoids. The most prominent membrane structure in the etioplast is the prolamellar body, which is a complex network of interconnected tubular membranes having the appearance of a crystal lattice (Figure 3). The prothylakoid membranes of the etioplasts contain 85 to 90% of the protochlorophyllide.[9] Etioplasts have most of the components found in chloroplasts, such as DNA fibrils, carotenoid pigments, 70S ribosomes, plastoglobuli, starch grains, and most of the photosynthetic enzymes. The ultrastructure of the chromoplast is quite different from other plastids. Most of the internal membranes are missing, but they are replaced with crystals and/or globules of carotenoids, lipids, and sometimes starch.

The ultrastructure of algal chloroplasts often differs substantially from the chloroplasts of angiosperms. For instance, organization of the grana in algae is usually quite different from those of higher plants. Some algae do not have proplastids and consequently do not develop

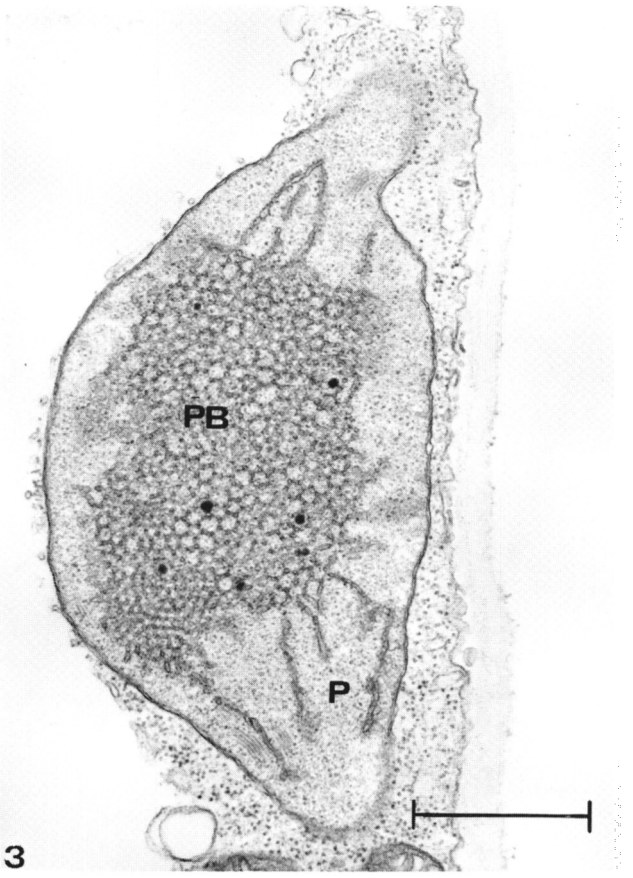

FIGURE 3. Etioplast from wheat seedling contains a prolamellar body (PB) and plastid ribosomes (P). Scale marker equals 1 μm.

chloroplasts in the same manner as higher plants. Schiff[10] proposed that the formation of thylakoid membranes in *Euglena* from proplastids requires the simultaneous availability of the membrane components and that this is achieved by an elaborate coregulation of the biosynthesis of these components to ensure that the correct amounts are usually present. When the synthesis of one set of components is inhibited (carotenoids), then the synthesis of other components (chlorophyll) are stopped and membrane assembly is halted. In higher plants, this does not appear to be the case. Frosch et al.[11] reported that chloroplasts can develop in the absence of carotenoids. Because of these differences between the algae and higher plants, investigators should be cautious about extrapolating their results describing the effect of herbicides on algae to higher plants. Stokes et al.[12] observed that algal cells were more tolerant to organic herbicides than higher plants.

III. CHLOROPLAST DEVELOPMENT

The formation of mature chloroplasts in higher plants involves a complex set of processes requiring synthesis and assembly of pigments, proteins, and other membrane components in fully functioning organelles. This development of chloroplasts may occur by several pathways: (1) conversion of proplastids to chloroplasts under normal conditions of illumination, (2) light-induced development of etioplasts into chloroplasts, and (3) conversion of

chromoplasts to chloroplasts. In many cases, photosynthesis is not required for chloroplast development. Proplastids contain only rudimentary thylakoids; however, when development commences, the thylakoids extend, flatten, and tend to become evenly spaced throughout the stroma. The thylakoids appear to be derived from various membranous invaginations, vesicles, and tubules that are associated with the plastid envelope.[13]

Upon illumination, the prolamellar bodies of the etioplasts break down and grana develop. The conversion of etioplasts to chloroplasts is the most often studied pathway. This pathway should not be used as a model to study other types of plastid development, since the chronological age of the plastid determines what biochemical events take place during its sequence of development. Once the chloroplast becomes fully developed, the chloroplast structure remains stable, if maintained under constant environment. However, some turnover of individual chloroplast components may occur.

IV. EFFECTS OF SELECTED HERBICIDES ON CHLOROPLAST DEVELOPMENT

A. Introduction

Most of the herbicides used to control weeds in field crops affect the chloroplast in some manner. See Table 7 in Chapter 1 for a listing of herbicides that affect chloroplast structure and photosynthesis. The effectiveness of these herbicides to interfere with chloroplast development usually depends upon the stage of growth of the plant when the herbicides are applied. Some herbicides are more effective when applied preemergence, whereas others are more effective when applied postemergence. Several "bleaching herbicides" such as norflurazon, metflurazon, fluridone, SAN 9785, amitrole, dichlormate, difunon, and pyrichlor are effective inhibitors of chloroplast development when applied preemergence. New growth appearing after treatment becomes chlorotic, whereas preexisting green tissue remains unaffected.[14] These herbicides interfere with plastid development by inhibiting specific reactions involved in the biosynthesis of carotenoids. In the absence of carotenoids, photooxidation of the chlorophyll pigments and peroxidation of other membrane components occurs, leading to thylakoid destruction and chlorosis. Certain of the "bleaching herbicides", e.g., norflurazon, can also directly inhibit photosynthesis, as well as plastid development, but at different concentrations. The diphenyl ethers and paraquat (bipyridylium salts), another group of "bleaching herbicides", also affect plastid development in some plant systems, but they are more effective as herbicides when applied to mature etioplasts or chloroplasts that contain carotenoid pigments. They induce the destruction of preexisting chloroplast pigments and thylakoid components in the presence of light and oxygen by peroxidation reactions.

A group of herbicides called the photosystem II inhibitors (see Chapter 1) have been shown to alter chloroplast development in growing tissue, but they appear to be more effective as herbicides when applied postemergence on mature green tissue. They cause the photodestruction of preexisting pigments and thylakoids of mature, green chloroplasts by inhibiting the photosynthetic electron-transport chain.

B. Inhibitors of Carotenoid Synthesis

Herbicides that inhibit carotenoid synthesis during plastid development do not affect preexisting carotenoids. The ultrastructural effects of norflurazon (SAN 9789) and metflurazon (SAN 6706) both on light- and dark-grown seedlings have been investigated by several researchers.[11,15-18] Their observations were generally similar, even though several plant species and herbicide concentrations were used in their investigations. Metflurazon has been shown to be converted to norflurazon by plants and it is suggested that the herbicidal effects produced by metflurazon were caused by norflurazon.[19] The ultrastructural changes of the

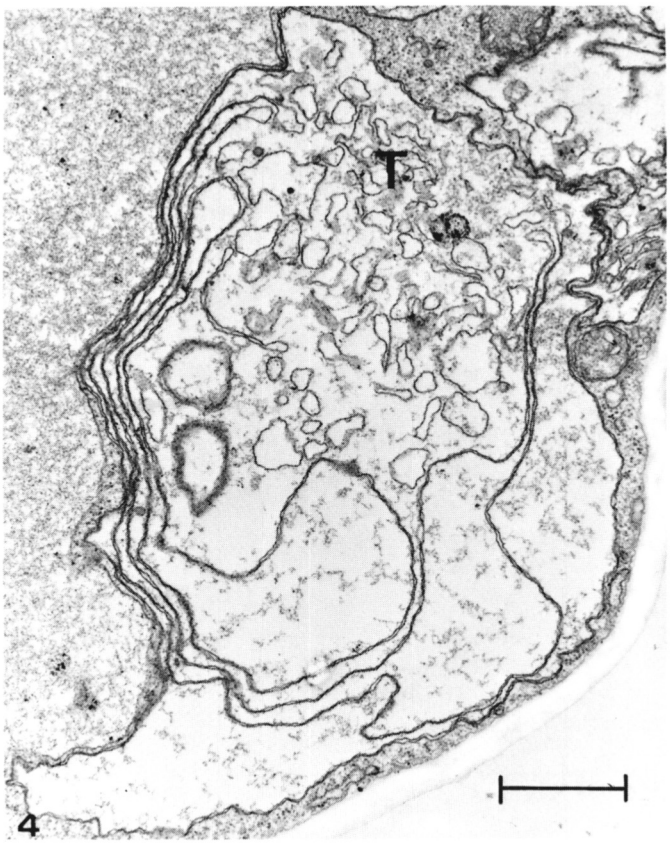

FIGURE 4. Plastid from metflurazon-treated wheat seedling showing an aggregate of disorganized Thylakoids (T) and loss of chloroplast ribosomes. Scale marker equals 1 μm.

chloroplast produced by these herbicides were dependent on herbicide concentrations (100 to 1 μ*M*) and the light intensities under which the seedlings were grown. Generally, the effect was observed 3 to 7 days after herbicide application. Plastids of seedlings treated with either herbicide and grown under high light intensities lacked grana and 70S ribosomes, but contained a few thylakoids that were unassociated, unbranched, usually long, and arranged parallel to the edge of the plastid envelope (Figure 4).[15-18,20] When wheat seedlings (*Triticum aestivum*) were grown under weak red light in the presence of norflurazon, the plastids lacked grana, exhibited a decreased chlorophyll *b*/*a* ratio, and contained a reduced number of prolamellar bodies.[21] When wheat seedlings were grown under weak white light (1 fc) for a week, the plastids of the untreated plants contained prolamellar bodies, ribosomes, and some thylakoids, whereas plastids of treated plants had prolamellar bodies and ribosomes, but lacked thylakoids.[15-17] In far-red light, which is inefficiently absorbed by chlorophyll, development of the plastid ultrastructure, synthesis of ribulose bisphosphate carboxylase, and synthesis of chlorophyll in white mustard seedlings (*Sinapis alba*) were not affected by norflurazon.[11] The concentration of the herbicides, as well as the intensity of light under which the plants were grown, affected the plastid structure. Light-grown barley seedlings (*Hordeum vulgare*) treated with 100 μ*M* of metflurazon had plastids which were severely disrupted, whereas seedlings treated with 10 μ*M* had plastids which were only slightly damaged.[18]

The etioplasts of metflurazon- and norflurazon-treated, dark-grown wheat and bean (*Phas-*

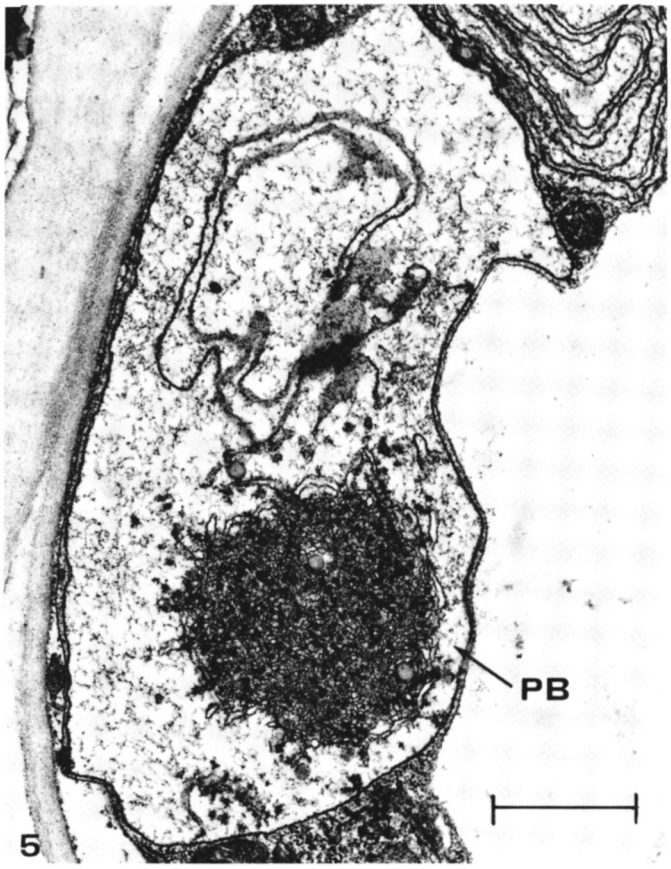

FIGURE 5. Chloroplast from metflurazon-treated wheat seedling grown in dark 6 days and then exposed to 12 h of high light intensity. Plastid contains a disorganized prolamellar body (PB). Scale marker equals 1 μm.

eolus vulgaris) seedlings were morphologically similar to etioplasts of untreated seedlings, having prolamellar bodies and ribosomes.[15,22] Etioplasts of norflurazon-treated wheat seedlings had larger but fewer plastoglobuli than controls.[21] The plastoglobuli were not osmiophilic and appeared transparent under the electron microscope. When dark-grown wheat seedlings were exposed to high light intensities for 12 h, the untreated plants became green, whereas the metflurazon-treated ones became white, and the prolamellar bodies of this treated tissue changed into an aggregated unit of a highly disorganized structure, and the stroma was then devoid of ribosomes (Figure 5).[15]

The effect of norflurazon and metflurazon on the ultrastructure of *Euglena* and *Chlorella* chloroplasts was reported to be quite different from the effect on higher plants. In contrast to higher plants, proplastids of dark-grown *Euglena* have thylakoids and noncrystalline prolamellar bodies. These internal thylakoids were reduced to small segments of incomplete membranes by norflurazon.[23] Because the cells were not exposed to light, this effect was not caused by photooxidation. Treatment of *Chlorella* with 30 μ*M* norflurazon or metflurazon stimulated the biosynthesis of plastid constituents, fatty acids, chlorophyll, and carotenoids. The ultrastructural analysis of the treated *Chlorella* cells showed that the chloroplast membranes were closely packed and that large segments of the pyrenoids were absent.[24]

The preemergence application of pyrazon and SAN 9785, two substituted pyridazinones, also affected plastid development in bean seedlings, but the chlorotic action was not as

marked as with norflurazon or metflurazon. Grana formation in chloroplasts was inhibited, thylakoids became swollen and perforated, ultimately disintegrating, and lipid globules increased in size and number following treatment of bean seedlings with pyrazon.[25] Ultrastructural study of *Vicia faba* seedlings treated with SAN 9785 showed that the plastids contained some grana, but had very few intergranal thylakoids.[26] In the most severely affected tissue, thylakoids were dilated and the number of granal units was considerably reduced.

The herbicides fluridone, dichlormate, difunon, pyrichlor, amitrole, and haloxydine have very different structures than the pyridazinone herbicides, yet they also inhibit the synthesis of carotenoids and the ultrastructural effects on plastids are very similar to those of norflurazon and metflurazon. Bartels and Watson[17] reported that the preemergence treatment of wheat with fluridone (1 to 100 μM) caused chlorotic seedlings to emerge under high light intensity after 7 days. The plastids of the light-grown seedlings lacked chloroplast ribosomes and normal ultrastructure, whereas dark-grown seedlings had plastid ribosomes and prolamellar bodies. When these 6-day-old, dark-grown treated wheat seedlings were exposed to high light intensities for 16 h, the etioplasts changed into a disorganized structure devoid of ribosomes and grana (similar to Figure 5).

Amitrole also inhibited the development of plastids and blocked the formation of grana. If membranes were present in the plastids, they were irregularly arranged.[27-29] The formation of 70S chloroplast ribosomes, internal membranes, and fraction I protein in developing etioplasts was not affected by amitrole preemergence treatment.[30-31] Plastids of root meristem cells in flax (*Linum usitatissimum*)[32] and the alga *Scenedesmus*[33] did not show any definite pattern of ultrastructure disorganization after amitrole treatment. Cells in the elongation zone of the amitrole-treated flax roots showed major structural damage following the rupture of the vacuolar membrane.

Difunon also affected the development of plastids in several species. Although the plastids from different tissues of several herbicide-treated grass seedlings possessed normal envelopes, they contained no thylakoids. This was true for plastids from the parenchyma, bundle sheath, and guard cells. Despite the absence of normal internal plastid membranes, the plastids had lipid globules and large starch grains.[34] Haloxydine and pyricolor, which are structurally similar to hydroxypyridine-type herbicides, interfered with normal chloroplast development by inhibiting carotenoid synthesis.[14,35] The conversion of etioplasts to chloroplasts was inhibited in haloxydine-treated barley seedlings, but the herbicide had no effect on etioplast development in the dark.[36]

The effects of dichlormate on wheat seedlings resembled those of amitrole and the pyridazinone herbicides.[37] Plastids from preemergence treated seedlings grown in the light lacked the normal membrane system of grana and intergranal thylakoids. Ribosomes and only a few scattered membranes were visualized in the plastids. Plastids from treated, dark-grown seedlings appeared to be similar to plastids of untreated seedlings.

Two preemergence herbicides, ioxysome and bromoxysome, produced albino leaves in developing oat (*Avena sativa*) seedlings.[38] The plastids of the chlorotic tissue lacked recognizable grana and intergranal thylakoids, but associated with the plastids were globules composed of lipid-like material. Fedtke[4] has suggested that these two herbicides interfere with carotenoid synthesis, but their mechanism of action is still unknown.

Several biochemical studies have reported that the pyridazinone herbicides caused loss of carotenoids and the accumulation of two colorless substances identified to be phytoene and phytofluene (reviewed by Ridley[14]). To explain these effects, it is suggested that the herbicides acted to inhibit the enzyme phytoene desaturase, which desaturates phytoene. Recently, this theory has received support from the work of Clarke et al.[39] who found that norflurazon inhibited the phytoene desaturation reaction of the carotenoid pathway in a cell-free carotenogenic system from the blue-green alga, *Aphanocapsa*. Not all of the "bleaching herbicides" inhibit carotenoid synthesis at this step in the biosynthetic pathway. Ridley[14]

in his review reported that the herbicides amitrole, dichlormate, and pyrichlor inhibited carotenoid synthesis at another site in the pathway (see Chapter 4 for further discussion).

The basis for understanding how ultrastructural changes occur during plastid development in the absence of carotenoids lies in the role that the carotenoids play in the photoprotection of chlorophyll and chloroplast membrane components. Chlorophyll molecules absorb light and become electronically excited. If this excitation energy is not dissipated through normal photosynthetic pathways, the chlorophyll transforms into a triplet excited state. Normally, the longer-lived triplet chlorophyll can return to ground state by passing on energy to carotenoids. In seedlings treated with "bleaching herbicides", the carotenoids are absent and the newly synthesized chlorophylls pass their excited energy to ubiquitous molecular oxygen, producing singlet oxygen or superoxide radicals.[40,41] Singlet oxygen and superoxides are highly disruptive, especially to membranes, and can rapidly destroy thylakoids, resulting in the formation of plastids lacking internal membranes. However, in the absence of chlorophyll and light, etioplast formation occurs normally and without disruption. The importance of carotenoids lies in providing a means of preventing chlorophyll oxidation and membrane lipid peroxidation by toxic oxygen species.[42]

The inhibition of linolenic acid formation by the pyridazinone herbicides could also affect the ultrastructural development of the chloroplast, since it is an essential constituent of the chloroplast membranes.[43] In *Euglena* and *Chlorella*, the bleaching and loss of chloroplast internal lamellae induced by norflurazon and metflurazon treatment does not appear to be caused by photooxidative destruction mechanisms, as was described for higher plants. The inhibition of carotenoid synthesis in algal cells was accompanied by cell-directed disassembly of the photosynthetic membranes, resulting in bleaching.[23,44]

C. Inhibitors of Photosynthetic Electron Transport

Several urea derivatives (*s*-triazines, bromacil, and bentazon) which inhibit photosystem II reactions at the secondary electron acceptor B have been shown to alter the greening and development of etioplasts and proplastids into normal chloroplasts. Many of the ultrastructural modifications caused by these herbicides are similar and suggest a common mode of action. In their ultrastructural study of plastid development in detached etiolated bean leaves, Klein and Neuman[45] showed that monuron at 10 μM had no effect on the initial stages of plastid development in the light, but only affected later stages. After 2 h, the number of grana in the chloroplast of treated plants was reduced, but the individual grana were bigger and contained more thylakoids. Diuron also did not inhibit grana formation and fusion of the thylakoids during greening of etiolated bean leaves.[46] Wergin and Potter[47] reported that fluometuron at the concentration of 80 $\mu g/m\ell$ modified the later steps of chloroplast development in mesophyll tissue of 7-day-old velvetleaf (*Abutilon theophrasti*) cotyledons. The formation of the prolamellar bodies and the subsequent formation into primary thylakoids were not prevented, whereas fusion of thylakoid membranes into normal grana was altered, resulting in plastids composed of linear and circular membranes (Figure 6). High concentrations of fluometuron reduced the number and length of stroma thylakoids and promoted more elongated grana with fewer compartments. Light-grown wheat seedlings germinated in 6 ppm methabenzthiazuron had chloroplasts which contained increased proportions of grana to stroma thylakoids and the chlorophyll *a*/*b* ratio was decreased.[4] The chloroplast ultrastructure of the treated seedlings resembled that obtained by shade adaptation.

Bentazon at 100 μM has recently been shown to modify the differentiation of etioplasts into chloroplasts of 3-day-old radish seedlings (*Raphanus sativus*).[48] Light-induced transformation of the etioplasts was not affected, however, and after 5 days of illumination the plastids had two different types of morphology. Some chloroplasts resembled chloroplasts found in shade-adapted plants, having increased grana stacking, decreased number of stroma thylakoids, increased grana size, and increased grana-to-stroma ratios. Other chloroplasts

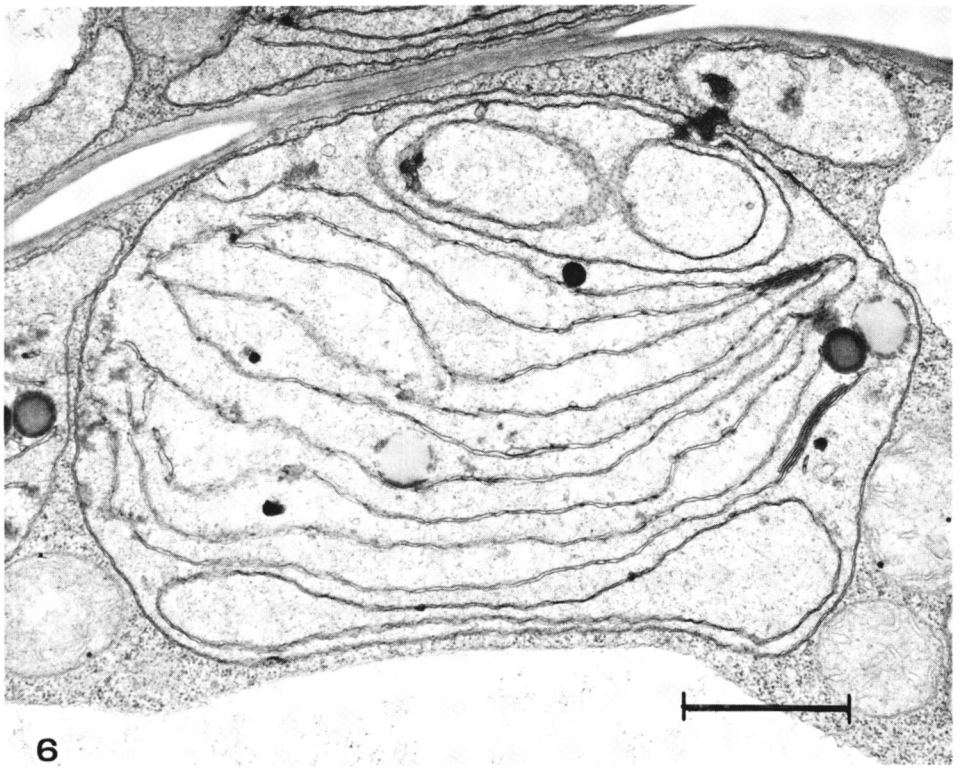

FIGURE 6. Enlarged chloroplast from fluometuron-treated velvetleaf whose grana are replaced by linear and circular membranes. Scale marker equals 1 μm. (From Wergin, W. P. and Potter, J. R., *Pestic. Biochem. Physiol.*, 5, 265, 1975. With permission.)

showed signs of senescence, having only a few grana stacks per plastid section, and the thylakoids of the grana were swollen and extended. Plastoglobuli and starch failed to accumulate in both types of plastids in the treated seedlings.[49]

Bromacil, another type of electron-transport inhibitor, caused an inhibition of grana and thylakoids in the developing plastids of oat seedlings.[50] The number of loculi per granum was reduced, but the length of the granum was increased, along with some swelling of the membrane system. The ultrastructural effects of pyrazon, also a photosystem II inhibitor, on chloroplast development were discussed in the previous section.

The treatment of *Euglena* cells with diuron resulted in a series of ultrastructural changes.[51] For the first week, the cells treated with 25 μ*M* diuron had normal thylakoids, whereas 2 weeks later, the thylakoids became swollen and vesiculated. After 3 weeks, these cells were able to recover and chloroplasts appeared normal. *Euglena* cells grown in 250 μ*M* diuron had chloroplasts that were severely damaged and were unable to recover at any time.

In summary, the electron-transport inhibitors appeared to affect chloroplast development in three phases. In the initial phase, no ultrastructural modifications were observed, but after a brief period, the chloroplast thylakoids were assembled into a few large grana resembling "shade-type" chloroplasts. In the last phase, the chloroplast followed a senescence pattern of degeneration. How might these herbicides cause this pattern of structural events to occur? Fedtke[4] suggested that these herbicides inhibited electron-transport flow at the time the electron chain becomes competent during the development of the plastid, resulting in de-

creased CO_2 fixation and photosynthate production. The low carbohydrate supply induced a regulatory response and plants responded by forming shade-type chloroplasts. With continued inhibition of photosynthesis, however, the chloroplasts may generate toxic substances via triplet chlorophyll of PS II and finally degenerate into plastids like those found in senescent plants. This scenario may describe how most electron inhibitors act on chloroplast development, except for fluometuron, which may act by inhibition of carotenoid synthesis.

D. Inhibition of Chloroplast Development by Other Selected Herbicides

Glyphosine, at 10 μM, a plant growth regulator and a compound chemically similar to glyphosate, has been reported to interfere with chloroplast development in duckweed (*Lemna gibba*)[52] and maize (*Zea mays*).[53] The new fronds of treated duckweed were white and the plastids of the chlorotic tissue contained only a few internal membrane structures and chloroplast ribosomes. Fedtke[4] reported that glyphosine may be converted into glyphosate by the plants and that the observed effect may be caused by glyphosate.

The auxin-herbicide 2,4-D, at a concentration of 1 mM, sprayed on 4-day-old etiolated radish seedlings, altered the development of etioplasts into chloroplasts.[54] The thylakoids were flattened and tightly appressed to each other with very little space between adjacent membranes. The chloroplasts also appeared smaller than the ones in control plants. Within the time of the 2,4-D treatment, no deterioration of the chloroplast membranes occurred, but rather the constituents of photosynthetic units were modified.[55] The 2,4-D probably does not act directly on chloroplast development and the ultrastructural modification of chloroplasts results from a secondary action of the herbicide on cell growth.

Newly formed organelles of apical meristem tissue of metolachlor-treated sorghum (*Sorghum bicolor*) seedlings were difficult to see with the electron microscope, having a ghost-like appearance.[56] The ultrastructural modifications of the cell structures may be brought about by the herbicide inhibiting lipid synthesis or by the herbicide altering the membrane structure. Permeability studies have shown that the integrity of the cellular membranes was modified by the metolachlor. Consequently, electron microscopic fixatives and stains, which normally bind to membrane components, might not be able to do so in these altered membranes and the membranous cell structures would be weakly discernible.

Although the diphenylether, diclofop-methyl, has been shown to be most effective as a herbicide when applied to mature chloroplasts of light-grown plants, Brezeanu et al.[57] reported that proplastid development in growing oat seedlings was also altered by this herbicide. Proplastids of the basal region of treated oat seedlings contained abnormal internal membranes and some granular material resulting from altered plastid development, rather than from the destruction of membranes already formed.

V. EFFECTS OF SELECTED HERBICIDES ON MATURE CHLOROPLASTS

A. Introduction

Approximately one-half of the available herbicides function by inhibiting some aspect of photosynthesis, thereby altering preexisting chloroplast structures. These inhibitors can be subdivided into three groups, according to their site of action. The first group includes the chemically and structurally unrelated herbicides of ureas, symmetrical triazines, triazinones, some phenyl-pyridazinones, and certain phenol compounds, bentazon, and some diphenylethers.[41] These electron-transport inhibitors appear to act on the B/plastoquinone region of the photosynthetic electron-transport chain (see Chapter 1). The second group includes the bipyridyl herbicides which accept electrons on the reducing side of photosystem I.[58] The addition of one electron to a bipyridylium ion gives rise to a free radical, which is believed to react with molecular oxygen to form a superoxide radical. The third group is composed of herbicides that do not affect photosynthesis directly, but rather modify the chloroplast

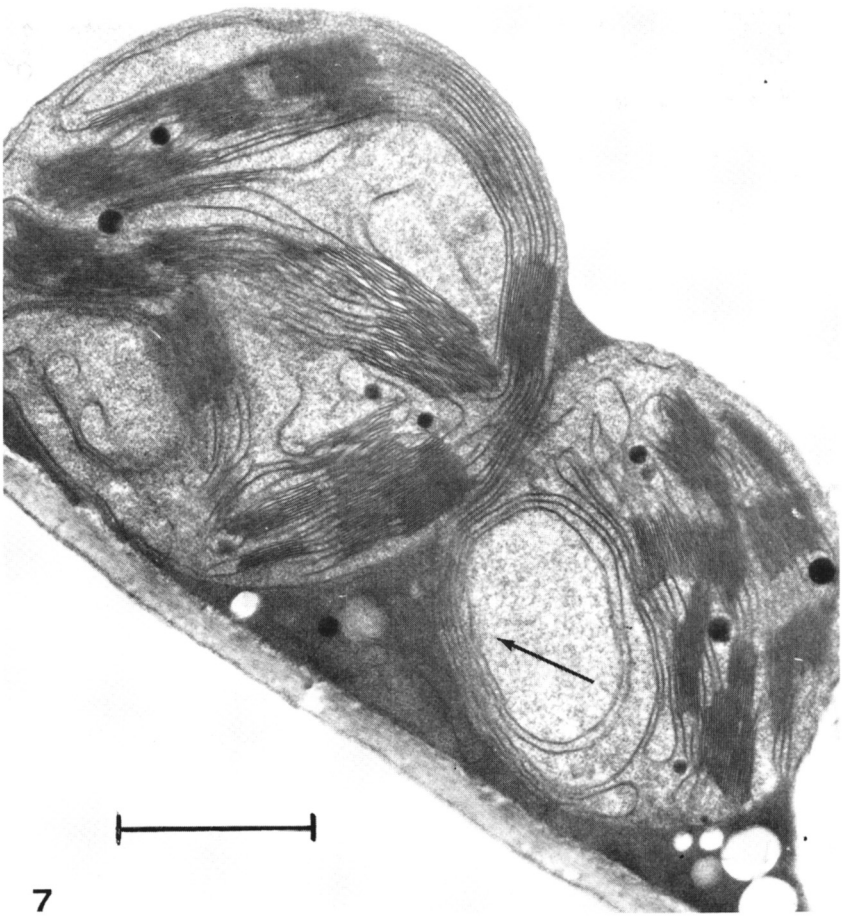

FIGURE 7. Swollen chloroplast after 120 hours monuron treatment at 0.2 W m^{-2} showing a breakdown of the grana and the formation of a concentric membrane configuration (arrowed). Scale marker equals 1 μm. (From Pallett, K. E. and Dodge, A. D., *Plant Cell Environ.*, 3, 183, 1980. With permission.)

structure by some direct or indirect biochemical mechanism. Some selected diphenylether herbicides cause the destruction of chloroplast membranes by lipid peroxidation reactions.[59]

B. Inhibitors of Photosynthetic Electron Transport

Postemergence treatment of 7-day-old, light-grown (30 W·m^{-2}) flax cotyledons with several electron-transport herbicides (bromacil, metribuzin, or monuron at 1 mM) for 96 h produced chlorotic leaves with damaged chloroplasts.[60] Within 24 h, the green chloroplasts appeared swollen and lacked starch grains. Subsequent deterioration of the chloroplast structure included the swelling of the granal and intergranal membranes and, lastly, the rupture of the chloroplast envelope. After 96 h when the leaves were completely chlorotic, the chloroplast membrane system and other cytoplasmic inclusions were mixed with vacuolar contents. The sequence of ultrastructural changes in the chloroplasts of flax plants grown under a lower light intensity (5 W·m^{-2}) was similar to that of plants grown under high light intensity, except that the changes occurred more slowly. Cotyledons exposed to a very low intensity of light (0.2 W·m^{-2}) in the presence of monuron contained chloroplasts that were spherical in shape and the thylakoids of the chloroplasts were arranged in concentric and myelinoid configurations (Figure 7).[61] The effect of ioxynil on the chloroplast structure was

different from the other mentioned herbicides. Tbe chloroplasts appeared oval-shaped and contained numerous membrane-bound vesicles in the stroma resulting from the swelling of the thylakoids.[60]

Vecchi[62] studied the effect of low concentrations (10 μM) of diuron on chloroplasts of mature green leaf discs from C_3 tobacco (*Nicotiana glutinosa*) and C_4 maize plants. The thylakoids of mesophyll cells from the treated discs of both species appeared more appressed than normal. The bundle sheath chloroplasts of treated leaf discs of maize had an increased number of thylakoids stacked into what appeared to be grana-like structures. Also, in tobacco chloroplasts several vesicles appeared at the chloroplast periphery, whereas in maize, the chloroplast peripheral reticulum disappeared.

Hatzios and Penner[63] studied the effect of the urea herbicide, buthidazole, at concentrations of 0.56 to 1.12 kg/ha, on chloroplasts of 20-day-old, light-grown maize plants. The mesophyll chloroplasts of treated plants appeared swollen within 24 h and the chloroplast membranes were completely disrupted after 96 h. The chloroplasts of the bundle sheath cells lacked starch grains.

In vitro treatment of isolated chloroplasts with selected electron transport inhibitors caused a disruption of the ultrastructure of the chloroplasts.[64] When suspensions of chloroplasts isolated from untreated maize seedlings were treated with linuron, the thylakoids of the grana became separated and large vesicles developed from the swollen thylakoids. Accompanying the disorganization of the chloroplast thylakoids was the loss of plastid pigments. The carotenoids, particularly the carotenes, decreased prior to the chlorophyll loss.[60,64]

The treatment of 14-day-old, light-grown green leaves of cocklebur (*Xanthium pensylvanicum*) with bentazon at 500 $\mu g/m\ell$ caused an ultrastructure change of the mature chloroplasts.[65] No obvious ultrastructural alterations occurred within 5 h of treatment; however, after that time the internal structures of the chloroplasts changed. The intergranal thylakoids and grana became dilated and the envelope appeared convoluted.

Atrazine affected the ultrastructure of the chloroplasts from mature, light-grown beans,[66] barnyardgrass (*Echinochloa crusgallis*),[67] and soybean (*Glycine max*).[68] Chloroplasts disintegrated when the treated plants were kept in the light, whereas there was no effect upon the chloroplasts of plants that were kept in the dark. Ultrastructural changes of the chloroplasts in the treated, light-grown beans and barnyardgrass included: (a) changing of the shape of the chloroplast, (b) disappearance of starch from stroma, (c) swelling of the intergranal and granal thylakoids, until the whole lamellar system became disorganized, and (d) finally, the disintegration of the chloroplast envelope (Figure 8). In barnyardgrass, these ultrastructural changes occurred as early as 2 hours after application of the herbicide.[67] In soybean, atrazine did not produce the same chloroplast disruptions as described for beans and barnyardgrass.[68] Early effects on chloroplast ultrastructure involved a reduction in the number of grana, disruption of intergranal thylakoids, and unstacking of grana. The effects of sublethal herbicidal concentrations of atrazine on the ultrastructure of chloroplasts of *Lemna minor* were quite different from those described for lethal herbicidal concentrations.[69] The number of grana per chloroplast section increased, whereas the number of thylakoids per grana decreased and these grana formed a very dense network in the stroma. Many osmiophilic globules accumulated in the stroma.

The triazine herbicides do not affect the ultrastructure of the various cellular organelles equally. Normally, cellular organelles other than the chloroplast begin to deteriorate only after the chloroplast envelope and tonoplast have ruptured.[60] The mitochondria of atrazine-treated barnyardgrass were not affected either by the duration of the treatment or by the concentration of the atrazine that would disrupt the chloroplasts.[67] Nuclei appeared to resist rupture altogether.

Application of herbicidal concentrations of atrazine to *Chlorella vulgaris* did not cause

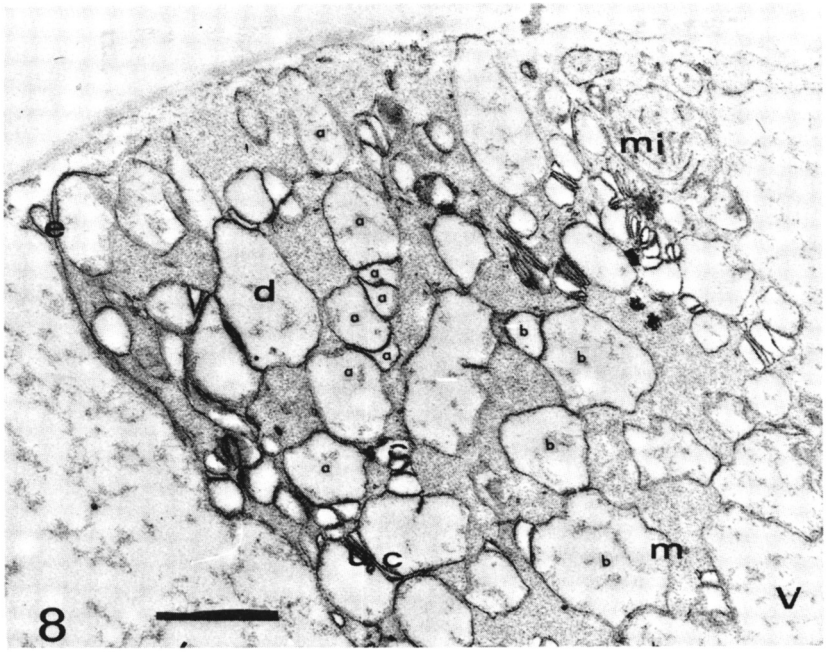

FIGURE 8. Chloroplast from green bean leaf treated with atrazine showing extensive swelling of grana and disruption of membranes of chloroplast envelope. Scale marker equals 1 μm. a,b,d,c, swollen grana; e, plastid envelope; m, margin; mi, mitochondrion; μc, unexpanded compartment; v, vacuole. (From Ashton, F. M., Gifford, E. M., and Bisalputra, T., *Bot. Gaz.*, 124, 336, 1963. With permission.)

abnormalities in the structure of chloroplasts or other organelles, but only prevented increases in chlorophyll levels and the accumulation of starch.[70]

To reconstruct chronologically the ultrastructural changes that occur in plants treated with electron-transport inhibitors requires an understanding of how chlorophyll absorbs light energy and how it dissipates this energy during photosynthesis. Normally, light energy absorbed by the chlorophyll is transferred to the photosynthetic reaction centers and any extra energy is quenched by the carotenoid pigments. Carotenoids can quench either the triplet chlorophyll or the singlet oxygen generated by the chlorophyll and thereby protect the chloroplasts from damage. If the electron flow between the two photosystems is inhibited by a herbicide and light energy absorbed by the chlorophyll can be transferred to the reaction centers, this protective mechanism involving carotenoids would be gradually overloaded and photodestruction of the chloroplast constituents would occur. Carotenoid pigments would be destroyed first, followed by chlorophyll.[60] The excitation energy of the triplet chlorophyll may be transferred to oxygen with the formation of singlet oxygen. The triplet chlorophyll and/or singlet oxygen would then oxidize or abstract hydrogen from the fatty acids of the membrane lipids. These lipid peroxidation reactions are autocatalytic and would spread to other membrane lipids of the chloroplast and cellular structures. These reactions would promote membrane destruction and chlorophyll loss, resulting in the swelling and disorganization of the thylakoids and other cellular membranes, as observed under the electron microscope.[42]

C. Diverters of Photosynthetic Electron Transport

Treatment of plants with paraquat in the light resulted in the ultrastructural changes in green mesophyll cells that were similar in all species studied. The first ultrastructural change

induced by paraquat in honey mesquite (*Prosopis juliflora*) was the rapid disintegration of the plasmalemma, followed by the disruption of the chloroplast membrances and loss of chloroplast turgor.[71] In 10-day-old flax plants, paraquat at100 μM caused a rapid disruption of the tonoplast and other cellular membranes, followed by the breakdown of chloroplast thylakoids with the accumulation of osmiophilic plastoglobuli.[72] Harvey and Fraser[73] studied the ultrastructural effects of paraquat at 100 μM on photosynthetic cells of 12-day-old tolerant and susceptible perennial ryegrass (*Lolium perenne*). The ultrastructural alterations resulting from herbicidal treatment of susceptible cultivars were confined to chloroplasts. The primary damage took the form of vertical and horizontal fusion between stroma and grana thylakoids, creating a honeycomb effect. With continued treatment, the thylakoids appeared extensively swollen, the stroma lost ribosomes, and finally the thylakoids appeared to have disintegrated. Cytoplasmic damage became evident when the chloroplasts were extensively swollen. Paraquat treatment failed to damage the chloroplast of tolerant cultivars, except for the appearance of a few, small, irregularly shaped patches of dense substances in the stroma. Dodge and Lawes[74] reported that within 3 to 4 days after treatment, chloroplast swelling and thylakoid distortion preceded tonoplast and plasmalemma breakdown in flax, maize, and marigold (*Chrysanthemum segetum*) treated with 100 μM diquat or morfamquat. Apparently, the thylakoid system was more susceptible to these herbicides than the tonoplast and plasmalemma were. In the alga *Chlorella* diquat treatment caused the loss of mitochondria and nuclei before the thylakoid and pyrenoid alteration took place.[12]

A sequence of biochemical events which could lead to the ultrastructural alteration of the chloroplast and other cellular structures begins with the bipyridyl herbicide competing for electrons at the reducing side of photosystem I. These herbicides accept electrons from photosystem I and form free radicals, which then react with molecular oxygen to form superoxide radical and H_2O_2.[75] Hydrogen peroxide and superoxide radical produce hydroxyl radicals and lipid peroxides. These reactive species are produced only in the light and H_2O_2 has a sufficiently long lifetime to migrate from the chloroplast to other cellular structures, where it can oxidize and damage the unsaturated fatty acids of the membranes. These peroxidation reactions cause a loss of membrane integrity, resulting in the rapid deterioration and destruction of the cellular organelles.[58]

D. Light-Requiring Herbicides

Some diphenylethers have a light requirement for full herbicidal activity. Incubation of cucumber cotyledons (*Cucumis sativus*) treated with 1 μM of acifluorfen-methyl in the dark for 6 h resulted in no ultrastructural damage.[59] However, massive cellular and membrane damage was apparent in the treated tissue within 45 min following exposure to light. Significant structural damage occurred to the chloroplast envelope, tonoplast, and plasma membrane (Figure 9). Brezeanu et al.[57] studied the effects of postemergence application of diclofop-methyl on the ultrastructure of mesophyll cells of wheat and wild oat (*Avena fatua*) seedlings. At the latter stages of injury, chloroplasts appeared spherical in shape and the entire lamellar system was disorganized, appearing as many vesicles in the stroma. Increased numbers of plastoglobuli and starch grains were apparent in the chloroplasts. The extent of damage in the chloroplasts and cells was frequently more severe in the wild oats than in wheat cells. The nucleus and mitochondria were not altered, but numerous myelinoid figures appeared in the cytoplasm of the treated seedlings. Treatment of cell suspensions from root-derived callus tissue of *Triticum monococcum* with diclofop-methyl were examined with the electron microscope during their growth cycle and senescence.[76] The extent of ultrastructural change depended upon the concentration of the herbicide and total exposure time. At low concentrations (4 μM) for 12 h, the internal membranes of the plastids resembled myelinoid figures. At higher concentrations and longer exposure times, plastids contained only remnants of membrane structure and plastoglobuli, but were filled with granular material.

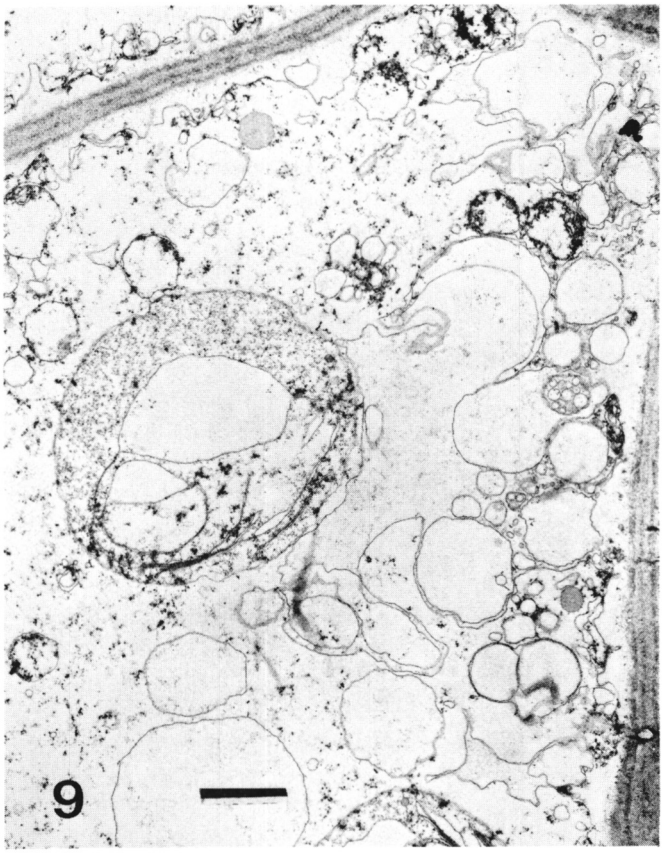

FIGURE 9. Cucumber cotyledon cell treated with acifluorfen-methyl followed by an exposure to high light showing general membrane disruption. Scale marker equals 1 μm. (From Orr, G. L. and Hess, F. D., *Plant Physiol.*, 69, 502, 1982. With permission.)

Although the ultrastructural appearance of the chloroplasts and cell structures of diphenylether herbicide-treated plants resembles the structural appearance of bipyridyl- and electron-transport inhibitor-treated plants, the mechanism of action of each appears to be different.[59] Light absorbed by the carotenoids appears to activate the diphenylether molecules which, in turn, results in the production of toxic oxygen species. However, the site of radical production and the sequence of membrane peroxidation may differ from that of most bipyridyliums.

E. Other Herbicides

The application of auxin-like herbicides to green mesophyll cells of several higher plant species produced changes or alterations of the chloroplast ultrastructure. Hallam[77] reported that within 8 h after 2,4-D treatment (1 m*M*) of light-grown bean leaves, the chloroplasts were distorted and filled with electron-dense granules and small vesicles. The leaf cells appeared to be plasmolyzed and light was required for these structure changes to occur. A similar effect was observed when fully expanded leaves of light-grown tobacco plants were injected with 2,4-D.[78] The concentration of 2,4-D used in these experiments was very high. Grana of chloroplasts were extensively swollen and large plastoglobuli accumulated in the chloroplasts. Treatment of *Rubus procerus* leaves with 2,4,5-T produced ultrastructural changes in mesophyllic cells similar to those produced by 2,4-D. Seventy-two hours after

treatment, grana of the chloroplast became indistinct and cytoplasm contained granular material. One week after treatment, there was an almost complete loss of subcellular structure.[79] The alga *Chlorella pyrenoidosa* responded to 2,4-D in a way that was ultrastructurally similar to that of higher plants.[80] Picloram, although chemically unrelated to phenoxy herbicides, exerts similar effects on the chloroplast's ultrastructure. Ayling[81] reported that picloram caused chloroplast enlargement and swelling of internal thylakoids, followed by disintegration of chloroplasts in needles of *Pinus radiata* and leaf discs of *Eucalptus viminalis*.

The application of glyphosate to mature green leaf tissue of several plant species caused ultrastructural damage to the mesophyll cells as early as 24 h, whereas visible damage, such as yellowing of the leaves, was observed much later. Campbell et al.[82] observed that chloroplasts of quackgrass (*Agropyron repens*) were changed by glyphosate treatment. These changes ranged from partial to complete disruption of the chloroplast envelope, swelling of the thylakoids of the grana, the accumulation of plastoglobuli and a decrease of starch content. Treatment of green leaves of velvet mesquite, white mustard seedlings, and duckweed produced similar ultrastructural alterations of the chloroplasts as with quackgrass.[83-85] Chloroplasts appeared rounded and swollen. Thylakoids of the chloroplasts were swollen and some of the thylakoids appeared as myelin figures. These defects were intensified with time until a complete disruption of the chloroplast and cellular structures occurred. In the liverwort, *Pellia epiphylla*, Pihakaski and Pihakaski[86] found that glyphosate caused swelling of the chloroplast envelope and the thylakoids, similar to changes observed in higher plants. In addition, bulges were formed at the surface of the chloroplast envelope and they were filled with stromal granular material. Besides acting on the chloroplast, glyphosate also caused swelling of the endoplasmic reticulum with subsequent formation of vesicles, swelling of mitochondria, and wrinkling of plasmalemma. These ultrastructural observations indicated that this herbicide has a general affect on all the cell structures. Haloxydine, which blocks carotenoid synthesis and plastid development, also affected preexisting chloroplasts. The effect of haloxydine ranged from some swelling to complete breakdown of the chloroplast.[36]

The primary site of action of herbicides 2,4-D, 2,4,5-T, and glyphosate remains obscure. At this time, no theory or scheme adequately explains how the inhibition of specific biochemical reactions by these herbicides causes morphological changes in plants. The ultrastructural changes induced by these herbicides in the plant cells resemble in many respects those changes that occur during normal senescence. Senescence of the plants follows a characteristic pattern. The RNA and ribosome population of the cell decrease, chloroplast stroma disappears, internal membrane systems disintegrate, and osmiophilic globules increase in number and size. The endoplasmic reticulum swells, vesiculates, and disappears, as do the dictyosomes.[87] As mentioned earlier, blockage of a step in a metabolic pathway can lead to eventual reduction and stoppage of other processes essential to the maintenance of ultrastructural integrity. This mechanism may be common to senescence and herbicides which interfere with metabolic pathways, resulting in a common sequence of ultrastructural deterioration.

VI. MICROTUBULES AND HERBICIDES

A. Introduction

Microtubules are filamentous, proteinaceous subcellular structures present in virtually all eukaryotic cells. In higher plants, spindle microtubules are functional elements of the mitotic apparatus, determining the plane of cell division. Cortical microtubules residing in the cortical cytoplasm are involved in orientation of cellulose microfibril deposition in growing cells and thus regulate cell wall morphogenesis. The protein subunits of the microtubules are referred to as tubulin, which itself is a dimer. The assembly and disassembly of microtubules

appears to be under the control of the microtubule-organizing centers (MTOC) located at opposite ends of the cell. In the higher plants, the MTOC is an electron-dense area of the cytoplasm from which the microtubules are assembled. Generally, the microtubules are observed only with the electron microscope.[88] Many cytological studies of dinitroaniline- and phenylcarbamate-treated roots showed that mitosis was disrupted and that the typical mitotic aberrations of chromosomes and enlarged amoeboid nuclei followed this treatment. The type of mitotic figure prevailing in the treated roots seemed to depend on the plant species and concentration of the herbicide used.[89] The results of these studies suggested that the herbicides were acting on the microtubules in a manner similar to the action of colchicine. Abnormal chromosome configurations were often caused by an effect on the spindle apparatus. This effect was attributed to an abnormal function of or an absence of the spindle microtubules.

B. Microtubules and Dinitroaniline Herbicides

An electron microscope study by Jackson and Stetler[90] showed that endosperm cells of the African blood lily (*Haemanthus katherinae*) treated with 50 ppb of trifluralin for a short time (15 min to 2 h) contained microtubules, but the number was reduced, especially in the cell plate interzonal area of the cell. They reported that endoplasmic reticulum was absent from the treated endosperm cells and plastid and mitochondrion morphology were altered. Bartels and Hilton[91] studied the effects of trifluralin and oryzalin at concentrations of 10 μM on the root tip cells of germinating corn and wheat seedlings. Their ultrastructural study showed that spindle microtubules and cell plate material of the center part of the cell were lost after 3 h of herbicidal treatment, whereas the cortical microtubules persisted a little while longer. Chromosomes in cells arrested at telophase remained in two distinct groups located at each end of the root cell, with cell plate vesicles and spindle microtubules absent in the inter-zone between daughter chromosomes (Figure 10). The rate of microtubule loss depended on the type of herbicide and the length of exposure to the herbicide. In the roots of treated wheat seedlings, endoplasmic reticulum was present, but swollen, whereas mitochondrion and plastid morphology appeared normal. The cell plate region lacked small or large vesicles.

The fine structural study of cotton roots by Hess and Bayer[92] showed that cells treated with trifluralin contained polyploid nuclei that were highly lobed. Since root meristem cells may be in many different stages of mitosis, the application of trifluralin to the root meristem will stop mitosis of the individual cells at different stages of division by destroying or disrupting the microtubules. With the mitosis sequence unable to proceed, the nuclear envelope reforms, yielding various nuclear patterns characteristic of the stage at which mitosis was arrested (Figure 11). Some cotton root meristem cells treated with trifluralin contained a few microtubules, but the ones located near the chromosomes were abnormally oriented.

Algal cells have been reported to respond to trifluralin in a similar manner as higher plants. In the algal *Oocystis solitaria* trifluralin and oryzalin caused the loss of microtubles and induced abnormal cell wall development, resulting in altered microfibril arrangements in the cell wall.[93]

C. Microtubules and Phenyl Carbamates

In dividing liquid endosperm cells of African blood lily treated with 50 ppm of propham, the spindle microtubules lost their parallel alignment and became oriented in radical arrays.[94] The microtubules' orientation was not random, but rather they were aligned in a multiple spindle.[94] Cortical microtubules of wheat and maize root tips treated with propham were not oriented parallel to each other, as in untreated cells. Spindle microtubules were observed at metaphase in propham-treated cells, but the number was reduced and they were more difficult to observe than in control cells.[91] Brower and Hepler[95] reported that cortical mi-

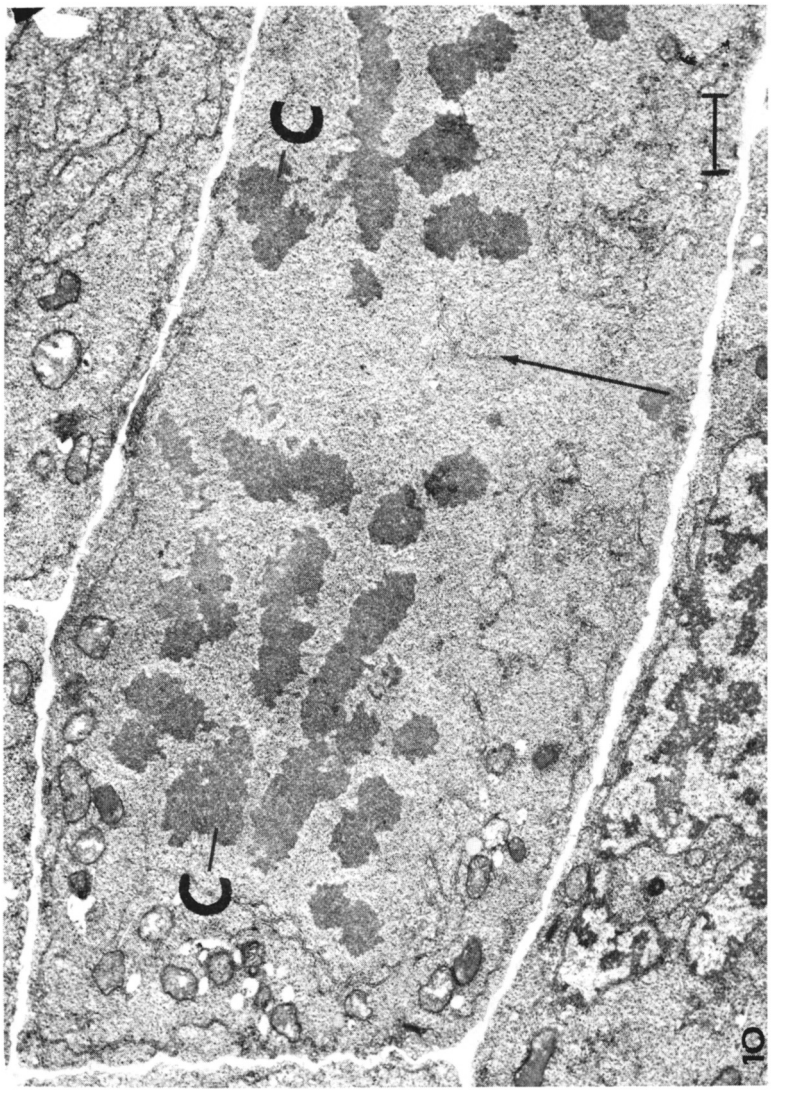

FIGURE 10. Wheat root cell in telophase treated with trifluralin showing no cell plate or microtubules (see arrow). Scale marker equals 1 μm. C, chromosome.

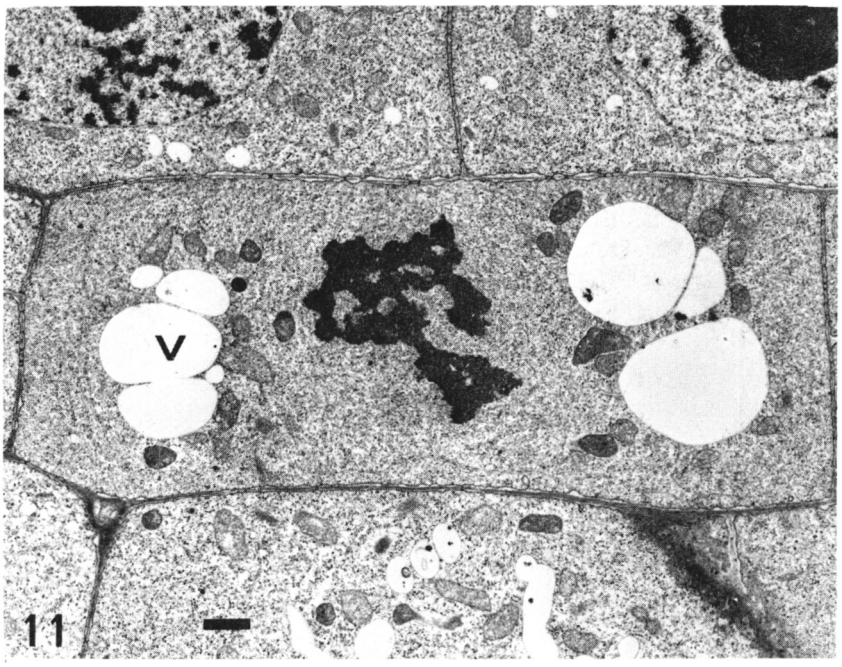

FIGURE 11. Nucleus of cotton root cell treated with trifluralin. Arrested metaphase division figures as a result of microtubule disappearance at late prophase or metaphase. Scale marker equals 1 μm. V, vacuole. (From Hess, D. and Bayer, D., *J. Cell Sci.*, 15, 429, 1974. With permission.)

crotubules, which run parallel to the wall of xylem cells, were almost totally eliminated in the roots of propham-treated wheat and onion (*Allium cepa*) seedlings. This treatment totally eliminated the secondary wall pattern of the root xylem cells.

In the green alga *Oedogonium cardiacum*, Coss and Pickett-Heaps[96] observed that propham prevented the assembly of microtubules, increased the number of functional polar bodies, and affected the orientation of the microtubules. Propham and chlorpropham treatment of the alga *Ochromonas* altered normal assembly of the microtubules, but did not affect the preexisting microtubules.[97]

Treatment of *Euglena* with 100 μM propham caused an inhibition of cell growth and produced several morphological disorders after 48 h treatment.[98] Several polymorphic bodies present in the cytoplasm appeared to be aggregates of stored microtubular protein or tubulin. Microtubules of zoospores from the alga *Pediostrum* are involved in extension of horns on the peripheral cells of the algal colony. Marchant[99] reported propham interfered with development of these horns, suggesting the MTOC was affected.

D. Microtubules and Other Herbicides

Hogetsu et al.[100] reported that dichlobenil reversed the effects of GA and kinetin on stem elongation of azuki bean (*Vigna angularis*) epicotyl cells in a way similar to the action of colchicine in these cells. Because colchicine is known to disrupt cell microtubules, they suggested that dichlobenil may also be disrupting wall microtubules. An ultrastructural examination of the dichlobenil-treated epicotyl revealed that this herbicide did not disrupt the wall microtubules.

Pronamide also caused the loss of cortical and spindle microtubules from wheat and maize roots. The ultrastructural appearance of the root cells was similar to dinitroaniline herbicide-treated root cells.[91]

The mechanism of how these herbicides caused the loss or disorientation of microtubules

is still unclear. Trifluralin may bind to tubulin, and thereby prevent microtubule polymerization of the tubulin pool within the cell. Hess[101] reported that trifluralin bound to flagellar tubulin obtained from the alga *Chlamydomonas* and prevented flagellar regeneration. The binding of trifluralin or any dinitroaniline herbicides to higher plant microtubules has not been tested because plant tubulin has not been isolated and purified to the degree necessary for binding reactions. Dinitroaniline herbicides may also act directly on the morphology of the MTOC, i.e., endoplasmic reticulum membranes that appear to be involved in the transport of tubulin to and from sites of polymerization. Endoplasmic reticulum was swollen and distorted in trifluralin-treated roots of corn and wheat.[91] The disruption of normal endoplasmic reticulum function, such as regulation of calcium and metabolite levels at the MTOC may explain the loss of microtubules. Calcium appears to be important in microtubule formation.

Propham, a carbamate herbicide, appears to work by another mechanism, as compared to the dinitroaniline herbicides. Coss and Pickett-Heaps[96] suggested that propham acts on the MTOC of the alga *Oedogonium*. Improper arrangement of the microtubules would disrupt cell division.

VII. OTHER CELLULAR STRUCTURES AS MODIFIED BY SELECTED HERBICIDES

Applicaton of sublethal concentrations of several *s*-triazines (atrazine, simazine, and terbutryn) to bush bean (*Phaseolus vulgaris*) cotyledons caused a doubling of the cisternae of the rough endoplasmic reticulum, increased levels of cytoplasm ribosomes, and increased numbers of protein bodies.[102] Wu et al.[103] also reported that simazine increased the size and shape of the protein bodies of pea (*Pisum sativum*) and maize. Bleckman et al.[104] observed a proliferation of rough endoplasmic reticulum in velvet mesquite and cat claw acacia (*Acacia greggii*) treated with picloram.

The application of 2,4-D to callus of *Haworthia variegata* was found to induce tumor-type growth. An ultrastructural examination of the 2,4-D treated callus showed that endoplasmic reticulum appeared dilated and the mitochondria had more frequent dilation of cristae and vesicle formation than the controls. The number of free ribosomes and polysomes appeared greater in the 2,4-D-induced tumor cells.[105] Mitochondria appear to be more sensitive to 2,4-D than to other herbicides. George[106] and Ladonin and Spesivtsev[107] observed changes in the mitochondrial ultrastructure of plants treated with 2,4-D.

The action of sublethal concentrations of triazine and 2,4-D herbicides on plants may be a consequence of their hormonal-like activity. Fedtke[4] reports that these herbicides stimulated growth and exhibited plant growth regulatory activity in several plant assay systems. The endoplasmic reticulum and mitochondria may be modified in the herbicide-treated plants to facilitate new growth.

Paraquat, besides acting on the chloroplast, also affected the ultrastructure of nonphotosynthetic cells. Application of this herbicide to transpiration streams of young slash pine (*Pinus elliotti*) trees significantly increased the biogenesis of oleoresin in cells of the xylem symplast.[108] Cells near the site of paraquat application showed extreme damage to membrane integrity, depletion of starch, and loss of cellular organization. The change in membrane permeability caused by paraquat may free previously compartmentalized cellular enzymes and substrates to provide additional sources of carbon for oleoresin biogenesis. The treatment of callus cultures of slash pine with paraquat modified the ultrastructure of the cells. Suspension cells treated with low concentrations of paraquat resembled untreated cells, whereas cells treated at higher concentrations contained broken tonoplasts, degenerated endoplasmic reticulum, few ribosomes, and granular, dispersed groundplasm. These culture cells, unlike the stem tissue, failed to produce oleoresin under the influence of paraquat. Paraquat may

be activated by the xylem symplast and thus disrupt the cellular membranes through peroxidation reactions.

The formation of cell walls in oat roots and tobacco protoplasts has been reported to be inhibited by bromacil[50] and dichlobenil.[109] Newly formed cell walls fail to develop, resulting in root cells and protoplasts containing multiple nuclei.[50,109] No hypothesis has been proposed to explain how bromacil and dichlobenil inhibit cell wall synthesis.

Several herbicides have been shown to modify the epicuticular wax deposition on the leaf surface of several plant species,[110] as reviewed by Hess in Chapter 8.

VIII. CONCLUSIONS AND FUTURE RESEARCH

This chapter has dealt with the effects of selected herbicides on the ultrastructure of developing plastids, mature green photosynthesizing chloroplasts, the microtubular system, and endoplasmic reticulum. The challenge of this review has been the attempt to translate primary herbicidal perturbations into the mode of action that leads to an expressed ultrastructural modification. This chapter has tried, whenever possible, to arrange known biochemical effects of a herbicide into a sequence of cause-response relationships, with the ultrastructural appearance of plants forming the end of the sequence.

It is evident that considerable amounts of information are required about herbicidal action before we fully understand the relationship between the ultrastructural appearance of a treated cell and the biochemical changes induced by the herbicides. For some classes of herbicides, this sequence of events from the biochemical level to the morphological level may be clear, whereas with other classes of herbicides these events are still obscure.

Many of the cited ultrastructural studies of herbicide-treated tissue revealed that ultrastructural changes associated with the herbicide-induced injury generally involved membrane damage and deterioration. Consequently, more information and future research are still needed about how the herbicides interact with membranes and membrane-associated enzymes. Because organelles are composed of or bounded by membranes, an understanding of membrane structure, function, origin, and regulation will provide new insights into how herbicides may influence the ultrastructure of the organelles and other cellular structures.[111] The internal components of a membrane may be visualized by the freeze-etching electron microscope technique. Some of these internal components have been shown to perform specific aspects of photosynthesis. A fruitful area of future research would be to examine membranes of herbicide-treated plants with this technique to determine what membrane components might be altered by the herbicide treatment. The localization of herbicide-induced reactions in plant organelles and membranes could be studied by the underutilized techniques of plant cytochemistry.[75]

In addition to studying membranes, more research needs to be performed on the ultrastructure of certain organelles. Very few studies have investigated the effect of herbicides on the proplastids of the meristematic tissue of plants. The initial effect of a herbicide that blocks chloroplast development would be on the proplastids.

A promising area for future research would be the ultrastructural examination of herbicide-treated C_4 plants. Malakondaiah and Fang's[112] biochemical studies showed that certain herbicides affected the photosynthesis of bundle sheaths differently than mesophyll cells of some C_4 plants. Presently, only a few ultrastructural studies have been performed to determine if the structure of bundle sheath and mesophyll chloroplasts may be modified similarly or differently by a herbicide.

In any future ultrastructural research dealing with herbicide-treated tissue, a simultaneous examination of tissue, both biochemically and ultrastructurally, should be included before, during, and following treatment. This would enhance the probability of finding causal relationships between biochemical and structural effects.

NOTE ADDED IN PROOF

Since this chapter was written, several important papers describing the effect of sethoxydim, ioxynil, and bromoxynil on chloroplast development and biogenesis have been published. Chloroplast swelling, decrease in starch grains, and thylakoid disruption prior to cellular destruction were ultrastructural effects caused by ioxynil and bromoxynil.[113] Sethoxydim inhibits chloroplast biogenesis at all stages of development from proplastids to prochloroplasts, young and mature chloroplasts. Thylakoid synthesis, thylakoid multiplication, grana formation, and chloroplast replication are all affected by sethoxydim.[114].

REFERENCES

1. **Anderson, J. L. and Thomas, W. W.,** The effect of herbicides on the ultrastructure of plant cells, *Residue Rev.*, 47, 167, 1973.
2. **Linck, A. J.,** Effect on cytology and fine structure of plant cells, in *Herbicides: Physiology, Biochemistry, Ecology,* Vol. 1, 2nd ed., Audus, L. M., Ed., Academic Press, New York, 1971, chap. 2.
3. **Ashton, F. M. and Crafts, A. S.,** *Mode of Action of Herbicides,* 2nd ed., John Wiley & Sons, New York, 1981, 525.
4. **Fedtke, C.,** *Biochemistry and Physiology of Herbicide Action,* Springer-Verlag, New York, 1982, 202.
5. **Moreland, D. E., St. John, J. B., and Hess, F. D.,** *Biochemical Responses Induced by Herbicides,* American Chemical Society, Washington, D.C., 1982, 274.
6. **Kirk, J. T. O. and Tilney-Bassett, R. A. E.,** *The Plastids: Their Chemistry, Structure, Growth, and Inheritance,* Elsevier/North-Holland, Amsterdam, 1978, 960.
7. **Gunning, B. E. S. and Steer, M. W.,** *Ultrastructure and the Biology of Plant Cells,* Edward Arnold, London, 1975, 312.
8. **Singer, S. J. and Nicolson, G. L.,** The fluid mosaic model of the structure of cell membranes, *Science,* 175, 720, 1972.
9. **Lütz, C.,** Development and ageing of etioplast structures in dark grown leaves of *Avena sativa* (L.), *Protoplasma,* 108, 83, 1981.
10. **Schiff, J. A.,** Photocontrol of chloroplast development in *Euglena*, in *Chloroplast Development,* Vol. 2, Akoyunoglou, G. and Argroudi-Akoyunoglou, J. H., Eds., Elsevier/North Holland, New York, 1978, 747.
11. **Frosch, S., Jabben, M., Bergfeld, R., Kleinig, H., and Mohr, H.,** Inhibition of carotenoid biosynthesis by herbicide SAN 9789 and its consequences for the action of phytochrome on plastogenesis, *Planta,* 145, 497, 1979.
12. **Stokes, D. M., Turner, J. S., and Markus, K.,** The effects of the diquat on the metabolism of *Chlorella vulgaris*. 2. Effect of diquat in the light on chlorophyll bleaching and plastid structures, *Aust. J. Biol. Sci.*, 23, 265, 1970.
13. **Whatley, J. M., Hawes, C. R., Horne, J. C., and Kerr, J. D. A.,** The establishment of the plastid thylakoid system, *New Phytol.*, 90, 619, 1982.
14. **Ridley, S.,** Carotenoids and herbicide action, in *Carotenoid Chemistry and Biochemistry,* Britton, G. and Goodwin, T. W., Eds., Pergamon Press, Oxford, 1982, 353.
15. **Bartels, P. G. and Hyde, A.,** Chloroplast development in 4-chloro-5-(dimethylamino)-2-α,α-α-trifluoro-m-tolyl-3(2H)-pyridazinone (Sandoz 6706) treated wheat seedlings, *Plant Physiol.*, 45, 807, 1970.
16. **Devlin, R. M., Kisiel, M. J., and Karczmarczyk, S. J.,** Chlorophyll production and chloroplast development in norflurazon-treated plants, *Weed Res.*, 16, 125, 1976.
17. **Bartels, P. G. and Watson, C. W.,** Inhibition of carotenoid synthesis by fluridone and norflurazon, *Weed Sci.*, 26, 198, 1978.
18. **Blume, D. E. and McClure, J. W.,** Developmental effects of Sandoz 6706 on activities of enzymes of phenolic and general metabolism in barley shoots grown in dark or under low or high intensity of light, *Plant Physiol.*, 65, 238, 1980.
19. **Strang, R. H. and Rogers, R. L.,** Behavior and fate of two phenyl pyridazinone herbicides in cotton, corn, and soybean, *J. Agric. Food Chem.*, 22, 1119, 1974.
20. **Duke, S. O., Vaughn, K. C., and Duke, S. H.,** Effect of norflurazon on light-increased extractable nitrate reductase activity in soybean *(Glycine max)* seedlings, *Plant Cell Environ.*, 5, 155, 1982.

21. **Axelsson, L., Dahlin, C., and Ryberg, H.,** The function of carotenoids during chloroplast development, V. Correlation between carotenoid content, ultrastructure, and chlorophyll b to chlorophyll a ratio, *Physiol. Plant.*, 55, 111, 1982.
22. **Pardo, A. D. and Schiff, J. A.,** Plastid and seedling development in SAN-9789 (4-chloro-5-(methylamine)-2-(α,α,α-trifluoro-m-tolyl)-3-(2H)-pyridazinone) treated etiolated bean seedlings, *Can. J. Bot.*, 58, 25, 1980.
23. **Vaisberg, A. and Schiff, J. C.,** Events surrounding the early development of Euglena chloroplasts. 7. Inhibition of carotenoid biosynthesis by the herbicide SAN 9789 (4-chloro-5(methylamino)-2-(α,α,α,-trifluoro-m-tolyl)-3(2H)-pyridazinone)) and its developmental consequences, *Plant Physiol.*, 57, 260, 1976.
24. **Herczeg, T., Lehoczki, E., Rojik, I., Vass, I., Farkas, T., and Szalay, L.,** Stimulatory effects of pyridazinone herbicides on *Chlorella*, *Plant Sci. Lett.*, 19, 285, 1980.
25. **Anderson, J. L. and Schaelling, J. P.,** Effect of pyrazon on bean chloroplast ultrastructure, *Weed Sci.*, 18, 455, 1970.
26. **Khan, M. U., Lem, N. W., Chandorkar, K. R., and Williams, J. P.,** Effects of substituted pyridazinones (SAN 6706, SAN 9774, SAN 9785) on glycerolipids and their associated fatty acids in the leaves of *Vicia faba* and *Hordeum vulgare*, *Plant Physiol.*, 64, 300, 1979.
27. **Bartels, P. G.,** Effect of amitrole on the ultrastructure of plastids in seedlings, *Plant Cell Physiol.*, 6, 227, 1965.
28. **DeKock, P. C. and Innes, A. M.,** The effect of amitrol on duckweed, *Can. J. Bot.*, 48, 1285, 1970.
29. **Guillot-Salomon, T.,** Action du 3-amino-1,2,4-triazole sur l'ultrastructure des chloroplastes des mais, *C. R. Acad. Sci. Ser. D*, 262, 2510, 1966.
30. **Bartels, P. G., Matsuda, K., Siegel, A., and Weier, T. E.,** Chloroplastic ribosome formation: inhibition by 3-amino-1,2,4-triazole, *Plant Physiol.*, 42, 736, 1967.
31. **Bartels, P. G. and Weier, T. E.,** The effect of 3-amino-1,2,4-triazole on the ultrastructure of plastids of *Triticum vulgare* seedlings, *Am. J. Bot.*, 56, 1, 1969.
32. **Arntzen, C. J., Linck, A. J., and Cunningham, W. P.,** The effect of 3-amino-s-triazole on flax root apices, *Bot. Gaz.*, 131, 14, 1970.
33. **Castelfranco, P. and Bisalputra, T.,** Inhibitory effect of amitrole on *Scenedesmus quadricauda*, *Am. J. Bot.*, 52, 222, 1965.
34. **Sankhla, N. and Ziegler, H.,** Plastid development in response to a new herbicide, EMD-IT-5914, *Naturwissenschaften*, 60, 157, 1973.
35. **Geronomino, J. and Herr, J. W.,** Ultrastructural changes of tobacco chloroplasts induced by pyriclor, *Weed Sci.*, 18, 48, 1970.
36. **Dodge, A. D. and Lawes, G. B.,** Some effects of the herbicide haloxydine on the structure and development of chloroplasts, *Ann. Bot.*, 36, 315, 1972.
37. **Bartels, P. G. and Pegelow, E. J.,** The action of sirmate (3,4-dichlorobenzyl methylcarbamate) on chloroplast ribosomes of *Triticum vulgare* seedlings, *J. Cell. Biol.*, 37, 1, 1968.
38. **Sarma, R., Flood, A. E., and Wain, R. L.,** Studies on plant growth-regulating substances. XLVII. Ultrastructural studies on emergent leaves of *Avena sativa* treated with 3,5(diiodo and dibromo)-4-hydroxyphenylmethyl sulphone, *Ann. Appl. Biol.*, 86, 429, 1977.
39. **Clarke, I. E., Sandmann, G., Bramley, P. M., and Böger, P.,** Carotene biosynthesis with isolated photosynthetic membranes, *FEBS Lett.*, 140, 203, 1982.
40. **Britton, G.,** Carotenoids biosynthesis — a target for herbicide activity, *Z. Naturforsch.*, 34c, 979, 1979.
41. **Sandmann, G. and Böger, P.,** Mode of action of herbicidal bleaching, in *Biochemical Responses Induced by Herbicides*, Moreland, D. C., St. John, J. B., and Hess, F. D., Eds., American Chemical Society, Washington, D.C., 1982, 111.
42. **Halliwell, B.,** The toxic effects of oxygen on plant tissue, in *Superoxide Dismutase*, Vol. 1, Oberley, L. W., Ed., CRC Press, Boca Raton, Fla., 1982, chap. 5.
43. **St. John, J. B. and Hilton, J. L.,** Structure versus activity of substituted pyridazinones as related to mechanism of action, *Weed Sci.*, 24, 579, 1976.
44. **Tantawy, M. M. and Grimme, L. H.,** The bleaching induced by the phenyl pyridazinone herbicide metflurazon in *Chlorella* as a metabolic and reversible process, *Pest. Biochem. Physiol.*, 18, 304, 1982.
45. **Klein, S. and Neuman, J.,** The greening of etiolated bean leaves and the development of chloroplast fine structure in the absence of photosynthesis, *Plant Cell Physiol.*, 7, 115, 1966.
46. **Butler, W. L., DeGreef, J., Roth, T. F., and Oelze-Karow, H.,** The influence of carbonylcyanide-m-chlorophenylhydrazone and 3-(3,4-dichlorophenyl)-1,1-dimethylurea on the fusion of primary thylakoids and the formation of crystalline fibrils in bean leaves partially greened in far-red light, *Plant Physiol.*, 49, 102, 1972.
47. **Wergin, W. P. and Potter, J. R.,** Effects of fluometuron on the ultrastructural development, chlorophyll accumulation and photosynthetic competence in developing velvetleaf seedlings, *Pest. Biochem. Physiol.*, 5, 265, 1975.

48. **Meier, D. and Lichtenthaler, H. K.,** Ultrastructural development of chloroplasts in radish seedlings grown at high and low light conditions and in the presence of herbicide bentazon, *Protoplasma*, 107, 195, 1981.
49. **Meier, D. and Lichtenthaler, H. K.,** Special senescence stages in chloroplast ultrastructure of radish seedlings induced by the photosystem II-herbicide bentazon, *Protoplasma*, 110, 138, 1982.
50. **Ashton, F. M., Cutter, E. G., and Huffstutter, D.,** Growth and structural modification of oats induced by bromacil, *Weed Res.*, 9, 198, 1969.
51. **Calvayrac, R., Bomsel, J. L., and Laval-Martin, D.,** Analysis and characterization of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)-resistant Euglena. I. Growth, metabolic and ultrastructural modifications during adaptation to different doses of DCMU, *Plant Physiol.*, 63, 857, 1979.
52. **Slovin, J. P. and Tobin, E. M.,** Glyphosine, a plant growth regulator, affects chloroplast membrane proteins, *Biochim. Biophys. Acta*, 637, 177, 1981.
53. **Croft, S. M., Arntzen, C. J., Vanderhoef, L. N., and Zettinger, C. S.,** Inhibition of chloroplast ribosome formation by N,N-bis(phosphonomethyl)glycine, *Biochim. Biophys. Acta*, 335, 211, 1974.
54. **Nadakavakaren, M. J. and McCracken, D. A.,** Effect of 2,4-dichlorophenoxyacetic acid on the structure and function of developing chloroplasts, *Planta*, 137, 65, 1977.
55. **McCracken, D. A., Ort, D. R., and Nadakavukaren, M.,** The effects of 2,4-dichlorophenoxyacetic acid altered chloroplast development on photosynthesis, *Physiol. Plant.*, 52, 285, 1981.
56. **Ebert, E.,** Herbicidal effects of metolachlor (2-chloro-N(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide) at the cellular level in sorghum, *Pest. Biochem. Physiol.*, 13, 227, 1980.
57. **Brezeanu, A. G., Davis, D. G., and Shimabukuro, R. H.,** Ultrastructural effects and translocation of methyl-2-(4-(2,4-dichlorophenoxy)-phenoxy)propanoate in wheat (*Triticum aestivum*) and wild oat (*Avena fatua*), *Can. J. Bot.*, 54, 2038, 1976.
58. **Dodge, A. D.,** The role of light and oxygen in the action of photosynthetic inhibitor herbicides, in *Biochemical Responses Induced by Herbicides*, Moreland, D. E., St. John, J. B., and Hess, F. D., Eds., American Chemical Society, Washington, D.C., 1982, chap. 4.
59. **Orr, G. L. and Hess, F. D.,** Mechanism of action of diphenyl ether herbicide acifluorfen-methyl in excised cucumber (*Cucumis sativus*), Light activation and the subsequent formation of lipophilic free radicals, *Plant Physiol.*, 69, 502, 1982.
60. **Pallett, K. E. and Dodge, A. D.,** Studies into the action of some photosynthetic inhibitor herbicides, *J. Exp. Bot.*, 31, 1051, 1980.
61. **Pallett, K. E. and Dodge, A. D.,** Modifications of chloroplasts of flax cotyledons treated with monuron: myelinoid figures formed under low light conditions, *Plant Cell Environ.*, 3, 183, 1980.
62. **Vecchi, L. D.,** Effect of 3,3(3,4 dichlorophenyl) 1,1-dimethyl urea on chloroplast ultrastructure of *Nicotiana glutinosa* and *Zea mays*, *Caryologia*, 33, 93, 1980.
63. **Hatzios, K. K. and Penner, D.,** Some effects of buthidazole on corn photosynthesis, respiration, and anthocyanin formation, and leaf ultrastructure, *Weed Sci.*, 28, 97, 1980.
64. **Muschinek, G. Y., Garab, I., Mustardy, L. A., and Faludi-Daniel, A.,** The mechanism of linuron phytotoxicity in maize, *Weed Res.*, 19, 101, 1979.
65. **Potter, J. R. and Wergin, W. P.,** Role of light in bentazon toxicity to cocklebur: physiology and ultrastructure, *Pest. Biochem. Physiol.*, 5, 458, 1975.
66. **Ashton, F. M., Gifford, E. M., and Bisalputra, T.,** Structural changes in *Phaseolus vulgaris* induced by atrazine. II. Effects on fine structure of chloroplasts, *Bot. Gaz.*, 124, 336, 1963.
67. **Hill, E. R., Patala, E. C., and Vengris, J.,** Atrazine induced ultrastructural changes of barnyardgrass chloroplasts, *Weed Sci.*, 16, 377, 1968.
68. **Sun, C. N. and Adams, R. S.,** Effects of the phosphorus-manganese-atrazine interaction in soybean plants, *J. Agric. Food Chem.*, 19, 325, 1971.
69. **Beaumont, G. A., Lord, W. J., and Grenier, G.,** Physiological effects of sublethal rates of atrazine on *Lemna minor*, V. Influence on the ultrastructure of the chloroplasts, *Can. J. Bot.*, 58, 1571, 1980.
70. **Ashton, F. M., Bisalputra, T., and Risley, E. B.,** Effect of atrazine on *Chlorella vulgaris*, *Am. J. Bot.*, 52, 217, 1966.
71. **Baur, J. R., Bovey, R. W., Baur, P. S., and El-Seify, Z.,** Effects of paraquat on the ultrastructure of mesquite mesophyll cells, *Weed Res.*, 9, 81, 1969.
72. **Harris, N. and Dodge, A. D.,** The effect of paraquat on flax cotyledon leaves: changes in fine structure, *Planta*, 104, 201, 1972.
73. **Harvey, B. M. and Fraser, T. W.,** Paraquat tolerant and susceptible perennial ryegrasses: effects of paraquat treatment on carbon dioxide uptake and ultrastructure of photosynthetic cells, *Plant Cell Environ.*, 3, 107, 1980.
74. **Dodge, A. D. and Lawes, G. B.,** Some effects of the herbicides diquat and morfamquat on the fine structure of leaf cells, *Weed Res.*, 14, 45, 1974.
75. **Vaughn, K. C. and Duke, S. O.,** *In situ* localization of the sites of paraquat action, *Plant Cell Environ.*, 6, 13, 1983.

76. **Davis, D. G. and Brezeanu, A.,** An ultrastructural study of *Triticum monococcum* cell suspension cultures during aging and after treatment with the herbicides diclofop-methyl (methyl-2-(4-(2',4',dichlorophenoxy)phenoxy) propanoate, *Can. J. Bot.*, 57, 2006, 1979.
77. **Hallam, N. D.,** The effect of 2,4-D and related compounds on the fine structure of the primary leaves of *Phaseolus vulgaris*, *J. Exp. Bot.*, 21, 1031, 1970.
78. **White, J. A. and Hemphill, D. D.,** An ultrastructural study of the effects of 2,4-D on tobacco leaves, *Weed Sci.*, 20, 478, 1972.
79. **Bretherton, G. and Hallam, N. D.,** The movement of 2,4,5-trichlorophenoxyacetic acid into the leaves of *Rubus procerus* P. J. Muell and its effect on chloroplast ultrastructure, *Weed Res.*, 19, 307, 1979.
80. **Bertagnolli, B. L. and Nadakavukaren, M. J.,** Effect of 2,4-D on the fine structure of *Chlorella pyrenoidosa* Chick., *J. Phycol.*, 6, 98, 1970.
81. **Ayling, R. D.,** Ultrastructural changes in leaf and needle segments treated with herbicides containing picloram, *Weed Res.*, 16, 301, 1976.
82. **Campbell, W. F., Evans, J. O., and Reed, S. C.,** Effect of glyphosate on chloroplast ultrastructure of quackgrass mesophyll cells, *Weed Sci.*, 24, 22, 1976.
83. **Hull, H. M., Beckman, C. A., and Morton, H. L.,** Some effects of glyphosate on the foliar ultrastructure of velvet mesquite seedlings, *Proc. West. Soc. Weed Sci.*, 30, 18, 1977.
84. **Uotila, M., Evjen, K., and Iversen, T.-H.,** The effects of glyphosate on the development and cell ultrastructure of white mustard (*Sinapis alba* L.) seedlings, *Weed Res.*, 20, 153, 1980.
85. **Hoagland, R. E. and Paul, R.,** Ultrastructural effects of glyphosate on *Lemna gibba*, *Abstr. Weed Sci. Soc. Amer.*, No. 170, Weed Science Society of America, Champaign, Ill., 1978, 78.
86. **Pihakaski, S. and Pihakaski, K.,** Effects of glyphosate on ultrastructure and photosynthesis of *Pellia epiphylla*, *Ann. Bot.*, 46, 133, 1980.
87. **Butler, R. D. and Simon, E. W.,** Ultrastructural aspects of senescence in plants, *Adv. Gerontol. Res.*, 3, 72, 1971.
88. **Gunning, B. E. S. and Hardham, A. R.,** Microtubules, *Annu. Rev. Plant. Physiol.*, 35, 651, 1982.
89. **Parka, S. J. and Soper, O. F.,** The physiology and mode of action of the dinitroaniline herbicides, *Weed Sci.*, 25, 79, 1977.
90. **Jackson, W. G. and Stetler, D. A.,** Regulation of mitosis, IV. An *in vitro* and ultrastructural study of effects of trifluralin, *Can. J. Bot.*, 51, 1513, 1973.
91. **Bartels, P. G. and Hilton, J. L.,** Comparison of trifluralin, oryzalin, pronamide, propham and colchicine treatments on microtubules, *Pest. Biochem. Physiol.*, 3, 462, 1973.
92. **Hess, D. and Bayer, D.,** The effect of trifluralin on the ultrastructure of dividing cells of the root meristem of cotton *(Gossypium hirsutum)*, *J. Cell. Sci.*, 15, 429, 1974.
93. **Robinson, D. G. and Herzog, W.,** Structure, synthesis and orientation of microfibrils. III. A survey of the action of microtubule inhibitors on microtubules and microfibril orientation in *Oocystis solitaria*, *Cytobiologie*, 15, 463, 1977.
94. **Hepler, P. K. and Jackson, W. T.,** Isopropyl-N-phenylcarbamate affects spindle microtubule orientation in dividing endosperm cells of *Haemanthus katherinae* Baker, *J. Cell Sci.*, 5, 727, 1969.
95. **Brower, D. L. and Hepler, P. K.,** Microtubules and secondary wall deposition in xylem: the effects of isopropyl N-phenyl carbamate, *Protoplasma*, 87, 91, 1976.
96. **Coss, R. A. and Pickett-Heaps, J. D.,** The effects of isopropyl N-phenyl carbamate on the green alga *Oedogonium cardiacum*, *J. Cell Biol.*, 63, 84, 1974.
97. **Brown, D. L. and Bouck, G. B.,** Microtubule biogenesis and cell shape in *Ochromonas*. III. Effects of the herbicidal mitotic inhibitor iso-propyl N-phenylcarbamate on shape and flagellum regeneration, *J. Cell Biol.*, 61, 514, 1974.
98. **Vannini, G. L., Mares, D., and Dall''Olia, G.,** Structural alterations in *Euglena gracilis* exposed to the herbicide isopropyl-N-phenylcarbamate (IPC), *Protoplasma*, 111, 189, 1982.
99. **Marchant, H. J.,** Microtubular determination of cell shape during colony formation by alga *Pediastrum*, *Protoplasma*, 98, 1, 1979.
100. **Hogetsu, T., Shiboaka, H., and Shimokoriyama, M.,** Involvement of cellulose synthesis in actions of gibberellin and kinetin on cell expansion, 2,6-dichlorophenozonitrite as a new cellulose-synthesis inhibitor, *Plant Cell Physiol.*, 15, 389, 1974.
101. **Hess, F. D.,** Determining causes and categorizing types of growth inhibition induced by herbicides, in *Biochemical Responses Induced by Herbicides*, American Chemical Society, Washington, D.C., 1982, chap. 11.
102. **Singh, B., Campbell, W. F., and Salunkhe, D. K.,** Effects of s-triazines on protein and fine structure of cotyledons of bush beans, *Am. J. Bot.*, 59, 568, 1972.
103. **Wu, M. T., Singh, B., and Salunkhe, D. K.,** Effects of s-triazines on protein synthesis in leaves of pea (*Pisum sativum*) and sweet corn (*Zea mays* L.) and on ultrastructure of pea cotyledons, *Experientia*, 28, 1002, 1972.

104. **Bleckman, C. A., Hull, H. M., and Morton, H. L.,** Ultrastructural effects of formulated picloram on leaflets of velvet mesquite and catclaw acacia, *Weed Res.*, 19, 225, 1979.
105. **Majumdar, S. K. and Sullender, J. S.,** The fine structure of 2,4-D induced tumor cells of *Haworthia variegata*, *Phyton*, 35, 145, 1977.
106. **George, K.,** Degeneration of mitochondria on herbicidal treatment, *Indian J. Exp. Biol.*, 9, 481, 1971.
107. **Ladonin, V. F. and Spesivtsev, L. G.,** The effect of halophenoxy acids on the biochemical composition, structure, and function of pea mitochondria, *Agrokhimiya*, 8, 115, 1971.
108. **Birchem, R. and Brown, C. L.,** Ultrastructure of paraquat-treated slash pine (*Pinus elliottii* engelm), *Am. J. Bot.*, 66, 1208, 1979.
109. **Meyer, Y. and Werner, H.,** Chemical inhibition of cell wall formation and cytokinesis but not nuclear division in protoplasts of *Nicotiana tobacum* L. cultivated *in vitro*, *Planta*, 142, 253, 1978.
110. **Still, G. G., David, D. G., and Zander, G. L.,** Plant epicuticular lipids: alteration by herbicidal carbamates, *Plant Physiol.*, 46, 307, 1970.
111. **Moreland, D. E.,** Mechanism of action of herbicides, *Annu. Rev. Plant Physiol.*, 31, 597, 1980.
112. **Malakondaiah, N. and Fang, S. C.,** Influence of monuron on photosystem II and light-dependent $^{14}CO_2$ fixation in isolated cells of C_3 and C_4 plants, *Pest. Biochem. Physiol.*, 9, 33, 1978.
113. **Sanders, G. E., Cobb, A. H., and Pallett, K. E.,** Physiological changes in *Matricaria inodora* following ioxynil and bromoxynil treatment, *Z. Naturforsch.*, 39c, 505, 1984.
114. **Lichtenthaler, H. K. and Meier, D.,** Inhibition by sethoxydim of chloroplast biogenesis, development and replication in barley seedlings, *Z. Naturforsch.*, 39c, 115, 1984.

Chapter 4

EFFECTS OF HERBICIDES ON NONPHOTOSYNTHETIC BIOSYNTHETIC PROCESSES

Stephen O. Duke

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I. INTRODUCTION

Few herbicides are known to inhibit a synthetic process by directly inhibiting the activity of an enzyme involved in that synthetic process. Thus, most literature dealing with herbicidal effects on plant constituent synthesis describes secondary or tertiary effects due to herbicidal effects on other processes, such as photosynthesis or respiration. Only the more important literature of this type will be included in this review. Emphasis will be on those few cases in which direct inhibition of a synthetic enzyme activity by a herbicide has been demonstrated.

Why do so few herbicides have as their primary mechanism of action the inhibition of enzymes of synthetic pathways? One obvious reason is that animals and plants have many metabolic pathways and enzyme types in common, so that many enzyme inhibitors with excellent herbicidal properties are also quite toxic to mammals (e.g., aminooxyacetic acid). Generally, the more fundamental the metabolic pathway, the less likely the enzymes of that pathway are to have drastically changed through evolution, decreasing the chance of inhibitor specificity for plant enzymes. Thus, in the cases of enzyme-inhibitor herbicides of which we are aware, the herbicides block pathways or synthetic steps not found in mammals. Also, phytoxicity through inhibition of enzyme activity is sometimes quite difficult to prove. The inhibited enzyme may not have been previously described. Indeed, discovery and further understanding of several enzymes have been facilitated through use of herbicidal inhibitors. Multiple pathways or isozymes may limit the phytotoxic efficacy of some highly specific enzyme inhibitors, so that many potent enzyme inhibitors may not be efficacious herbicides.

In the cases of carotenoid or chlorophyll synthesis inhibitors, the visible clues and the available knowledge of these pathways have been helpful in elucidating the mechanisms of action. Herbicides which cause such obvious effects may be more likely to be selected for further commercial development. In many other cases, however, where symptoms develop slowly, secondary and tertiary effects can mask the primary effect, making discovery of the primary mechanism difficult.

II. NITROGEN METABOLISM

A. Nitrogen Reduction

In many agricultural situations nitrogen in the form of nitrate is used to increase crop yields. Nitrate application can also influence weed seed populations (Volume I, Chapter 2), weed/crop competition, and herbicide efficacy. Sodium chlorate is the only herbicide known that directly influences nitrate reductase (EC 1.6.6.1) activity. Chlorate ion competitively inhibits nitrate reductase, but it exerts its phytotoxicity through reduction of chlorate to the acutely toxic chlorite ion.[1]

Nitrite reduction by the plastidic enzyme, nitrite reductase (EC 1.7.99.3), is dependent on photosynthetic electron flow to ferredoxin. Reduced ferredoxin donates electrons to nitrite (see Chapter 1). Klepper hypothesized that many photosynthesis inhibitors might act by preventing nitrite reduction, resulting in accumulation of nitrite which can form nitrous acid and deaminate both free and protein amino groups.[2-4] Accumulation of nitrite in plants treated with photosynthetic inhibitors herbicides such as metribuzin (Figure 1) indicates that there is little coordination between the cytoplasmic enzyme nitrate reductase and the plastidic enzyme, nitrite reductase.[5] In fact, the measurement of nitrite accumulation has been suggested as an assay for photosynthesis inhibitors.[6] Treatment of plants with combinations of photosystem II inhibitors and certain other herbicides (e.g., dicamba) causes synergistic increases in nitrite accumulation (Figure 1). Klepper speculated that this effect was due to an increase in in vivo nitrate reductase activity caused by the nonphotosynthesis inhibitor, perhaps through an increase in NADH availability.[4] Earlier work in which respiratory inhibitors paused accumulation of nitrite in darkness supported this hypothesis.[5]

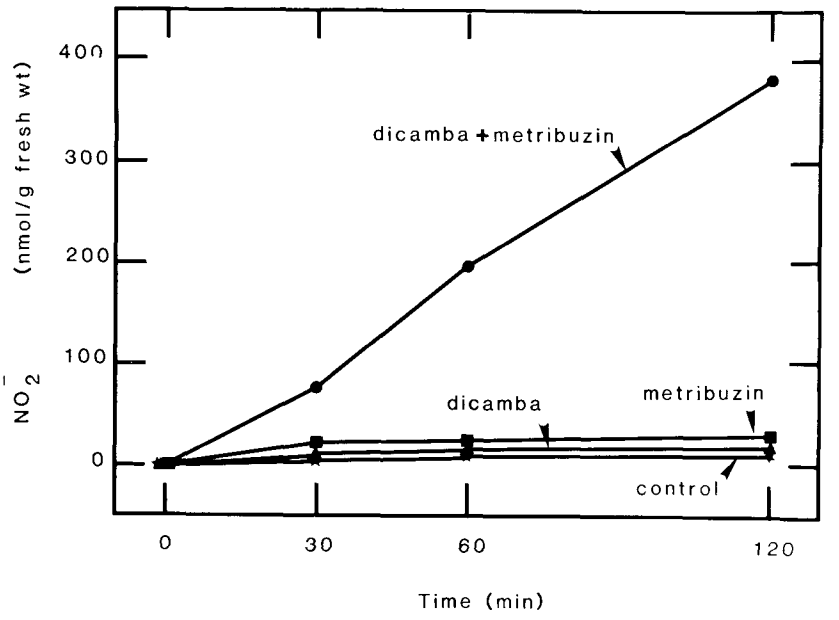

FIGURE 1. Accumulation of nitrite in illuminated wheat leaves as influenced by treatment with dicamba (900 μM) and metribuzin (930 μM) or both combined. Redrawn from Klepper.[4]

On the other hand, there is evidence that the accumulation of nitrite caused by treatment with photosynthesis or oxidative phosphorylation inhibitors is not phytotoxic.[7] This may be due to the sequestering of nitrite in cytoplasmic vesicles or the vacuole, thus protecting other cellular components (Figure 2).[8] Nitrite sequestering is apparently quite effective, even in tissues in which nitrate reductase activity has been greatly increased by herbicide treatment.[7,8]

A large body of literature exists on the effects of various herbicides on in vivo and extractable nitrate reductase activity.[9-16] The results are quite varied, depending on the assay, the herbicide, the species, the developmental stage of the treated tissue, and the growing conditions of the plant. Thus, generalizations are difficult to make, however, a few results are relatively consistent. Herbicides that stop chloroplast development (such as pyridazinones) greatly reduce light induction of nitrate reductase activity in monocots.[13,16] These same compounds, however, cause superinduction of nitrate reductase in some dicot tissues,[9] although the superinduced enzyme is a different isozyme than that normally induced.[17] Photosynthesis inhibitors generally have no effect or slightly increase nitrate reductase activity in green tissues.[11,12,18] There is no convincing evidence that any herbicide exerts its phytotoxic effects solely through either direct or indirect effects on reduction of nitrate.

If Klepper's[2-5] views are correct, direct or indirect inhibition of nitrite reductase should be an effective site of herbicide action. However, no herbicides are known to act at this site.

B. Amino Acid Metabolism

1. Glyphosate Inhibition of Aromatic Amino Acid Synthesis

The most important herbicide thought to exert its herbicidal effect through direct inhibition of amino acid synthesis is glyphosate. Recent reviews have discussed this subject in detail.[19,20]

Inhibition of aromatic amino acid synthesis was first hypothesized to be the mode of action of glyphosate by Jaworski.[21] The hypothesis was based upon the finding that aromatic amino acid supplements to the media used for growing duckweed (*Lemna gibba* L.) or

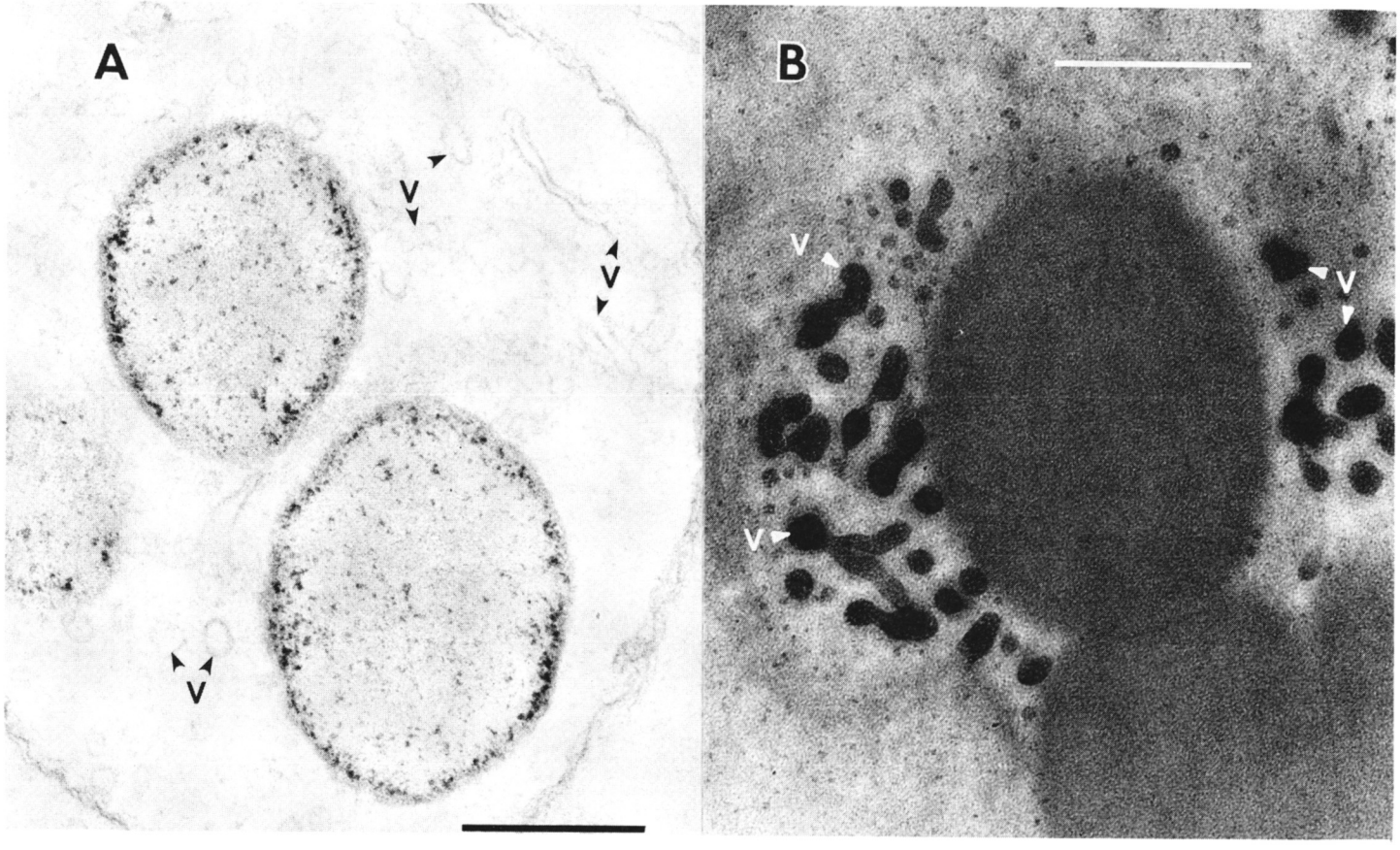

FIGURE 2. (A) Cytoplasmic vesicles (v) in norflurazon-bleached soybean cotyledons. (B) Cytoplasmic vesicles (v) stained for nitrite. Bars = 1 μm. Micrographs courtesy of K. C. Vaughn.

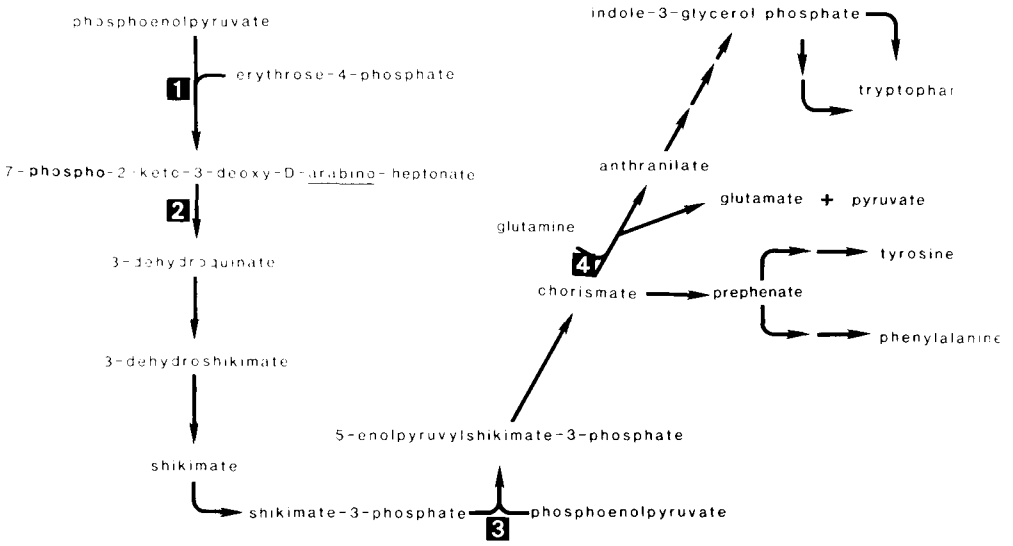

FIGURE 3. Sites of action of glyphosate in the synthesis of aromatic amino acids. Enzymes 1 (phospho-2-keto-3-deoxy-heptonate aldolase), 2 (dehydroquinate synthase), and 4 (anthranilate synthase) are weakly inhibited and enzyme 3 (EPSP-synthase) is strongly inhibited. Shikimate accumulates in glyphosate-treated tissues.

Rhizobium japonicum could alleviate inhibition of growth caused by glyphosate. Later studies showed that in most affected tissues glyphosate causes lowered free pools of aromatic amino acids.[20,22,24] Duke and Hoagland hypothesized that glyphosate might exert its phytotoxicity by greatly increasing phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) activity (see Section VI.B for further discussion).[25] Although initial data supported this hypothesis, later results revealed that glyphosate effects on PAL activity were indirect.

Later work in Amrhein's laboratory established that glyphosate is a potent inhibitor of 5-enolpyruvylshikimate-3-phosphate synthase (EPSP synthase; EC 2.5.1.19) (Figure 3).[26-29] Further work has shown that inhibition of EPSP synthase is competitive with respect to phosphoenolpyruvate and uncompetitive with respect to shikimate-3-phosphate.[30] Inhibition at this site leads to accumulation of very high levels of shikimic acid in glyphosate-treated tissues.[28,29,31] Accumulation of shikimate is primarily in the vacuole.[32] Elevated EPSP synthase levels lead to some degree of tolerance of higher plant (e.g., *Corydalis sempervirens* Pers.) tissue cultures or bacterial (e.g., *Aerobacter aerogenes*) cultures to glyphosate.[29,33] *A. aerogenes*[34] and *Salmonella typhimurium*[35] strains with glyphosate-insensitive EPSP-synthases are totally insensitive to glyphosate. Furthermore, the gene for glyphosate-insensitive EPSP-synthase has been cloned and transferred to *E. coli*.[35] No other enzyme involved in aromatic amino acid synthesis has been shown to be as effectively inhibited as EPSP synthase, however, glyphosate has also been demonstrated to be a weak inhibitor of phospho-2-keto-3-deoxy-heptonate aldolase (EC 4.1.2.15), 3-dehydroquinate synthase (EC 4.6.1.3), and anthranilate synthase (EC 4.1.3.27).[36-38]

Despite these findings, there is evidence that glyphosate's phytotoxicity to intact, higher plants is not entirely due to inhibition of aromatic amino acid synthesis. Although the inhibitory effects of glyphosate on growth have been alleviated with supplemental amino acids in fungi, bacteria, and higher plant cell cultures, this has rarely been the case with intact higher plants, except for the case of *Arabidopsis thaliana* L.[21,22,29,39,40] Glyphosate has approximately 90% of its growth-inhibiting influence on soybean seedlings in which aromatic amino acid levels were substantially increased by either inhibiting PAL activity[41] or by supplying exogenous aromatic amino acids to the roots.[42] In these studies, the root-

fed aromatic amino acids were readily absorbed and incorporated into protein and secondary phenolic compounds.[42] This work was at the plant organ level, however, so whether or not the increased aromatic amino acids arrived at the intercellular or intracellular site(s) affected by glyphosate could not be determined.

2. Nonaromatic Amino Acids

Few commercially used herbicides are known to directly affect nonaromatic amino acid metabolism. Benzadox is a weak in vitro amino transferase inhibitor; however, it may be metabolized in vivo to the very potent amino transferase inhibitor aminooxyacetic acid (AOA).[43] AOA has been patented as a herbicide,[44] and is a strong inhibitor of all pyridoxyl phosphate-requiring enzymes.[45] Selectivity of benzadox is apparently due to differences in the ability of different species to metabolize the compound to AOA.

Similarities in the effects of AOA and glyphosate on profiles of free amino acids indicate that glyphosate may also influence the activity of some aminotransferases.[46] Not all amino acids are affected similarly by both compounds. In these cases the effect of glyphosate generally predominates over that of AOA when plants are treated with both compounds. This suggests that glyphosate and AOA may interact at a common site.

A buildup of free glutamine and glutamate in glyphosate-treated wheat plants led Nilsson to hypothesize that glyphosate is a transaminase inhibitor.[24] Others have found glyphosate to cause similar effects on free amino acid profiles in tissues of other species. No systematic survey has been made of in vitro effects of glyphosate on the activities of plant transaminases.

Killmer et al. speculated that the high levels of glutamine found in glyphosate-treated tissue could be an indirect effect resulting from the unregulated flow of carbon into shikimate.[47] This could deplete the cell of respiratory substrates and substrates for ammonia assimilation, leading to the accumulation of glutamine and ammonia. They found that intermediates of the tricarboxylic acid cycle, particularly α-ketoglutarate, succinate, and malate, would partially reverse the inhibitor effects of glyphosate on growth of carrot tissue cultures. Glutamate and aspartate were also quite effective in ameliorating glyphosate effects in that system.

Amitrole inhibits imidazoleglycerol phosphate dehydratase (EC 4.2.1.19), an enzyme involved in histidine synthesis in both microorganisms and higher plants.[47,49] Exogenous histidine can greatly reduce the herbicidal effect of amitrole in microorganisms, but not in higher plants. In higher plants histidine content is not reduced and the enzyme substrate content is not increased by the herbicide.[50,51] Therefore, inhibition of histidine synthesis does not appear to be a primary site of action of this herbicide in higher plants.

Tabtoxin is a naturally occurring fungal toxin that is extremely phytotoxic,[52,53] although it is not a commercial herbicide. Its phytoxicity is due to inhibition of glutamine synthetase (EC 6.3.1.2) resulting in the accumulation of ammonia at toxic levels. Exploitation of the chemistry of fungal toxins such as tabtoxin could lead to the production of new classes of herbicides.

Other specific inhibitors of enzymes of amino acid metabolism are known which are not used as commercial herbicides. For instance, methionine sulfoximine inhibits glutamine synthetase and, thus, strongly disrupts nitrogen and carbon metabolism of higher plants.[54,55]

Several herbicides have been reported to alter the extractable activities of enzymes associated with amino acid synthesis. For instance, extractable glutamine synthetase activity from cucumber seedlings is reduced by atrazine, chlortoluron, MCPA, and dichlobenil.[56] The extractable activities of alanine aminotransferase (EC 2.6.1.2),[57] glutamine synthetase, and glutamate synthase have been reported to be increased by treatment of tissues with *s*-triazines.[58]

C. Other Effects on Nitrogen Metabolism

Urease (EC 3.5.1.5) activity is inhibited by many urea herbicides (e.g., diuron, monuron, linuron).[59] Whether or not this causes significant metabolic damage, however, is not known.

The inhibition of urate oxidase (EC 1.7.3.3) by nonherbicidal *s*-triazines has been demonstrated.[60] It is possible that herbicidal *s*-triazines or some of their metabolic derivatives might produce a similar effect.

Folic acid or its coenzyme form, tetrahydrofolic acid, serves as an intermediate carrier of hydroxyl methyl, formyl, or methyl groups in a large number of enzymatic reactions such as the metabolism of amino acids, purines, and pyrimidines in plants. Folate and its analogs are strong antagonists of asulam-inhibited growth.[61-62] Asulam reduces accumulation of folates and reduces activity of dihydropteroate synthase (EC 2.5.1.15), an enzyme involved in folate synthesis, activity in vitro.[63] Thus, there is strong evidence that asulam's primary mechanism of action is through direct inhibition of folic acid synthesis.

III. NUCLEIC ACID AND PROTEIN SYNTHESIS

In virtually all cases in which a herbicide has been suggested to directly inhibit nucleic acid or protein synthesis, no definitive proof has been produced. Most studies have measured only protein or nucleic acid content or incorporation of radiolabeled precursors into protein or nucleic acids in an in vivo system with no attempt to separate organelle from nuclear-controlled synthesis of nucleic acids or proteins. Because the majority of cellular energy is expended on macromolecule synthesis, any herbicide that inhibits respiration or photosynthesis should rapidly influence synthesis of nucleic acids and proteins. Herbicides that inhibit amino acid synthesis, such as glyphosate, have rapid and profound effects on protein synthesis. Thus, secondary effects of almost every herbicide have been demonstrated on protein and/or nucleic acid synthesis. A number of investigations have screened herbicides for their relative effectiveness in inhibiting protein and/or nucleic acid synthesis in intact tissues,[64-66] however, such comparisons are difficult to interpret because of different mechanisms of action, differential uptake and translocation, and differential susceptibility of the species tested.

Rapid effects on cell division and on incorporation of nucleotides into DNA do not necessarily indicate a direct herbicide effect on DNA synthesis. This is well illustrated by the case of chlorsulfuron (DPX 4189). In early work with chlorsulfuron it was shown to inhibit cell division and to significantly reduce DNA synthesis within 1 h.[67] This led some to speculate that there could be a direct effect on DNA synthesis.[68] Later work, however, showed that there was no inhibitory effect of chlorsulfuron on DNA synthesis by isolated plant nuclei, nor on the enzymes DNA polymerase (EC 2.7.7.7) and thymidine kinase (EC 2.7.1.21).[69] This indicates that chlorsulfuron has no direct effect on DNA synthesis.

Difenzoquat is a more likely candidate for a herbicide that directly affects DNA synthesis. Incorporation of ^{14}C-thymidine was inhibited about 50% within 15 min in the apical meristems of wheat shoots by 0.1 m*M* difenzoquat.[70] This effect was not caused by inhibition of labeled thymidine uptake and was followed by inhibition of cell division and growth. Several other herbicides that rapidly inhibit cell division (e.g., the dinitroanilines) interfere with mitotic spindle formation and/or function.[71,72] This topic is discussed further in Chapter 3.

No commercially available herbicides are known to directly affect nucleic acid or protein synthesis. However, MDMP, an unmarketed herbicide, has been reported to interfere directly with protein synthesis in higher plants by interference with 80S ribosomes.[73] Only nuclear-coded protein synthesis is directly affected by this herbicide.[74]

Herbicides which cause photobleaching of chloroplasts (e.g., some pyridazinones — see next section and Chapter 3) also secondarily cause photodestruction of 70S ribosomes.[75,76] This effect has been useful in determining whether certain plastid proteins are nuclear- or

plastid-coded.[76] This photobleaching effect is, however, far removed from the primary site of these herbicides.

In summary, direct effects of commercial herbicides on nucleic acid or protein synthesis do not exist or have yet to be discovered. Chemicals that affect such fundamental and evolutionarily conservative processes as these are unlikely to be herbicidally selective and are likely to be toxic to mammals as well. The plastid is therefore the most logical site for a commercially useful herbicide that directly inhibits nucleic acid or protein synthesis.

IV. PHOTOSYNTHETIC PIGMENTS

A. Chlorophyll

Although many herbicides cause rapid and profound chlorosis, few are known to have any direct effects on chlorophyll synthesis. In many studies chlorosis has been assumed to result from inhibited chlorophyll synthesis, without adequate proof. In almost all cases, herbicide-caused chlorosis is actually due to bleaching caused by loss of protection from carotenoids (e.g., pyridazinones) or by overproduction of toxic oxygen species (e.g., bipyridiniums). A rapid, but seldom used, method of separating bleaching from effects (direct or indirect) on synthesis of chlorophyll is to examine the effect of the chemical on protochlorophyll(ide) accumulation in the dark. This can be very easily done using in vivo techniques.[77]

Of the herbicides that influence chlorophyll synthesis listed by Wolf,[78] only amitrole appears to have a direct effect. Amitrole has been shown to cause accumulation of geranylgeraniol-containing chlorophyll and decreases in phytol, indicating that phytol synthesis is inhibited.[79,80] Despite these findings, Grumbach and Bach[81] showed that diuron and bentazon reduced ^{14}C-acetate incorporation into chlorophylls to a greater extent than did amitrole. No enzymatic site of inhibition has been directly demonstrated for any of these herbicides.

Accumulation of the chlorophyll precursor 5-aminolevulinic acid (ALA) is inhibited by glyphosate.[82] Subsequent conversion of ALA into chlorophyll, however, is not affected by glyphosate.[83] Cole[20] has speculated that the cause of inhibited ALA synthesis is a reduction of α-ketoglutarate. Because ALA is a precursor of all porphyrins, such an effect could result in decreases in porphyrin enzymes such as the cytochromes and catalase, as well as reduced chlorophyll synthesis. Indeed, Abu-Irmeileh and Jordan[84] measured a significant reduction in catalase activity in glyphosate-treated purple nutsedge (*Cyperus rotundus* L.) leaves within 3 h.

Aminoxyacetate, the metabolic product of benzadox (see Section II.B.2), inhibits the transaminase that converts 4,5-dioxovaleric acid to ALA.[85] If glyphosate is a transaminase inhibitor, as has been speculated,[24,46] this site may be susceptible to glyphosate.

The unmarketed herbicide DTP inhibits protochlorophyll(ide) accumulation.[86] It prevents conversion of 5-aminolevulinic acid into vinyl pheoporphyrin and promotes formation of uro-, copro-, and protoporphyrins, thus inhibiting protochlorophyll(ide) synthesis. It was suggested that the mechanism of this effect is through DTP donation of protons, perhaps by acid liberation of Mg from Mg-porphyrins.

The thiocarbamate herbicide EPTC causes increased protochlorphyll(ide) synthesis in etiolated tissues,[87] perhaps because of greater substrate availability caused by inhibition of other pathways such as lipid metabolism.

Several nonherbicide chemicals interfere with chlorophyll synthesis directly (e.g., levulinic acid)[88] or reduce protochlorophyll(ide) accumulation in healthy, etiolated tissues (e.g., tentoxin).[77] Inhibition of chlorophyll synthesis has not been fully exploited as a site of herbicide action.

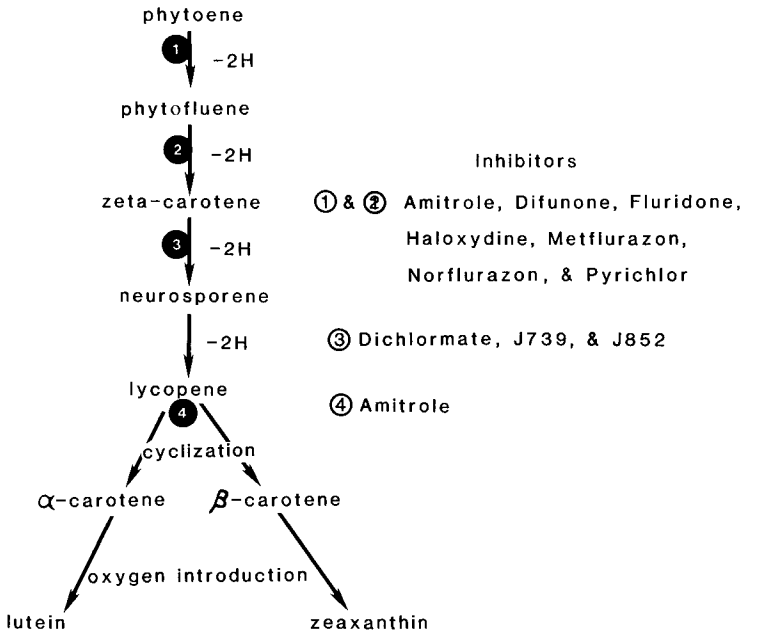

FIGURE 4. Sites of action of carotenoid synthesis-inhibiting herbicides.

B. Carotenoids

Many of the so-called "bleaching" herbicides cause their effects by inhibiting carotenoid synthesis or inducing carotenoid destruction. This topic has been reviewed by others recently.[68,89-91] Loss of carotenoids leads to damage from singlet oxygen generated from triplet chlorophyll, both of which are normally quenched by carotenoids. Singlet oxygen oxidizes chloroplast membranes and other plastid components, resulting in the "bleaching" effect of these compounds. These herbicides are generally most effective in developing chloroplasts in which carotenoid synthesis rates are high.

The herbicides that inhibit carotenoid synthesis can be divided into those that result in phytoene accumulation and those that cause zeta-carotene buildup (Figure 4). As discussed by Ridley,[90] there may be other affected sites in the synthetic pathway; however, this cannot presently be directly examined because in vitro assays for the desaturase enzymes involved in carotenoid synthesis are not available.

Certain pyridazinone herbicides such as norflurazon result in accumulation of high levels of phytoene and greatly reduced carotenoid and xanthophyll accumulation.[75,91,92] Phytofluene accumulation is enhanced to a lesser extent.[93] The actual site of action of pyridazinones may be the chloroplast envelope, because there is growing evidence that carotenoids, at least from phytoene onward, are synthesisized there.[94,95] Approximately 20% of the phytoene accumulating in the plastids of metflurazon- or amitrole-treated radish seedlings in darkness accumulates in the envelope.[96] Nuclear-coded protein synthesis is apparently required for pyridazinone inhibition of carotenoid synthesis.[97] This indicates that the herbicide may interact with a carotenoid-synthesizing enzyme before it is incorporated into the plastid envelope, or it may prevent the posttranslational processing of an enzyme required for proper incorporation into the envelope. Indeed, Ridley and Ridley[92] concluded that metflurazon does not enter the plastid in treated tissues and that the herbicide-susceptible, phytoene-synthesizing enzyme is protected from the herbicide after becoming a functional plastid enzyme.

Fluridone also inhibits carotenoid synthesis and causes accumulation of phytoene and

phytofluene.[98] Structure-activity similarities with norflurazon indicate the mechanisms of action of these herbicides are identical.[90] Herbicides with structures less related to pyridazinones than fluridone that block carotenoid synthesis at the phytoene or phytofluene desaturation site are difunone,[90] 4-hydroxypyridines (such as haloxydine and pyrichlor),[90,99] and amitrole.[99]

A second group of carotenoid synthesis inhibitors are those that block zeta-carotene desaturation, resulting in accumulation of high zeta-carotene levels. These include the *N*-methyl carbamate, dichlormate[99] and the 6-methyl pyrimidines, J739 and J852[90] (structures shown in Reference 90). The third site of herbicide interaction with carotenoid synthesis is that of lycopene cyclization. Lycopene accumulation has been noted in wheat[79] and radish[100] treated with amitrole.

V. LIPIDS AND WAXES

Effects of herbicides on lipid synthesis have been recently reviewed by Fedtke,[68] St. John,[101] and Rivera and Penner.[102] Lipids are essential to the integrity and functioning of membranes, the proper function of lipoprotein enzymes, and modulation of the activity of certain other enzymes. There are mitochondrial, plastidic, and cytoplasmic lipids, each synthesized through an independent pathway. Thus, the number of potential sites of herbicide action is large. Simply shifting a pathway to alter the relative proportions of the different lipid components of a membrane can have profound effects on the physiology of a plant.[101]

Several substituted pyridazinones alter the galactolipid composition of higher plants as well as the fatty acid composition of specific galactolipids.[103] The major effect on galactolipids (the major lipid of plastid membranes) is that they are more saturated following pyridazinone treatments, apparently mostly due to inhibition of the desaturation of linoleic to linolenic acid. This effect is even seen in nonphotosynthetic root tips,[104] indicating that it is independent of photosynthesis. It has been firmly established that pyridazinone effects on lipid synthesis are not secondary effects due to photobleaching or to effects on photosynthesis.[101]

In general, substituted pyridazinones which inhibit carotenoid synthesis also inhibit linolenic acid production, however, some exist (e.g., BAS 13 338) which have no effect on carotenoid synthesis, but strongly inhibit linoleic acid desaturation.[103-105] Some pyridazinones preferentially alter the fatty acids of monogalactosyl diglyceride (MGDG) compared to digalactosyl diglyceride (DGDG), while others affect both galactolipids in a similar fashion.[101-103] Metflurazon decreases ^{14}C-acetate incorporation into *t*-Δ3-hexadecanoate, whereas Sandoz 9785 (same as BAS 13 338) does not.[106]

Desaturation of the linoleic acid component of phospholipids is inhibited by BAS 13 338 in root tissues. Dehydrogenation of the C16:0 to C16:1 fatty acids and the linoleic to linolenic acid of phosphatidylglycerol is inhibited by San 9785 and metflurazon in barley and broad bean leaves.[107] Phospholipids of nonplastid cellular components were unaffected.

The enzymes involved in desaturation of fatty acids of the plastid are thought to reside in the plastid envelope.[108] Only lipids of the plastid have been found to be affected by pyridazinones. As discussed in the previous section, desaturation reactions involved in the synthesis of carotenoids, which are also thought to occur in the plastid envelope, are also inhibited by pyridazinones. Thus, it appears that the major site of activity of substituted pyridazinones are the desaturase enzymes of the plastid envelope. A major task remaining is to characterize these enzymes and to determine the precise mechanism of inhibition. As mentioned earlier, there is reason to believe that pyridazinones may inhibit the posttranslational processing of nuclear-coded desaturases. Processing of nuclear-coded proteins of the plastid seems to be a potentially vulnerable and plant-specific site for herbicide action. The

chlorosis-causing mycotoxin, tentoxin, apparently has such a mode of action,[109] in addition to its effects on photophosphorylation.[110]

The effects of thiocarbamates, particularly EPTC, on lipid synthesis have been well studied. EPTC, diallate, butylate, pebulate, and vernolate inhibit incorporation of ^{14}C-acetate into the lipids of isolated spinach chloroplasts.[111,112] Similar results have been found in other systems, including non-green red beet root tissue.[113] Thiocarbamates also reduce the levels and change the composition of epicuticular waxes.[114,115] Their effects on lipid synthesis are numerous, including inhibition of phospholipid synthesis in roots,[116] fatty acid desaturation,[113] incorporation of unsaturated fatty acids into complex lipids,[116] alkane synthesis in epicuticular waxes,[117] galactolipid synthesis,[118] linolenic acid synthesis,[118] and deposition of *n*-nonocosane and *n*-nonocosan-15-one on cabbage leaf surfaces.[115]

Thiocarbamates also strongly reduce gibberellic acid (GA) levels in affected tissues,[119] apparently through the inhibition of a step leading to kaurene synthesis.[120] The inhibited step appears to be the cyclization of geranylgeranyl pyrophosphate to kaurene. The relationship of thiocarbamate effects on GA and lipid synthesis are not known, however, Wilkinson[120] has speculated that the lipid effects are an indirect effect of reductions in GA synthesis.

Prevention of the effects of EPTC on lipid and GA synthesis with a protectant or "safener" has yielded some insight into the mechanism of action of thiocarbamates.[115,120,122] The protectant diclormid (*N,N*-diallyl-2,2-dichloroacetamide, also known as R-25788 and DDCA) reduces the effect of EPTC on gibberellin,[120] phytol,[120] and neutral and polar lipids[121,122] synthesis. Diclormid has been shown to effectively reverse EPTC effects only in maize.

Evidence exists to support the view that thiocarbamate herbicides are converted to sulfoxides in the plant and these sulfoxides then react with the critical sulfhydryl group(s) of target enzymes (Figure 5).[68] Three possible sites of dichlormid interference with this mechanism are possible: (1) interference with oxidation of thiocarbamates; (2) increasing levels of glutathione and glutathione-*S*-transferase, resulting in conjugation of the thiocarbamate sulfoxide with glutathione;[123] and (3) competition with the thiocarbamate for the herbicide's primary site of action.[120,121] There is no evidence for the first of these mechanisms and, in fact, diclormid apparently increases this pathway.[124] In maize cell cultures, EPTC has been shown to affect lipid synthesis well before diclormid has a measurable effect on glutathione levels, indicating that diclormid may act at the EPTC primary site of action.[125-127] It is possible, however, that diclormid increases glutathione turnover before the pool levels are increased. Nevertheless, diclormid affects lipid synthesis and inhibits EPTC uptake before its effects on glutathione occur, indicating that its protective action involves a sequence of multilevel interactions.[127]

A number of other herbicides have been reported to influence lipid synthesis. The two substituted benzoic acids, dinoben and chloramben, appear to have contrasting effects on lipid synthesis.[128,129] Dinoben strongly inhibits lipid synthesis, whereas chloramben slightly stimulates lipid synthesis. However, chloramben will not reverse the effect of dinoben. Dinoben inhibits up to 99% of acetate and malonate incorporation into lipids; however, it has no effect on acetyl-CoA or malonyl-CoA incorporation. This was interpreted to be due to dinoben inhibition of the acetate and malonate thiokinase systems.[128] Chloramben stimulated the incorporation of all substrates tested into lipids, leading Muslih and Linscott[128] to hypothesize that its effects are due to stimulation of either acetyl-CoA carboxylase and/or fatty acid synthetase.

Lipid synthesis by isolated soybean or navy bean cells was found to be more sensitive than photosynthesis, protein production, or RNA synthesis to chlorsulfuron[130] and sethoxydim.[131] Although buthidazole and tebuthiuron are photosynthetic inhibitors, both inhibited lipid synthesis more strongly than protein or RNA synthesis.[132] These studies have not been

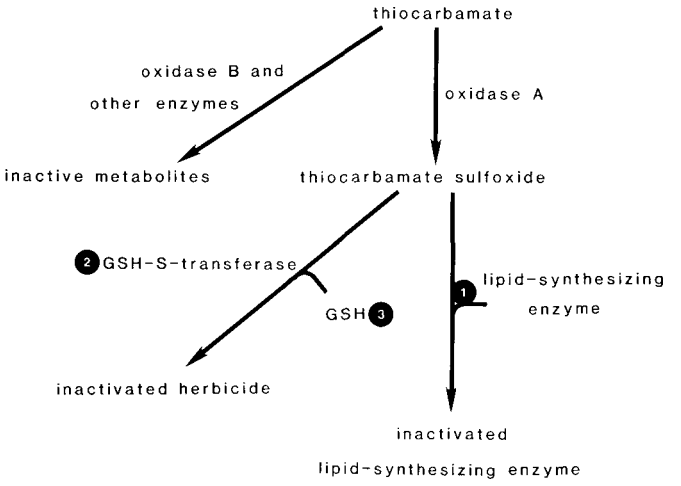

FIGURE 5. Theoretical mechanism of action of thiocarbamate herbicides and protection by diclormid.

verified by others, however, and no further studies on the effects of these herbicides on lipid synthesis have been conducted.

Sublethal levels of *s*-triazine herbicides cause complex changes in plastid lipid profiles,[101,133] resulting in profiles similar to those of the lipids of triazine-resistant mutants (see Chapter 7). This suggests that the effects of triazines on lipid synthesis are secondary in nature.

VI. SECONDARY COMPOUNDS

A. Introduction

Secondary plant compounds are those not directly involved in primary metabolic processes such as respiration, photosynthesis, nitrogen metabolism, carbohydrate metabolism, or protein synthesis. By definition, their role is secondary and, in many cases, their true role is unclear. Certain of them have been implicated, however, in allelopathy (see Volume I, Chapter 5), disease resistance, protection from ultraviolet light, insect resistance, and as growth regulators.[134-136] Because plant survival is only secondarily dependent on production of these compounds, inhibition of their synthesis has not been a sought after mechanism of herbicide action. In fact, most preliminary screening of compounds for herbicide activity is done in glass houses, an environment in which plants normally do not synthesize normal outdoor levels and profiles of many secondary compounds, and in which the protective properties of certain of these compounds may not be as important as in field situations. Also, phytotoxic symptoms due to interference with secondary plant metabolism might appear relatively slowly. These factors may explain why no herbicide is known to have a primary mechanism of action through inhibition of synthesis of secondary compounds. Many herbicides, however, have been reported to have dramatic effects on synthesis of various of these compounds as a consequence of their primary effects on other processes.

B. Aromatic Compounds

Most secondary aromatic compounds found in higher plants are phenolic compounds derived from phenylalanine or tyrosine. Indeed, the pivotal enzyme in the synthesis of these compounds is phenylalanine ammonia-lyase (PAL), the enzyme that deaminates both phenylalanine and tyrosine. Inhibition of PAL activity was suggested as a possible target for herbicidal action by Jangaard.[137,138] Results of his experiments are difficult to evaluate, however, due to the high rates of foliar-applied herbicides used (15 to 150 m*M*), the high (10%) acetone levels used with some herbicides, and the surfactants used. In a study of 16 herbicides, representing 14 herbicide classes, no direct, in vitro effects of any of the herbicides on PAL activity were found.[139] Although no known commercially marketed herbicide has been shown to have a direct influence on PAL activity, several strong inhibitors of PAL are known. One of these, aminoxyacetic acid (AOA), is patented as a herbicide (see Section II.B.2), however, most of its herbicidal properties are probably due to its greater effectiveness as an inhibitor of pyridoxal phosphate-requiring enzymes such as transaminases.[45,46]

The most specific inhibitor of PAL known, α-aminooxy-β-phenylpropionic acid (AOPP), is a very weak transaminase inhibitor, but a very effective PAL inhibitor.[140,141] With AOA the specificity is reversed.[141] Although AOPP has not been screened as a herbicide in the field, it inhibits growth only mildly at PAL-inhibiting concentrations in laboratory-grown plants over short periods.[41] Thus, specific inhibitors of PAL activity are likely to cause symptoms of phytotoxicity which develop slowly as those phenolic compounds necessary for plant development, such as lignin, fail to accumulate. An advantage of such a mechanism of action is that a specific inhibitor of PAL should be relatively safe to mammals, because PAL is found only in plants. There is no evidence, however, of specificity of AOPP or other inhibitors for PAL for certain plant species. Thus, selectivity of a PAL-inhibiting herbicide might be low.

No known stimulators of PAL activity are known. Greatly increased PAL activity could slow protein synthesis through depletion of aromatic amino acid pools, as well as through the production of phytotoxic levels of ammonia and secondary phenolics. Indeed, in some systems, strong induction of PAL activity has been associated with growth cessation.[142]

Many herbicides and other chemicals are known to strongly influence extractable PAL activity through indirect means. Hoagland and Duke found varying degrees of influence, from slight increases to strong decreases, of 16 herbicides from 14 herbicide classes on extractable PAL activity from soybean seedlings (Figure 6).[143] Increased PAL activity was generally correlated with anthocyanin accumulation, indicating that the extractable activities reflected in vivo activities. In the case of glyphosate, however, extractable PAL activity was strongly increased, while anthocyanin content was decreased. This effect is similar to that caused by AOPP.[41] The decreased synthesis of aromatic amino acids (PAL substrates, see Section II.B.1) caused by glyphosate leads to lowered synthesis of phenolic compounds which, in turn, probably results in decreased feed-back inhibition of PAL synthesis.[144] AOPP causes similar "superinduction" of PAL activity.[41] This phenomenon is not found in all species.[144,145] The contribution of indirect herbicidal effects on PAL activity to phytotoxicity, if any, is not known.

As mentioned earlier, some secondary compounds are involved in protection against pathogens. Glyphosate-reduced phytoalexin production has been correlated with increased susceptibility of soybean to pathogens.[146,147] Similar results have been obtained with AOPP.[148]

Not all secondary phenolic compounds are derived from tyrosine or phenylalanine. For instance, benzoic acids are derived from shikimate precursors.[149] Amrhein et al.[150] found glyphosate to caused a fourfold increase in the gallic acid content. This presumably arose from dehydroshikimic acid accumulation due to inhibition of EPSP synthase (see Section II.A.1). Alkaloid accumulation in opium poppy latex is also increased by glyphosate.[151] It

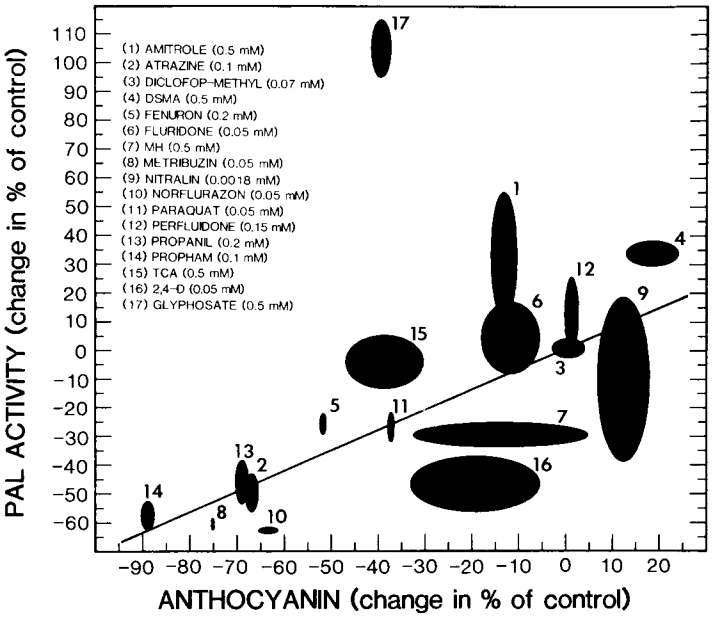

FIGURE 6. Correlation of extractable PAL activity with anthocyanin content of light-grown soybean seedlings 48 h after treatment with various herbicides. Data points are mean values bounded by ellipses with axes ± 1 SE for each mean. The diagonal line represents the linear regression line for all data points, excluding glyphosate. (From Hoagland, R. E. and Duke, S. O., *Weed Sci.*, 31, 845, 1983. With permission.)

was concluded, however, that this result was primarily due to inhibition of degradation of alkaloids.

Polyphenol oxidase (PPO; EC 1.10.3.1) has been thought to be involved in the synthesis of certain phenolic compounds by ortho-hydroxylating their precursors.[152] Although such reactions occur in vitro, there is good evidence that it does not occur in vivo in healthy tissues, due to substrate/enzyme separation and latency of the enzyme.[153,154] Nevertheless, PPO undoubtedly plays a role in host plant resistance to pathogens through the production of quinones from phenolic compounds in damaged cells.

Two groups have found glyphosate to increase extractable PPO in treated plants,[42,155] however, their assays could not distinguish between PPO and peroxidase (POD: EC 1.11.1.7). There is good evidence that POD involvement in phenolic metabolism has been mistaken for PPO involvement in the past.[154] If POD was mistaken for PPO,[42,155] the results of Lee[156,157] could be partially explained by their findings. Lee found glyphosate enhanced oxidative degradation of indole-3-acetic acid (IAA). IAA oxidase is known to be a form of POD, the activity of which is strongly influenced by secondary phenolic compounds. The effects of glyphosate on IAA metabolism and physiology described by Lee[156,157] and Baur[158] could therefore result from altered IAA-oxidase activity caused by glyphosate-caused reductions in secondary phenolic compounds.

Scarponi et al.[159] surveyed the effects of 18 herbicides at concentrations of 10 to 100 μM on in vitro POD activity, as measured by oxidative decarboxylation of tryptophan. They found only a slight (± 10%) inhibition or stimulation by a few of the compounds. The development of in vivo POD activity in cabbage seeds, however, was greatly retarded by 2,4-D.

Thiocarbamates have been hypothesized to cause part of their effects through increased peroxidase activity, resulting in growth-inhibiting increases in lignification.[160] EPTC has been reported to strongly inhibit polyphenol oxidase in vitro.[161] PPO is a copper enzyme and some thiocarbamates, particularly diethyldithiocarbamate, are known to be strong inhibitors of PPO, due to their copper-chelating ability.[162] Lipids also strongly influence PPO activity[162] and ETPC has strong influences on lipid synthesis (see Section V). Whether ETPC acts on PPO by its interaction with copper or lipids is not known, however, it is doubtful that the effect of EPTC on PPO is important in its mechanism of action, in that EPTC inhibits early growth and loss of all PPO activity due to tentoxin treatment has no effect on seedling growth.[153]

VII. CONCLUSIONS

Few herbicides are known to exert their major phytotoxic effects through initially inhibiting a nonphotosynthetic, enzyme-controlled synthetic process. The notable exceptions are (1) glyphosate, which inhibits aromatic amino acid synthesis; (2) the pyridazinones, which inhibit carotenoid synthesis; (3) the thiocarbamates, which inhibit lipid synthesis; (4) benzadox, which inhibits pyridoxyl phosphate-requiring enzymes; and (5) asulam, which inhibits folate synthesis. Of these, the characterization of the inhibition of a specific enzyme has only been achieved for glyphosate and asulam. There are many more potential enzymatic sites of herbicide action that involve synthetic processes. In a few cases, we may discover that already existing herbicides act at one or more of these sites. However, there are undoubtedly many unexploited sites that will be the targets of herbicides to be developed in the future.

EPILOGUE

Since this chapter went to press, several important findings have been made. The sulfonylurea herbicides chlorsulfuron and sulfometuron methyl have been shown to block the synthesis of valine and isoleucine by inhibiting acetolactate synthase (EC 4.1.3.18).[163,164] Mutants of higher plants which have acetolactate synthases that are insensitive to sulfonylureas are insensitive to the herbicides. Furthermore, feeding valine plus isoleucine completely overcomes growth retardation of pea roots and seedlings by chlorsulfuron, even at high herbicide concentrations.[164] Another class of herbicides, the imidazolinones, have also been reported to inhibit acetolactate synthase (= acetohydroxy acid synthase).[165] Feeding valine, leucine, plus isoleucine alleviated the toxicity of these herbicides.[166] Whether sulfonylureas and imidazolinones are acting at the same site on the enzyme has yet to be reported.

Further studies of the effects of glyphosate on EPSP synthase of higher plants have been reported,[167,168] but still no higher plant with a glyphosate-insensitive EPSP isozyme has been discovered. An analogue of glyphosate, SC-0024 (trimethylsufonium carboxymethylaminomethylphosphate) was found to have similar phytotoxic properties to glyphosate, yet feeding aromatic amino acids would not reverse its effects as they did the effects of glyphosate in the same system.[169]

Bialaphos (L-2-amino-4-(1-hydroxy)(methyl)phosphinyl)-butyryl-L-alanyl-L-alanine), a metabolic product of *Streptomyces*, was shown to be an effective herbicide due to metabolism to phosphinothricin (ammonium(3-amino-3-carboxypropyl)-methylphosphinate) in sensitive species.[170-172] Phosphinothricin is a potent inhibitor of glutamine synthetase from higher plants, causing accumulation of high levels of ammonia in treated plants. It is about 150 times more effective in inhibiting glutamine synthetase from mustard roots than is methionine sulfoximine.[172]

Several diphenyl ethers of the *m*-phenoxybenzamide type have been shown to inhibit

carotenoid synthesis in a manner similar to 2-phenylpyridazinones such as norflurazon.[173] Structure-activity relationships of *m*-phenoxybenzamides, as well as several previously unreported compounds, on carotenoid and phytoene accumulation were made and as it was concluded that inhibition of phytoene desaturase is dependent on portions of the herbicide molecule that resemble the segment of phytoene that is desaturated. This conclusion was supported by studies of cell-free carotene biosynthesis in which the same structure-activity relations were valid for inhibition of activity of "phytoene dehydrogenase".[175]

Two herbicides, oxadiazon (2-*tert*-butyl-4-(2,4-dichloro-5-isopropoxyphenyl)-Δ^2-1,3,4-oxadiazolin-5-one) and MK-616 (*N*-(4-chlorophenyl)-3,4,5,6-tetrahydrophthalimide) have been reported to interfere with chlorophyll synthesis, while having no effect on carotenoid synthesis.[176] They also cause decreases in cytochrome *c*-553 content.

The diphenyl ether herbicides acifluorfen and oxyfluorfen have been shown to greatly enhance production of certain aromatic amino acid-derived phenolic compounds, as well as pterocarpans and terpenoids.[177,178] These effects have been interpreted to be due to secondary reactions to stress. Chlorsulfuron has been shown to cause massive (more than 30-fold increases) accumulation of aromatic amino acid-derived phenolic acids.[179] The subject of chemical effects on phenolic metabolism is covered in a recent review.[180]

REFERENCES

1. **Hofstra, J. J.,** Chlorate toxicity and nitrate reductase activity in tomato plants, *Physiol. Plant.*, 41, 65, 1977.
2. **Klepper, L. A.,** A Mode of Action of Herbicides: Inhibition of the Normal Process of Nitrite Reductase, Res. Bull. 259, Agric. Exp. Stn., University of Nebraska, Lincoln, 1974.
3. **Klepper, L. A.,** Inhibition of nitrate reduction by photosynthetic inhibitors, *Weed Sci.*, 23, 188, 1975.
4. **Klepper, L. A.,** Effects of certain herbicides and their combinations on nitrate and nitrite reduction, *Plant Physiol.*, 64, 273, 1979.
5. **Klepper, L. A.,** Nitrite accumulation within herbicide-treated leaves, *Weed Sci.*, 24, 533, 1976.
6. **Decleire, M. and DeCat, W.,** Detection rapide de traces d'herbicides inhibiteurs de photosynthese par l'inhibition de la reduction des nitrites a la lumiere, *Z. Pflanzenphysiol.*, 82, 310, 1977.
7. **Fedtke, C.,** Formation of nitrite in plants treated with herbicides that inhibit photosynthesis, *Pestic. Sci.*, 8, 152, 1977.
8. **Vaughn, K. C. and Duke, S. O.,** Histochemical localization of nitrate reductase, *Histochemistry*, 72, 191, 1981.
9. **Duke, S. O., Vaughn, K. C., and Duke, S. H.,** Effects of norflurazon (San 9789) on light-increased nitrate reductase activity in soybean [*Glycine max* (L.) Merr.] seedlings, *Plant Cell Environ.*, 5, 155, 1982.
10. **Finke, R. L., Warner, R. L., and Muzik, T. J.,** Effect of herbicides on *in vivo* nitrate and nitrite reduction, *Weed Sci.*, 25, 18, 1977.
11. **Tweedy, J. A. and Ries, S. K.,** Effect of simazine on nitrate reductase in corn, *Plant Physiol.*, 42, 280, 1967.
12. **Aslam, M. and Huffaker, R. C.,** Effect of DCMU, simazine and atrazine on nitrate reductase in *Hordeum vulgare in vitro* and *in vivo*, *Physiol. Plant.*, 28, 400, 1973.
13. **Duke, S. O. and Duke, S. H.,** Photosynthetic independence of initial light-caused increase in extractable nitrate reductase activity from maize seedlings, *Plant Cell Physiol.*, 20, 1371, 1979.
14. **Peirson, D. R. and Elliott, J. R.,** Antagonistic effects of cyclohexanecarboxylic acid and 2,4-dichlorophenoxyacetic acid in *Phaseolus vulgaris:* nitrate reductase activity, *Z. Pflanzenphysiol.*, 96, 81, 1980.
15. **Lolas, P. C.,** Effect of herbicides alachlor, metolachlor and oryzalin on soybean (*Glycine max*) leaf nitrate reductase activity, *Weed Res.*, 20, 239, 1980.
16. **Deane-Drummond, C. E. and Johnson, C. B.,** Absence of nitrate reductase activity in San 9789 bleached leaves of barley seedlings (*Hordeum vulgare* cv. Midas), *Plant Cell Environ.*, 3, 303, 1980.
17. **Kakefuda, G., Duke, S. H., and Duke, S. O.,** Differential light induction of nitrate reductases in greening and photobleached soybean seedlings, *Plant Physiol.*, 73, 56, 1983.
18. **James, D. B. and Smith, S. M.,** The absence of any influence of light on *in vivo* nitrate reductase activity in leaf tissue of *Lolium perenne* L., *Ann. Bot.*, 46, 803, 1980.

19. **Hoagland, R. E. and Duke, S. O.,** Biochemical effects of glyphosate [*N*-(Phosphonomethyl)glycine], in *Biochemical Responses Induced by Herbicides,* ACS Symp. Ser. No. 181, American Chemical Society, Washington, D.C., 1982, chap. 10.
20. **Cole, D. J.,** Mode of action — a literature analysis, in *The Herbicide Glyphosate,* Grossbard, E. and Atkinson, D., Eds., Butterworths, Oxford, 1984, in press.
21. **Jaworski, E. G.,** Mode of action of *N*-phosphonomethyl-glycine: inhibition of aromatic amino acid biosynthesis, *J. Agric. Food Chem.,* 20, 1195, 1972.
22. **Hoagland, R. E., Duke, S. O., and Elmore, C. D.,** Effects of glyphosate on metabolism of phenolic compounds. II. Influence on soluble hydroxyphenolic compounds, free amino acid and soluble protein levels in dark-grown maize roots, *Plant Sci. Lett.,* 13, 291, 1978.
23. **Duke, S. O., Hoagland, R. E., and Elmore, C. D.,** Effects of glyphosate on metabolism of phenolic compounds. IV. Phenylalanine ammonia-lyase activity, free amino acids, and soluble hydroxyphenolic compounds in axes of light-grown soybeans, *Physiol. Plant.,* 46, 307, 1979.
24. **Nilsson, G.,** Effects of glyphosate on the amino acid content in spring wheat plants, *Swed. J. Agric. Res.,* 7, 153, 1977.
25. **Duke, S. O. and Hoagland, R. E.,** Effects of glyphosate on metabolism of phenolic compounds. I. Induction of phenylalanine ammonia-lyase activity in dark-grown maize roots, *Plant. Sci. Lett.,* 11, 185, 1978.
26. **Amrhein, N., Shab, J., and Steinrücken, H. L.,** The mode of action of the herbicide glyphosate, *Naturwissenshaften,* 67, 356, 1980.
27. **Steinrücken, H. C. and Amrhein, N.,** The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase, *Biochem. Biophys. Res. Commun.,* 94, 1207, 1980.
28. **Amrhein, N., Deus, B., Gehrke, P., and Steinrücken, H. C.,** The site of the inhibition of the shikimate pathway by glyphosate. II. Interference of glyphosate with chorismate formation *in vivo* and *in vitro, Plant Physiol.,* 66, 830, 1980.
29. **Amrhein, N., Johänning, D., Schab, J., and Schulz, A.,** Biochemical basis for glyphosate-tolerance in a bacterium and a plant tissue culture, *FEBS Lett.,* 157, 191, 1983.
30. **Boocock, M. R. and Coggins, J. R.,** Kinetics of 5-enolpyruvylshikimate-3-phosphate synthase inhibition by glyphosate, *FEBS Lett.,* 154, 127, 1983.
31. **Berlin, J. and Witte, L.,** Effects of glyphosate on shikimic acid accumulation in tobacco cell cultures with low and high yields of cinnamoyl putrescines, *Z. Naturforsch.,* 36C, 210, 1981.
32. **Holländer-Czytko, H. and Amrhein, N.,** Subcellular compartmentalization of shikimic acid and phenylalanine in buckwheat cell suspension cultures grown in the presence of shikimate pathway inhibitors, *Plant Sci. Lett.,* 29, 89, 1983.
33. **Rogers, S. G., Brand, L. A., Holder, S. B., Sharps, E. S., and Brackin, M. J.,** Amplification of the *aroA* gene from *Escherichia coli* results in tolerance to the herbicide glyphosate, *Appl. Environ. Microbiol.* 46, 37, 1983.
34. **Schulz, A., Sost, D., and Amrhein, N.,** Insensitivity of 5-enolpyruvylshikimate-3-phosphate synthase EC-2.5.1.19 to glyphosate confers resistance to this herbicide in a strain of *Aerobacter aerogenes, Arch. Microbiol.,* 137, 121, 1984.
35. **Comai, L., Sen, L. C., and Stalker, D. M.,** An altered *aroA* gene product confers resistance to the herbicide glyphosate, *Science,* 221, 370, 1983.
36. **Roisch, U. and Lingens, F.,** Effect of the herbicide *N*-(phosphonomethyl)glycine on the biosynthesis of aromatic amino acids, *Angew. Chem. Int. Ed. Engl.,* 13, 400, 1974.
37. **Roisch, U. and Lingens, F.,** The mechanism of action of the herbicide *N*-(phosphonomethyl)glycine. Its effect on the growth and the enzymes of aromatic amino acid biosynthesis in *Escherichia coli, Hoppe-Seyler's Z. Physiol. Chem.,* 361, 1049, 1980.
38. **Rubin, J. L., Gaines, C. G., and Jensen, R. A.,** Enzymological basis for the herbicidal action of glyphosate, *Plant Physiol.,* 70, 833, 1982.
39. **Gresshoff, P. M.,** Growth inhibition by glyphosate and reversal of its action by phenylalanine and tyrosine, *Aust. J. Plant Physiol.,* 6, 177, 1979.
40. **Haderlie, L. C., Widholm, J. M., and Slife, F. W.,** Effect of glyphosate on carrot and tobacco cells, *Plant Physiol.,* 60, 40, 1977.
41. **Duke, S. O., Hoagland, R. E., and Elmore, C. D.,** Effects of glyphosate on metabolism of phenolic compounds. V. L-α-aminoxy-β-phenylpropionic acid and glyphosate effects on phenylalanine ammonia-lyase in soybean seedlings, *Plant Physiol.,* 65, 17, 1980.
42. **Duke, S. O. and Hoagland, R. E.,** Effects of glyphosate on metabolism of phenolic compounds. VII. Root-fed amino acids and glyphosate toxicity in soybean (*Glycine max*) seedlings, *Weed Sci.,* 29, 297, 1981.
43. **Nakamoto, H., Ku, M. S. B., and Edwards, G. E.,** Inhibition of C_4 photosynthesis by (benzamidooxy)acetic acid, *Photosyn. Res.,* 3, 293, 1982.

44. U.S. Patent Office No. 3,162,525, Dow Chemical Company, Midland, Mich., 1964.
45. **John, R. A., Charteris, A., and Fowler, L. J.,** The reaction of amino-oxyacetate with pyridoxyl phosphate-dependent enzymes, *Biochem. J.*, 171, 771, 1978.
46. **Hoagland, R. E. and Duke, S. O.,** Effects of glyphosate on metabolism of phenolic compounds. VIII. Comparison of the effects of aminooxyacetate and glyphosate, *Plant Cell Physiol.*, 23, 1081, 1982.
47. **Killmer, J., Widholm, J., and Slife, F.,** Reversal of glyphosate inhibition of carrot cell culture growths by glycolytic intermediates and organic and amino acids, *Plant Physiol.*, 68, 1299, 1981.
48. **Hilton, J. L., Kearney, P. C., and Ames, B. N.,** The mode of action of the herbicide 3-amino-1,2,4-triazole (amitrole) inhibition of an enzyme of histidine biosynthesis, *Arch. Biochem. Biophys.*, 112, 544, 1965.
49. **Wiater, A., Klopotowski, T., and Bagdasarian, G.,** Synergistic inhibition of plant imidazoleglycerol phosphate dehydratase by aminotriazole and phosphate, *Acta Biochim. Pol.*, 18, 309, 1971.
50. **McWhorter, C. G. and Hilton, J. L.,** Alterations in amino acid content caused by 3-amino-1,2,4-triazole, *Physiol. Plant.*, 20, 30, 1967.
51. **Hilton, J. L.,** Inhibitions of growth and metabolism of 3-amino-1,2,4-triazole (amitrole), *J. Agric. Food Chem.*, 17, 182, 1969.
52. **Uchytil, T. F. and Durbin, R. D.,** Hydrolysis of tabtoxins by plant and bacterial enzymes, *Experientia*, 36, 301, 1980.
53. **Turner, J. G. and Debbage, J. M.,** Tabtoxin-induced symptoms are associated with the accumulation of ammonia formed during photorespiration, *Physiol. Plant Pathol.*, 20, 223, 1982.
54. **Berger, M. G. and Fock, H. P.,** Effects of methionine sulfoximine and glycine on nitrogen metabolism of maize leaves in the light, *Aust. J. Plant Physiol.*, 10, 187, 1983.
55. **Muhitch, M. J. and Fletcher, J. S.,** Influence of methionine sulfoximine on the photosynthesis of isolated chloroplasts, *Photosyn. Res.*, 4, 241, 1983.
56. **Decleire, M. and van Roey, G.,** Effets de Divers Herbicides sur l'Activite de la Glutamine Synthetase dans des Plantules de Concombre (*Cucumis sativus*), *Z. Pflanzenphysiol.*, 97, 361, 1980.
57. **Wu, M. T., Singh, B., and Salunkhe, D. K.,** Influence of foliar application of *s*-triazine compounds on fresh weight, dry weight, chemical composition, and enzymatic activity of pea and sweet corn seedlings, *J. Exp. Bot.*, 23, 793, 1972.
58. **Mohandas, G., Wallace, W., and Nicholas, D.J.D.,** Effects of atrazine on the assimilation of inorganic nitrogen in cereals, *Phytochemistry*, 17, 1021, 1978.
59. **Cerrelli, S., Nannipieri, P., Giovanni, G., and Perna, A.,** Jack bean urease inhibition by substituted ureas, *Pestic. Biochem. Physiol.*, 5, 221, 1975.
60. **Fridovich, I.,** The competitive inhibition of uricase by oxonate and by related derivatives of *s*-triazines, *J. Biol. Chem.*, 240, 2491, 1965.
61. **Killmer, J. L., Widholm, J. M., and Slife, F. W.,** Antagonistic effect of *p*-aminobenzoate or folate on asulam [methyl(4-aminobenzensulphonyl carbamate)] inhibition of carrot suspension cultures, *Plant Sci. Lett.*, 19, 203, 1980.
62. **Veerasekaran, P., Kirkwood, R. C., and Parnell, E. W.,** Studies of the mechanism of action of asulam in plants. I. Antagonistic interaction of asulam and 4-aminobenzoic acid, *Pestic. Sci.*, 12, 325, 1981.
63. **Veerasekaran, P., Kirkwood, R. C., and Parnell, E. W.,** Studies of the mechanism of action of asulam in plants. II. Effect of asulam on the biosynthesis of folic acid, *Pestic. Sci.*, 12, 330, 1981.
64. **Mann, J. D., Jordan, L. S., and Day, B. E.,** A survey of herbicides for their effect upon protein synthesis, *Plant Physiol.*, 40, 840, 1965.
65. **Moreland, D. E., Malhotra, S. S., Gruenhagen, R. D., and Shokraii, E. H.,** Effects of herbicides on RNA and protein synthesis, *Weed Sci.*, 17, 556, 1969.
66. **Ashton, F. M., de Villiers, O. T., Glenn, R. K., and Duke, W. B.,** Localization of metabolic sites of action of herbicides, *Pestic. Biochem. Physiol.*, 7, 122, 1977.
67. **Ray, T. B.,** The mode of action of chlorsulfuron: a new herbicide for cereals, *Pestic. Biochem. Physiol.*, 17, 10, 1982.
68. **Fedtke, C.,** *Biochemistry and Physiology of Herbicide Action*, Springer-Verlag, New York, 1982.
69. **Ray, T.,** The mode of action of chlorsulfuron: the lack of direct inhibition of plant DNA synthesis, *Pestic. Biochem. Physiol.*, 18, 262, 1982.
70. **Pallett, K. C. and Caseley, J. C.,** Differential inhibition of DNA synthesis in difenzoquat tolerant and susceptible United Kingdom spring wheat cultivars, *Pestic. Biochem. Physiol.*, 14, 144, 1980.
71. **Hess, F. D.,** Determining causes and categorizing types of growth inhibition induced by herbicides, in *Biochemical Responses Induced by Herbicides*, ACS Symp. Ser. No. 181, American Chemical Society, Washington, D.C., 1982, chap. 11.
72. **Strachan, S. D. and Hess, F. D.,** The biochemical mechanism of action of the dinitroaniline herbicide oryzalin, *Pestic. Biochem. Physiol.*, 20, 141, 1983.

73. **Baxter, R., Knell, V., Somerville, H. J., Swain, H. M., and Weeks, D. P.,** Effect of MDMP on protein synthesis in wheat and bacteria, *Nature (London) New Biol.*, 243, 139, 1973.
74. **Ellis, R. J.,** Inhibition of chloroplast protein synthesis by lincomycin and 2-(4-methyl-2,6-dinitroanilino)-*N*-methyl propionamide, *Phytochemistry*, 14, 89, 1975.
75. **Bartels, P. G. and Hyde, A.,** Chloroplast development in 4-chloro-5-(dimethylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3 (2H)-pyridazinone (Sandoz 6706)-treated wheat seedlings. A pigment, ultrastructural, and ultracentrifugal study, *Plant Physiol.*, 45, 807, 1970.
76. **Feierabend, J. and Schubert, B.,** Comparative investigation of the action of several chlorosis-inducing herbicides on the biogenesis of chloroplasts and leaf microbodies, *Plant Physiol.*, 61, 1017, 1978.
77. **Duke, S. O., Wickliff, J. L., Vaughn, K. C., and Paul, R. N.,** Tentoxin does not cause chlorosis in greening mung bean leaves by inhibiting photophosphorylation, *Physiol. Plant.*, 56, 387, 1982.
78. **Wolf, F. T.,** Effects of chemical agents in inhibition of chlorophyll synthesis and chloroplast development in higher plants, *Bot. Rev.*, 43, 395, 1977.
79. **Rüdiger, W., Benz, J., Lempert, U., Schoch, S., and Steffens, D.,** Hemmung der Phytol-Akkumulation mit Herbiziden. Geranylgeraniol-und Dihydrogeranylgeraniol-haltiges Chlorophyll aus Weizenkeimlingen, *Z. Pflanzenphysiol.*, 80, 131, 1976.
80. **Rüdiger, W. and Benz, J.,** Influence of aminotriazol on the biosynthesis of chlorophyll and phytol, *Z. Naturforsch.*, 34C, 1055, 1979.
81. **Grumbach, K. H. and Bach, T. J.,** The effect of PS II herbicides, amitrole and SAN 6706 on the activity of 3-hydroxy-3-methylglutaryl-coenzyme-A-reductase and the incorporation of [2-^{14}C] acetate and [2-^{3}H] mevalonate into chloroplast pigments of radish seedlings, *Z. Naturforsch.*, 34C, 941, 1979.
82. **Kitchen, L. M., Witt, W. W., and Rieck, C. E.,** Inhibition of chlorophyll accumulation by glyphosate, *Weed Sci.*, 29, 513, 1981.
83. **Kitchen, L. M., Witt, W. W., and Rieck, C. E.,** Inhibition of 5-aminolevulinic acid synthesis by glyphosate, *Weed Sci.*, 29, 571, 1981.
84. **Abu-Irmeileh and Jordan, L. S.,** Some aspects of glyphosate action in purple nutsedge *(Cyperus rotundus)*, *Weed Sci.*, 26, 700, 1978.
85. **Harel, E., Ne'eman, E., and Meller, E.,** Alternative routes for the synthesis of 5-amino-levulinic acid in maize leaves. I. Formation from 2-ketoglutarate via 4,5-dioxovaleric acid, *Plant Physiol.*, 72, 1056, 1983.
86. **Kawakubo, K., Shindo, M., and Konotsune, T.,** A mechanism of chlorosis caused by 1,3-dimethyl-4-(2,4-dichlorobenzoyl)-5-hydroxypyrazole, a herbicidal compound, *Plant Physiol.*, 64, 774, 1979.
87. **Weinberg, M. B. and Castelfranco, P. A.,** Effect of EPTC on plastid membrane constituents in germinating cucumber cotyledons, *Weed Sci.*, 23, 185, 1975.
88. **Beale, S. I. and Castelfranco, P. A.,** The biosynthesis of 5-aminolevulinic acid in higher plants. I. Accumulation of 5-aminolevulinic acid in greening plant tissue, *Plant Physiol.*, 53, 291, 1974.
89. **Britton, G.,** Carotenoid biosynthesis — a target for herbicide activity, *Z. Naturforsch.*, 34C, 979, 1979.
90. **Ridley, S. M.,** Carotenoids and herbicide action, in *Carotenoid Chemistry and Biochemistry*, Britton, G. and Goodwin, T. W., Eds., Pergamon Press, Oxford, 1982, 353.
91. **Sandmann, G. and Boger, P.,** Mode of action of herbicidal bleaching, in *Biochemical Responses Induced by Herbicides*, ACS Symp. Ser. No. 181, Moreland, D. E., St. John, J. B., and Hess, F. D., Eds., American Chemical Society, Washington, D.C., 1982, chap. 7.
92. **Ridley, S. M. and Ridley, J.,** Interaction of chloroplasts with inhibitors. Location of carotenoid synthesis and inhibition during chloroplast development, *Plant Physiol.*, 63, 392, 1979.
93. **Bartels, P. G. and McCullough, C.,** A new inhibitor of carotenoid synthesis in higher plants: 4-chloro-5-(dimethylamino)-2-α,α,α,(trifluoro-*m*-tolyl)-3-(2H)-pyridazinone (Sandoz 6706), *Biochem. Biophys. Res. Commun.*, 48, 16, 1972.
94. **Lütke-Brinkhaus, F., Liedvogel, B., Kreuz, K., and Kleinig, H.,** Phytoene synthase and phytoene dehydrogenase associated with envelope membranes from spinach chloroplasts, *Planta*, 156, 176, 1982.
95. **Camara, B., Bardat, F., and Moneger, R.,** Sites de biosynthese de carotenoides dans les chromoplastes de fruit de Poivon (*Capsicum annum* L.), *C. R. Acad. Sci. Paris*, 294, Ser III, 649, 1982.
96. **Grumbach, K. H. and Britton, G.,** Distribution of carotenoids in sub-cellular and sub-plastidic fractions of radish seedlings (*Raphanus sativus*) grown in the presence of bleaching herbicides, *Phytochemistry*, 22, 1937, 1983.
97. **Grumbach, K. H. and Drollinger, M.,** The effect of phytochrome and protein synthesis-inhibitors on the formation of chlorophylls and carotenoids in radish seedlings treated with photosystem II and bleaching herbicides, *Z. Naturforsch.*, 35C, 445, 1980.
98. **Bartels, P. G. and Watson, C. W.,** Inhibition of carotenoid synthesis by fluridone and norflurazon, *Weed Sci.*, 26, 198, 1978.
99. **Burns, E. R., Buchanan, G. A., and Carter, M. C.,** Inhibition of carotenoid synthesis as a mechanism of action of amitrole, dichlormate, and pyriclor, *Plant Physiol.*, 47, 144, 1971.

100. **Grumbach, K. H.,** Effect of San 6706, amitrole and photosystem II herbicides on chloroplast development, in *Proc. 5th Int. Photosynthesis Congr.*, Vol. 6, Akoyunoglou, G., Ed., Balaban International, Philadelphia, 1981, 625.
101. **St. John, J. B.,** Effects of herbicides on the lipid composition of plant membranes, in *Biochemical Responses Induced by Herbicides*, ACS Symp. Ser. 181, Moreland, D. E., St. John, J. B., and Hess, F. D., Eds., American Chemical Society, Washington, D.C., 1982, chap. 6.
102. **Rivera, C. M. and Penner, D.,** Effects of herbicides on plant cell membrane lipids, *Res. Rev.*, 70, 45, 1979.
103. **St. John, J. B.,** Manipulation of galactolipid fatty acid composition with substituted pyridazinones, *Plant Physiol.*, 57, 38, 1976.
104. **St. John, J. B. and Christiansen, M. N.,** Inhibition of linolenic acid synthesis and modification of chilling resistance in cotton seedlings, *Plant Physiol.*, 57, 257, 1976.
105. **Ashworth, E. N., Christiansen, M. N., St. John, J. B., and Patterson, G. W.,** Effect of temperature and BASF 13 338 on the lipid composition and respiration of wheat roots, *Plant Physiol.*, 67, 711, 1981.
106. **Davies, A. O. and Harwood, J. L.,** Effect of substituted pyridazinones on chloroplast structure and lipid metabolism in greening barley leaves, *J. Exp. Bot.*, 34, 1089, 1983.
107. **Khan, M.-U., Lem, N. W., Chandorkar, K. R., and Williams, J. P.,** Effects of substituted pyridazinones (San 6706, San 9774, San 9785) on glycerolipids and their associated fatty acids in the leaves of *Vicia faba* and *Hordeum vulgare*, *Plant Physiol.*, 64, 300, 1979.
108. **Douce, R. and Joyard, J.,** Structure and function of the plastid envelope, *Adv. Bot. Res.*, 7, 1, 1979.
109. **Vaughn, K. C. and Duke, S. O.,** Tentoxin stops the processing of polyphenol oxidase into an active enzyme, *Physiol. Plant.*, 257, 1984.
110. **Steele, J. A., Uchytil, T. F., Durbin, R. D., Bhatnager, P., and Rich, D. H.,** Chloroplast coupling factor 1: a species-specific receptor for tentoxin, *Proc. Natl. Acad. Sci. U.S.A.*, 73, 2245, 1976.
111. **Wilkinson, R. E. and Smith, A. E.,** Thiocarbamate inhibition of fatty acid biosynthesis in isolated spinach chloroplasts, *Weed Sci.*, 23, 100, 1975.
112. **Wilkinson, R. E. and Smith, A. E.,** Butylate, pebulate, and vernolate inhibition of plant fatty acid biosynthesis, *Phytochemistry*, 15, 841, 1976.
113. **Wilkinson, R. E. and Smith, A. E.,** EPTC altered beet disc betacyanin efflux and fatty acid synthesis, *Weed Sci.*, 24, 235, 1976.
114. **Flore, J. A. and Bukovac, M. J.,** Pesticide effects on the plant cuticle. I. Response of *Brassica olearacea* L. to EPTC as indexed by epicuticular wax production, *J. Am. Soc. Hortic. Sci.*, 99, 34, 1974.
115. **Leavitt, J. R. C., Duncan, D. N., Penner, D., and Meggitt, W. F.,** Inhibition of epicuticular wax deposition on cabbage by ethofumesate, *Plant Physiol.*, 61, 1034, 1978.
116. **Karunen, P. and Wilkinson, R. E.,** Influence of *S*-ethyl dipropylthiocarbamate (EPTC) on wheat root fatty acid composition, *Physiol. Plant.*, 35, 228, 1975.
117. **Kolattukudy, P. E. and Walton, T. J.,** The biochemistry of plant cuticular lipids, in *Progress in the Chemistry of Fats and Other Lipids*, Holman, R. T., Ed., Academic Press, New York, 1973, chap. 3.
118. **Wilkinson, R. E., Smith, A. E., and Michel, B.,** Alteration of soybean complex lipid biosynthesis by *S*-ethyl dipropylthiocarbamate, *Plant Physiol.*, 60, 86, 1977.
119. **Wilkinson, R. E. and Ashley, D.,** EPTC induced modification of gibberellin biosynthesis, *Weed Sci.*, 27, 270, 1979.
120. **Wilkinson, R. E.,** Gibberellin precursor biosynthesis inhibition by EPTC and reversal by R-25788, *Pestic. Biochem. Physiol.*, 19, 321, 1983.
121. **Ezra, G., Gressel, J., and Flowers, H. M.,** Effects of the herbicide EPTC and the protectant DDCA on incorporation and distribution of (2-^{14}C) acetate into major lipid fractions of maize cell suspension cultures, *Pestic. Biochem. Physiol.*, 19, 225, 1983.
122. **Wilkinson, R. E. and Smith, A. E.,** Reversal of EPTC-induced fatty acid synthesis inhibition, *Weed Sci.*, 23, 98, 1975.
123. **Lay, M.-M. and Casida, J. E.,** Involvement of glutathione and glutathione-*s*-transferases in the action of dichloroacetamide antidotes for thiocarbamate herbicides, in *Chemistry and Action of Herbicide Antidotes*, Pallos, F. M. and Casida, J. E., Eds., Academic Press, New York, 1978, 151.
124. **Leavitt, J. R. C. and Penner, D.,** In vitro conjugation of glutathione and other thiols with acetanilide herbicides and EPTC sulfoxide and the action of the herbicide antidote R-25788, *J. Agric. Food Chem.*, 27, 533, 1979.
125. **Ezra, G. and Gressel, J.,** Rapid effects of a thiocarbamate herbicide and its dichloroacetamide protectant on macromolecular synthesis and glutathione levels in maize cell cultures, *Pestic. Biochem. Physiol.*, 17, 48, 1982.
126. **Ezra, G., Krochmal, E., and Gressel, J.,** Competition between a thiocarbamate herbicide and herbicide protectants at the level of uptake into maize cells in culture, *Pestic. Biochem. Physiol.*, 18, 107, 1982.

127. **Ezra, G., Flowers, H. M., and Gressel, J.,** Rapid multilevel interactions of a thiocarbamate herbicide and its protectant in maize cell cultures, in *Pesticide Chemistry: Human Welfare and the Environment*, Vol. 3, *Mode of Action, Metabolism and Toxicology*, Matsunaka, S., Hutson, D. H., and Murphy, S. D., Eds., Pergamon Press, Oxford, 1983, 1225.
128. **Muslih, R. K. and Linscott, D. L.,** Regulation of lipid synthesis in soybeans by two benzoic acid herbicides, *Plant Physiol.*, 60, 730, 1977.
129. **Mann, J. D. and Pu, M.,** Inhibition of lipid synthesis by certain herbicides, *Weed Sci.*, 16, 197, 1968.
130. **Hatzios, K. K. and Howe, C. M.,** Influence of the herbicides hexazinone and chlorsulfuron on the metabolism of isolated soybean leaf cells, *Pestic. Biochem. Physiol.*, 17, 207, 1982.
131. **Hatzios, K. K.,** Effects of sethoxydim on the metabolism of isolated leaf cells of soybean [*Glycine max* (L.) Merr.], *Plant Cell Rep.*, 1, 87, 1982.
132. **Hatzios, K. K. and Penner, D.,** Localizing the action of two thiadiazoyl herbicidal derivatives, *Pestic. Biochem. Physiol.*, 13, 237, 1980.
133. **Grenier, G. and Beaumont, G.,** Effects physiologiques de l'atrazine à doses sublétales sur *Lemna minor*. VII. Incorporation d'-acetate-1,2-(^{14}C) dans les groupes de lipides et leurs acides gras, *Physiol. Plant.*, 57, 477, 1983.
134. **Swain, T.,** Phenolics in the environment, in *Biochemistry of Plant Phenolics*, Swain, T., Harborne, J. B., and Van Sumere, C. F., Eds., Plenum Press, New York, 1979, chap. 19.
135. **Hedin, P. A., Ed.,** *Plant Resistance to Insects*, ACS Symp. Ser. 208, American Chemical Society, Washington, D.C., 1983.
136. **Bell, E. A.,** The physiological role(s) of secondary (natural) products, in *The Biochemistry of Plants, Vol. 7, Secondary Plant Products*, Stumpf, P. K. and Conn, E. E., Eds., Academic Press, New York, 1981, chap. 1.
137. **Jangaard, N. O.,** The characterization of phenylalanine ammonia-lyase from several plant species, *Phytochemistry*, 13, 1765, 1974.
138. **Jangaard, N. O.,** The effect of herbicides, plant growth regulators and other compounds on phenylalanine ammonia-lyase activity, *Phytochemistry*, 13, 1769, 1974.
139. **Hoagland, R. E. and Duke, S. O.,** Effects of herbicides on growth and extractable phenylalanine ammonia-lyase activity in light- and dark-grown soybean (*Glycine max*) seedlings, *Weed Sci.*, 29, 433, 1981.
140. **Amrhein, N. and Godeke, K. H.,** α-Aminooxy-β-phenylpropionic acid, a potent inhibitor of L-phenylalanine ammonia-lyase *in vitro* and *in vivo*, *Plant Sci. Lett.*, 8, 313, 1977.
141. **Amrhein, N.,** Novel inhibitors of phenylpropanoid metabolism in higher plants, in *Regulation of Secondary Product and Plant Hormone Metabolism*, Luckner, M. and Schrieber, K., Eds., Pergamon Press, Oxford, 1979, 173.
142. **James, D. J. and Davidson, A. W.,** Phenylalanine ammonia-lyase and the regulation of cell division in Jerusalem artichoke (*Helianthus tuberosis* L.) callus cultures, *Ann. Bot.*, 40, 957, 1976.
143. **Hoagland, R. E. and Duke, S. O.,** Relationships between phenylalanine ammonia-lyase activity and physiological responses of soybean (*Glycine max*) seedlings to herbicides, *Weed Sci.*, 31, 845, 1983.
144. **Duke, S. O. and Hoagland, R. E.,** Effects of glyphosate on metabolism of phenolic compounds, in *The Herbicide Glyphosate*, Grossbard, E. and Atkinson, D., Eds., Butterworths, Oxford, 1984, in press.
145. **Holländer, H. and Amrhein, N.,** The site of inhibition of the shikimate pathway by glyphosate. I. Inhibition by glyphosate of phenylpropanoid synthesis in buckwheat (*Fagopyrum esculentum* Moench), *Plant Physiol.*, 66, 823, 1980.
146. **Holliday, M. J. and Keen, N. T.,** The role of phytoalexins in the resistance of soybean leaves to bacteria: effect of glyphosate on glyceollin accumulation, *Phytopathology*, 72, 1470, 1982.
147. **Keen, N. T., Holliday, M. J., and Yoshikawa, M.,** Effects of glyphosate on glyceollin production and the expression of resistance to *Phytophthora megasperma* f. sp. *glycinea* in soybean, *Phytopathology*, 72, 1467, 1982.
148. **Moesta, P. and Grisebach, H.,** L-2-aminooxy-3-phenylpropionic acid inhibits phytoalexin accumulation in soybean with concomitant loss of resistance against *Phytophthora megasperma* f. sp. *glycinea*, *Physiol. Plant Pathol.*, 21, 65, 1982.
149. **Gross, G. G.,** Phenolic acids, in *The Biochemistry of Plants Vol. 7, Secondary Plant Products*, Stumpf, P. K. and Conn, E. E., Eds., Academic Press, New York, 1981, chap. 11.
150. **Amrhein, N., Holländer-Czytko, H., Leifeld, J., Schulz, A., Steinrucken, H. C., and Topp, H.,** Inhibition of the shikimate pathway by glyphosate, in *Proceedings of Journées Internationales d'Etudes et Assemblies du Groupe Polyphenols*, Boudet, A. M. and Ranjera, R., Eds., Bulletin De Liaison, Vol. 11, Toulouse, France, 1983, 21.
151. **Vincent, P. G. and Engelke, B. F.,** personal communication, November, 1983.
152. **Butt, V. S.,** Oxidases in aromatic metabolism, *Recent Adv. Phytochem.*, 12, 433, 1979.
153. **Duke, S. O. and Vaughn, K. C.,** Lack of involvement of polyphenol oxidase in ortho-hydroxylation of phenolic compounds in mung bean seedlings, *Physiol. Plant.*, 54, 381, 1982.

154. **Vaughn, K. C. and Duke, S. O.,** Function of polyphenol oxidase in higher plants, *Physiol. Plant.*, 60, 106, 1984.
155. **Cole, D. J., Dodge, A. D., and Caseley, J. C.,** Some biochemical effects of glyphosate on plant meristems, *J. Exp. Bot.*, 31, 1665, 1980.
156. **Lee, T. T.,** Mode of action of glyphosate in relation to metabolism of indole-3-acetic acid, *Physiol. Plant.*, 54, 289, 1982.
157. **Lee, T. T.,** Promotion of indole-3-acetic acid oxidation by glyphosate in tobacco callus tissue culture, *J. Plant Growth Regul.*, 1, 37, 1982.
158. **Baur, J. R.,** Effect of glyphosate on auxin transport in corn and cotton tissues, *Plant Physiol.*, 63, 882, 1979.
159. **Scarponi, L., Tafuri, F., and Perucci, P.,** Effects of certain herbicides on the oxidative decarboxylation of tryptophan by peroxidase, *Pestic. Sci.*, 11, 446, 1980.
160. **Harvey, B. M. R., Chang, F. Y., and Fletcher, R. H.,** Relationship between *S*-ethyl dipropylthiocarbamate injury and peroxidase activity in corn seedlings, *Can. J. Bot.*, 53, 225, 1975.
161. **Wilkinson, R. E.,** Physiological response of lipid components to thiocarbamates and antidotes, in *Chemistry and Action of Herbicide Antidotes*, Pallos, F. M. and Casida, J. E., Eds., Academic Press, New York, 1978, 85.
162. **Mayer, A. M. and Harel, E.,** Polyphenol oxidases in plants, *Phytochemistry*, 18, 193, 1979.
163. **Chaleff, R. S. and Mauvais, C. J.,** Acetolactate synthase is the site of action of two sulfonylurea herbicides in higher plants, *Science*, 224, 1443, 1984.
164. **Ray, T. B.,** Site of action of chlorsulfuron. Inhibition of valine and isoleucine biosynthesis in plants, *Plant Physiol.*, 75, 827, 1984.
165. **Shaner, D. L., Anderson, P. C., and Stidham, M. A.,** Imidazolinone herbicides are inhibitors of acetohydroxy acid synthase, *Plant Physiol.*, 75S, 50, 1984.
166. **Shaner, D. L.,** personal communication, January 1984.
167. **Mousdale, D. M. and Coggins, J. R.,** Purification and properties of 5-enolpyruvylshikimate-3-phosphate synthase from seedlings of *Pisum sativum* L., *Planta*, 180, 78, 1984.
168. **Rubin, J. L., Gaines, C. G., and Jensen, R. A.,** Glyphosate inhibition of 5-enolpyruvylshikimate-3-phosphate synthase from suspension-cultured cells of *Nicotiana silvestris*, *Plant Physiol.*, 75, 839, 1984.
169. **Cooley, M. E. and Foy, C. L.,** Effect of aromatic amino acids on the growth inhibition of inflated duckweed (*Lemna gibba* L.) by SC-0224, *Weed Sci. Soc. Am. Abstr.*, Weed Science Society of America, Champaign, Ill., 1984, 79.
170. **Sekizawa, Y. and Takematsu, T.,** How to discover new antibiotics for herbicidal use, in *Pesticide Chemistry. Human Welfare and the Environment*, Vol. 2, *Natural Products*, Miyamoto, J. and Kearney, P. C., Eds., Pergamon Press, New York, 1983, 261.
171. **Leason, M., Cunliffe, D., Parkin, D., Lea, P. J., and Miflin, D. J.,** Inhibition of pea glutamine synthetase by methionine sulphoximine, phosphinothricin and other glutamate analogues, *Phytochemistry*, 21, 855, 1982.
172. **Wild, A. and Manderscheid, R.,** The effect of phosphinothricin on the assimilation of ammonia in plants, *Z. Naturforsch.*, 39C, 500, 1984.
173. **Lambert, R. and Böger, P.,** Inhibition of carotenogenesis by substituted diphenyl ethers of the *m*-phenoxybenzamide type, *Pest. Biochem. Physiol.*, 20, 183, 1983.
174. **Sandmann, G., Clarke, I. E., Bramley, P. M., and Böger, P.,** Inhibition of phytoene desaturase-the mode of action of certain bleaching herbicides, *Z. Naturforsch.*, 39C, 443, 1984.
175. **Bramley, P. M., Clarke, I. E., Sandmann, G., and Böger, P.,** Inhibition of carotene biosynthesis in cell extracts of *Phycomyces blakeleeanus*, *Z. Naturforsch.*, 39C, 460, 1984.
176. **Sandmann, G., Reck, H., and Böger, P.,** Herbicidal mode of action on chlorophyll formation, *J. Agric. Food Chem.*, 32, 868, 1984.
177. **Kömives, T. and Casida, J. E.,** Diphenyl ether herbicides: effects of acifluorfen on phenylpropanoid biosynthesis and phenylalanine ammonia-lyase activity in spinach, *Pest. Biochem. Physiol.*, 18, 191, 1982.
178. **Kömives, T. and Casida, J. E.,** Acifluorfen increases the leaf content of phytoalexins and stress metabolites in several crops, *J. Agric. Food Chem.*, 31, 751, 1983.
179. **Suttle, J. C., Swanson, H. C., and Schreiner, D. R.,** Effect of chlorsulfuron on phenylpropanoid metabolism in sunflower seedlings, *J. Plant Growth Regul.*, 2, 137, 1983.
180. **Duke, S. O.,** Chemical manipulation of phenolic compounds biosynthesis in higher plants, in *The Chemistry of Allelopathy*, Thompson, A. L., Ed., ACS Symp. Ser., American Chemical Society, Washington, D.C., in press, 1984.

Chapter 5

HERBICIDE EFFECTS ON MEMBRANE FUNCTIONS

Nelson E. Balke

TABLE OF CONTENTS

I. INTRODUCTION

Membranes are of paramount importance for the function of cells. The truth of this statement is evident by the fact that it is difficult to find any significant phenomenon in cell biology that is not correlated to membrane function in some way. This is as true for single-celled organisms as it is for highly differentiated ones. For example, in bacteria, which lack internal membranes, the plasma membrane takes over many organellar functions such as electron transport, oxidative phosphorylation, and protein synthesis.

With membranes being of critical importance to the metabolism of cells, any chemical that perturbs the structure or function of membranes can be expected to harm a plant cell and possibly cause toxicity. Herbicides are especially likely to act upon membranes because most herbicides are more soluble in lipophilic than hydrophilic solvents, and membranes contain abundant lipids. Thus, many herbicides can partition into the membranes of plant cells and might be expected to disrupt membranes. Also, herbicides may bind directly to proteins or lipids located in membranes and thus alter the function of these constituents and, in turn, the membrane.

In this chapter, I will discuss the effects of herbicides on membranes in plant cells. Because other chapters in this book discuss effects of herbicides on physiological processes occurring in the major membranous organelles of plant cells, my discussion will be restricted to effects on the plasma membrane and tonoplast. I will discuss the variety of ways by which herbicides are known to affect membranes and will propose some additional areas that are worthy of investigation. Although I have attempted to review all the literature on this topic, it is impossible to cite each relevant publication. Rather, I will present the most pertinent historical and recent research on the subject after discussing the structure and function of the plasma membrane and the tonoplast.

II. PLASMA MEMBRANE AND TONOPLAST

The plasma membrane (plasmalemma) and tonoplast are the two membranes that confine the cytoplasm in a plant cell. As boundaries between cytoplasm and cell wall, and cytoplasm and vacuole, these two membranes separate plasmatic and nonplasmatic compartments in a cell.[1] Both the plasma membrane and the tonoplast are important for controlling what chemical substances enter and leave the cytoplasm. The other functions of these two membranes relate to their primary function as barriers. Excellent reviews regarding the morphology, purification, composition, and function of the plasma membrane[2,3] and tonoplast (vacuoles)[4-6] are available. I will present a brief discussion of these topics because they are important for understanding how herbicides affect membrane functions; the above reviews should be consulted for details.

A. Morphology, Structure, and Composition

Both the plasma membrane and the tonoplast appear as tripartite dark-light-dark structures in transmission electron micrographs,[2,7] and average about 100 Å in thickness.[2,6] The two membranes react differently to some of the electron-dense stains used to visualize them.[7] The phosphotungstic acid-chromic acid staining procedure will preferentially stain the plasma membrane and not the tonoplast.[8,9] However, strict adherence to the technical protocol is required or else membranes in addition to the plasma membrane can be stained.[10] Freeze-fracture electron microscopy has revealed particles embedded in a matrix in the fracture faces of plasma membranes[2] and tonoplasts.[11] The particles are presumed to be proteins embedded in the lipid matrix. The particles are often distributed asymmetrically between the two sides of the membranes.[11]

Characterization of the composition and function of the plasma membrane and tonoplast

depends upon the purification of each membrane separately from other cellular components. Because of the various organellar membranes present in plant cells and the need to use harsh procedures to break the cell wall and release the membranes from the cell, it has not been possible to purify to homogeneity either the plasma membrane or the tonoplast from plant tissues. However, procedures have been developed which result in partial purification of the plasma membrane[3] and tonoplast[6] from tissues and protoplasts. A new technique using aqueous polymer two-phase systems to separate membranes based on their surface properties[12] shows promise for producing more homogeneous preparations of membrane vesicles.[13]

Chemically, both the plasma membrane and tonoplast consist of lipids, proteins, and carbohydrates. Phospholipids, glycolipids, sterols, and neutral lipids comprise the lipids of both membranes. The plasma membrane contains the higher sterol:phospholipid ratio, whereas the amount of carbohydrate is highest in the tonoplast. Protein comprises 35 to 40% of the weight of both membranes, but in general the plasma membrane contains more protein than does the tonoplast. The proteins are variously distributed in the membranes; some of them are peripheral, whereas others are integral proteins. The carbohydrates are present in the form of glycolipids, glycoproteins, and perhaps glucan polymers (in or on the plasma membrane).

The interaction and basic assembly of these membrane constituents are very important for the functions of the membranes. Current models for all membranes describe a fluid, lipid bilayer with proteins embedded in the bilayer (Figure 1). Interactions between lipids and proteins in the lipid matrix are essential for the expression of enzymatic activity by the proteins. Removal of lipids from integral proteins decreases their enzymatic activity, and addition of phospholipids to the extracted proteins restores activity.

The presence of enzymatic proteins in the plasma membrane and tonoplast are crucial to several functions of these membranes. The most important enzymes for solute transport are ATP phosphohydrolases (ATPases).[2,14,15] These enzymes hydrolyze cytoplasmic ATP to ADP and Pi; the released energy is believed to energize the movement of mineral ions and organic molecules across the plasma membrane and tonoplast. The majority of these ATPases require Mg^{2+} for activity and are stimulated by monovalent cations (especially K^+), however, some of them are stimulated by anions. It is not known if a particular membrane contains more than one ATPase. Possible mechanisms of operation of ATPases in solute transport are described later (Section II.B).

Several other enzymatic activities are associated with the plasma membrane. Glycosyltransferases that transfer sugar units from a nucleotide to proteins and lipids (and perhaps sterols) to form glycoproteins and glycolipids (and perhaps glycosterols) are located on some plasma membranes. A glucan synthetase, which synthesizes cell wall polymers, is especially prevalent. Tissues undergoing abscission and certain dicots contain cellulase activity in their plasma membranes. These enzymes may function in the catabolism of cellulose in cell walls. A protein that specifically binds the auxin-transport inhibitor, *N*-1-napthylphthalamic acid, is located in the plasma membrane. Also, proteins that bind pathogenic toxins such as helminthosporoside, produced by *Helminthosporium sacchari*, and fusicoccin, produced by *Fusicoccum amygdali*, exist in plasma membranes of susceptible plants.

Localization of enzymatic proteins in addition to ATPase in the tonoplast is an area of active research. Because methods to purify the tonoplast in large amounts have become available only recently, identification of tonoplast-associated proteins has lagged behind similar research with plasma membranes and other organellar membranes.

B. Functions

All known functions of the plasma membrane and tonoplast are related to their being barriers between the cytoplasm and nonplasmatic space (cell wall or vacuole).[1] Thus, the tonoplast can be expected to possess many functions in common with the plasma membrane

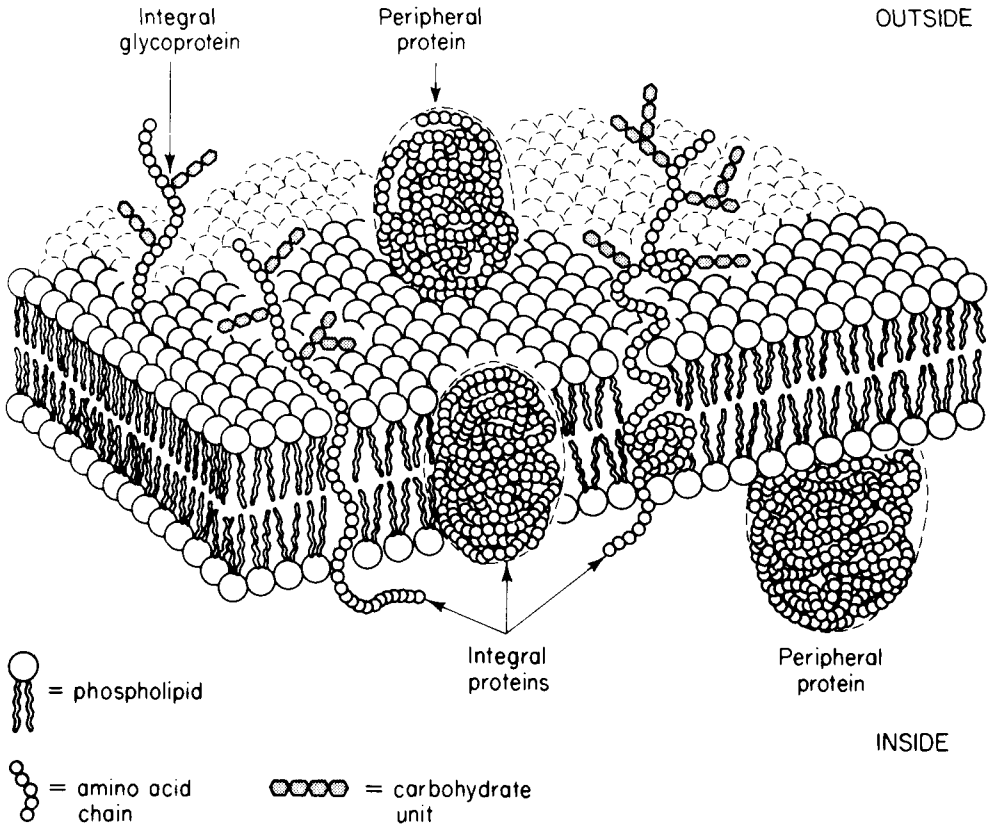

FIGURE 1. Fluid mosaic model of the plasma membrane of cells. Proteins are asymetrically distributed in the lipid bilayer. Carbohydrate units may be attached to protein or lipid but only on the outer surface. The tonoplast structure is similar except glycoproteins may not be present. (From *Biology of the Cell*, 2nd ed., by Stephen L. Wolfe. ©1981 by Wadsworth, Inc. Reprinted by permission of Wadsworth Publishing Company, Belmont, California, 94002.)

in addition to serving as a barrier. These functions include transport of solutes across the barrier, site for enzymatic activities, and receptor of hormonal and environmental stimuli. Active transport of mineral ions into the cytoplasm and the vacuole is of major importance to cells because: (1) a proper ionic environment must be maintained for metabolism and (2) high ionic concentration is required to produce high osmotic pressure, which drives cell enlargement, stomatal movement, and leaf movement.

Selective transport of both inorganic mineral ions and organic molecules into and out of the cytoplasm across membrane barriers is a major function of both the plasma membrane and the tonoplast. This transport results in accumulation of metabolically essential organic molecules and minerals in the cytoplasm, and sequestration of toxic molecules, excess organic constituents, and minerals in the vacuole. The fact that both membranes are hydrophobic, lipid bilayers necessitates the presence of various "carrier" molecules in the bilayers to facilitate movement of aqueous solutes through the barrier.

Several commonly-agreed-upon features of membrane transport are worthy of enumeration:[16]

1. Intracellular concentrations of anions, cations, and organic metabolites exceed their extracellular concentrations.
2. An electrical potential difference (PD) is maintained across the plasma membrane (and

perhaps the tonoplast) so that the cytoplasm is negatively charged as compared to the cell wall and vacuole. Part of this potential (diffusion potential) arises from different permeabilities of cations and anions through the membranes, but a major portion (electrogenic potential) is produced by an electrogenic pump. Ions diffuse through the membranes in response to both this PD and their concentration gradients across the membrane.

3. The electrogenic pump requires metabolic energy; thus, ion transport requires energy.
4. Plant cells extrude H^+ out of the cytoplasm across the plasma membrane and tonoplast.
5. The free energy of H^+ is higher outside than inside the cytoplasm. This free energy gradient (proton motive force) is used to drive the transport of certain mineral ions and organic solutes.

Based on these principles and the presence of ATPases in the plasma membrane and tonoplast, a model that describes the mechanisms of mineral and organic ion transport across the plasma membrane and tonoplast is proposed (Figure 2). This model is a compilation of previous models.[14,16,17] The central facet of this model is the presence of H^+-transporting ATPases in the plasma membrane and the tonoplast. Although these ATPase activities may not represent the same enzyme, they both transport H^+ out of the cytoplasm. The H^+ efflux at the plasma membrane produces an electrogenic potential. This PD plus the proton gradient supplies the driving force for movement of positively charged solutes into the cytoplasm from the cell wall. K^+ may move on the ATPase or on a separate carrier. Divalent cations may move by a carrier or may merely diffuse into the cytoplasm in response to the PD. Anions may be co-transported with H^+ into the cytoplasm or be transported in exchange for OH^- (HCO_3^-) movement out of the cytoplasm (counter-transport). Hexoses, and acidic and neutral amino acids are co-transported with H^+ on separate carriers. The basic amino acids are transported on a uniporter without balancing movements of H^+ or OH^- across the plasma membrane. Similar carriers are believed to move these solutes across the tonoplast into the vacuole, however, the details of those mechanisms are even less well understood than for the plasma membrane.

Another major function of the plasma membrane and tonoplast is reception of hormonal stimuli. Although hypotheses for the mechanisms of action of all the known hormones include actions on membranes,[18] the best evidence is available for IAA. IAA very quickly accelerates the extrusion of H^+ from tissue (especially coleoptiles) and there is some evidence that auxin-binding sites exist on the plasma membrane of cells.[19] Thus, auxin may stimulate the plasma membrane-associated ATPase that energizes H^+ extrusion and solute transport. Such hormone responsive sites might exist on the tonoplast as well. In addition, evidence is available in support of the transport of gibberellin A,[20] and IAA[21] into vacuoles. Thus, the tonoplast may regulate the cytoplasmic concentration of hormones.

The environmental stimuli of light and temperature are sensed by membranes. Both phytochrome[22,23] and the flavin-*b*-type-cytochrome complex, which absorbs blue light,[24,25] bind to or exist in plasma membranes. These photoreceptors are believed to release secondary messengers such as Ca^{2+} into the cytoplasm or induce changes in the enzymatic or the physical properties of the membrane. Also, membranes respond to temperature. Membrane lipids can undergo liquid-crystalline to gel phase transitions in response to temperature changes within the range of temperatures that support plant growth.[26] Because the fluidity of membranes is essential for life processes,[27] alteration of fluidity by changes in temperature can affect membrane functions. In response to temperature, changes can occur in membrane permeability, activation energy of membrane-associated enzymes, and membrane structure. Thus, the regulation of membrane functions by environmental stimuli may be as important as their regulation by hormones.

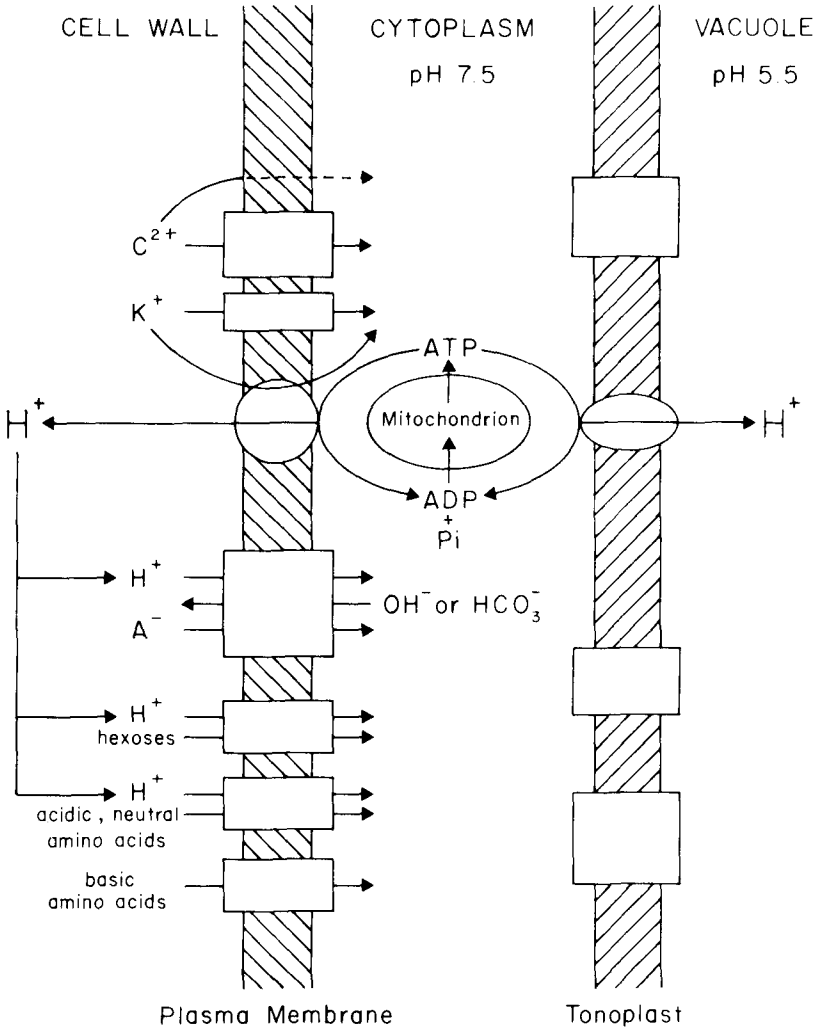

FIGURE 2. Schematic representation of current models describing possible mechanisms for coupling of energy to mineral, hexose, and amino acid absorption into plant cells. ATP produced in mitochondria is used by the plasma membrane and tonoplast to pump H^+ out of the cytoplasm. The resultant H^+ gradient and electrogenic transmembrane potential provide the driving force for absorption of various solutes. K^+ absorption across the plasma membrane may be mediated by the ATPase or a separate carrier protein. Divalent cation (C^{2+}) absorption may be via a carrier or by passive diffusion (represented by the broken line). Monovalent anion (A^-) absorption may be by co-transport with H^+ or by counter-transport with OH (or HCO_3^-). Hexoses and acidic or neutral amino acids move by co-transport on separate carriers, whereas basic amino acids move without an accompanying H^+ or OH^- (like K^+). Similar carriers are believed to transport solutes at the tonoplast, but detailed models have not been proposed. This model is a compilation of previous models.[14,16,17]

III. EFFECTS OF HERBICIDES ON MEMBRANE FUNCTIONS

A. Alteration of Membrane Permeability

One of the major ways that herbicides can affect membrane function is by altering the semipermeability of the membrane. Because the primary role of membranes in cells is to prevent diffusive movement of solutes, metabolites, and enzymes out of the cytoplasm,

disruption of membrane permeability may have significant and ultimately toxic effects on plant cells. Usually changes in membrane permeability are measured by determining the leakage of materials out of the cytoplasm in the presence of the perturbing chemical. However, the leakage of materials into the cytoplasm from either the cell wall or the vacuole may be as important.

Compiled in Table 1 are the herbicides that have been reported to increase the leakage of various solutes out of plant cells. This list contains all the major families of herbicides. In several classes, more chemicals could be cited, but for brevity only certain ones are included. Many different plant systems have been investigated, ranging from intact tissue to isolated protoplasts. Also, leakage of a variety of solutes (ions, pigments, organic metabolites) has been studied.

Leakage studies have employed primarily four techniques. Betacyanin leakage was the first method used to study leakage. However, this spectrophotometric technique is restricted to tissues that contain the pigment (usually beet storage roots are used) and is neither very sophisticated nor sensitive. For the second technique, electrolyte leakage is measured by changes in conductivity of solution bathing the tissue. This is the most common method but does not identify the specific ions leaking from the tissue. Radiolabel leakage, the third method, is very sensitive and can be selective, but the tissue must be "loaded" with the label prior to measuring the leakage. Either radiolabeled organic compounds or minerals can be used. For the former, often $^{14}CO_2$ is fixed photosynthetically by the tissue and thus ^{14}C is dispersed throughout many compounds. Leakage of particular types of ^{14}C-molecules can be followed if the eluted compounds are separated. On the other hand, leakage of monovalent or divalent metal ions (e.g., $^{86}Rb^+$ or $^{45}Ca^{2+}$) or specific labeled organic compounds can be monitored after introduction of these compounds into cells or tissues by a variety of methods. Use of $^{86}Rb^+$ is advantageous because it is absorbed rapidly by plant cells yet is not incorporated into organic molecules. Thus, unlike many minerals and most organic solutes, metabolism of Rb^+ does not influence either the absorption or the leakage of Rb^+. The last method, fluorescein leakage, is a relatively new technique. Tissue is loaded with fluorescein diacetate, which passively diffuses across the plasma membrane and is hydrolyzed to fluorescein by esterases in the cytoplasm. Unlike fluorescein diacetate, fluorescein is fluorescent and does not readily diffuse across membranes. Thus, the fluorometric detection of fluorescein leakage into extracellular solutions is a good, sensitive measure of membrane permeability.

Few studies (Table 1) attempted to determine if membrane permeability to more than one solute was increased. Paraquat, diquat, dinoseb, and oxyfluorfen increased leakage of both electrolytes and amino acids from bean leaves[36] as did dinoseb with wheat roots.[41] Oryzalin increased the leakage of predominantly the neutral products (sugars) from cotton mesophyll cells, although more anionic (organic acids and sugar phosphates) and cationic (amino acids) products also leaked from the cells.[40] In dichlone-treated *Chlorella* cells, practically all the ^{14}C-sucrose, alanine, glutamine, serine, and glycine leaked out, whereas glutamic, aspartic, succinic, and fumaric acids only partially leaked and no lipids leaked from the cells.[47] Acifluorfen-methyl increased the leakage of $^{86}Rb^+$, $^{36}Cl^-$, $^{45}Ca^{2+}$, 3-*O*-methyl-^{14}C-glucose, and ^{14}C-methylamine$^+$ from cucumber cotyledons.[43] For most of these herbicides that increased leakage, it is not known if permeability to all solutes was increased or if the damage was selective. Experiments to determine the selectivity of leakage would be valuable because the results would indicate the extent of membrane damage; e.g., larger molecules such as proteins would leak from cells only if a herbicide produced relatively large holes in the membrane.

Although most leakage studies only used one plant species, several compared two or more species. The extent of ^{32}P-solute leakage from plant roots in the presence of metolachlor was greater for onion than cotton or cucumber and was least for soybeans and corn.[30] Thus,

Table 1
EFFECTS OF HERBICIDES ON MEMBRANE PERMEABILITY AS MEASURED BY INCREASED LEAKAGE OF VARIOUS SOLUTES FROM PLANT TISSUES, CELLS, AND PROTOPLASTS

HERBICIDE FAMILY Chemical	Plant			Herbicide Treatment		
	Species[a]	System[b]	Leakage assay	Conc.	Time[c]	Ref.
ALIPHATICS						
Dalapon	LM	F	Electrolytes	100 μ*M*	12 h	28
AMIDES						
Alachlor	AS	MP	^{14}C-solutes	1 m*M*	1 h	29
Metolachlor	AC	R	^{32}P-solutes	100 μ*M*	3 d	30
Propanil	BV	SRD	Betacyanin	400 μ*M*	2 d	31
BENZONITRILES						
Dichlobenil	BV	SRD	Betacyanin	100 μ*M*	8 h	32
Ioxynil	NT	SCC	Fluorescein and electrolytes	100 μ*M*	2 h	33
	AP	SCC	Fluorescein	100 μ*M*	1 h	34
BIPYRIDILIUMS						
Paraquat	PV	L	Electrolytes	400 μ*M*	1 d	35
	PV	L	Electrolytes and amino acids	1 m*M*	3 h	36
	LM	F	Electrolytes	9 μ*M*	3 h	37
	LU	CoS	K^+	100 m*M*	3 h	38
CARBAMATES						
Barban	AS	MP	^{14}C-solutes	1 m*M*	1 h	29
	NT	SCC	Fluorescein and electrolytes	100 μ*M*	2 h	33
DINITROANILINES						
Dinitramine	GM	R	$^{86}Rb^+$	620 μ*M*	30 min	39
Oryzalin	GH	MC	^{14}C-solutes	100 μ*M*	1 h	40
DINITROPHENOLS						
Dinoseb	TA	R	Electrolytes	10 μ*M*	1 h	41
	PV	L	Electrolytes and amino acids	100 μ*M*	12 h	36
	LM	F	Electrolytes	1 μ*M*	12 h	28
	AP	SCC	Fluorescein	100 μ*M*	1 h	34
DNOC	AP	SCC	Fluorescein	100 μ*M*	1 h	34
DIPHENOXY-CARBOXYLIC ACIDS						
Diclofop-methyl	ZM	RT	Amino acids	50 μ*M*	12 h	40
	AF	L	Electrolytes	3 m*M*	12 h	42
DIPHENYL ETHERS						
Acifluorfen-methyl	CS	Co	$^{86}Rb^{+d}$	10 n*M*	3 h	43
Fluorodifen	AP	SCC	Fluorescein	100 μ*M*	1 h	34
PHENOLS						
PCP	NT	SCC	Fluorescein and electrolytes	1 μ*M*	2 h	33
PHENOXYS						
2,4-D	LM	F	Electrolytes	10 μ*M*	12 h	28
	PR	NS	Conductance	113 μ*M*	2 h	44
2,4-5-T	NT	SCC	Fluorescein and electrolytes	10 μ*M*	2 h	33
THIOCARBAMATES						
EPTC	BV	SRD	Betacyanin	950 μ*M* 2 m*M*	24 h 1 h	45
TRIAZINES						
Simazine	LM	F	Electrolytes	10 μ*M*	12 h	28

Table 1 (continued)
EFFECTS OF HERBICIDES ON MEMBRANE PERMEABILITY AS MEASURED BY INCREASED LEAKAGE OF VARIOUS SOLUTES FROM PLANT TISSUES, CELLS, AND PROTOPLASTS

HERBICIDE FAMILY Chemical	Plant		Leakage assay	Herbicide Treatment		Ref.
	Species[a]	System[b]		Conc.	Time[c]	
UREAS						
Linuron	LM	F	Electrolytes	10 μ*M*	12 h	28
Monuron	AP	SCC	Fluorescein	100 μ*M*	1 h	46
UNCLASSIFIED						
Amitrole	LM	F	Electrolytes	10 μ*M*	12 h	28
Dichlone	CP	C	^{14}C-solutes	3 μ*M*	15 min	47
Difenzoquat	TA	L	Electrolytes	28 m*M*	1 d	48
Glyphosate	LM	F	Electrolytes	100 μ*M*	12 h	28

[a] Abbreviations of plant species: AC, *Allium cepa* L.; AF, *Avena fatua* L.; AP, *Acer pseudoplatanus*; AS, *Avena sativa* L.; BV, *Beta vulgaris* L.; CP, *Chlorella pyrenoidosa*; CS, *Cucumis sativus* L.; GH, *Gossypium hirsutum* L.; GM, *Glycine max* (L.) Merr.; LM, *Lemna minor*; LU, *Linium usitatissimum* L.; NT, *Nicotiana tabacum* L.; PR, *Pinus radiata* D. Don; PV, *Phaseolus vulgaris* L.; TA, *Triticum aestivum* L.; ZM, *Zea mays* L.

[b] Abbreviations of plant systems: C, cells; Co, cotyledons; CoS, cotyledon slices; F, fronds; L, leaves; MC, mesophyll cells; MP, mesophyll protoplasts; NS, needle slices; R, roots; RT, root tips; SCC, suspension culture cells; SRD, storage root discs.

[c] Time represents either the duration of the assay or the minimal time to elicit the response. Abbreviations: d, day; h, hour; min, minute.

[d] Leakage of $^{36}Cl^-$, $^{45}Ca^{2+}$, 3-*O*-methyl-^{14}C-glucose and ^{14}C-methylamine$^+$ measured also.

susceptibility of the species to metolachlor-induced leakage correlated with susceptibility to phytotoxicity. The same correlation was reported for leakage induced by diclofop-methyl in wild oats and barley (susceptible) and wheat (tolerant).[42] Oryzalin-induced leakage was greater in cotton than in soybean mesophyll cells;[40] both of these crops are resistant to oryzalin in the field. More comparative experiments are needed to determine if leakage of solutes is a good physiological test for selectivity of herbicide phytotoxicity.

With most of the leakage studies (Table 1), leakage across the plasma membrane cannot be separated from leakage across the tonoplast. The intracellular location of the permeating solute must be known in order to differentiate leakage across the two membranes. Because betacyanin is compartmentalized in the vacuole, leakage of this pigment infers that permeability of both the plasma membrane and the tonoplast is increased. Mineral ions, most ^{14}C-solutes, and fluorescein are distributed in both the cytoplasm and the vacuole (see Wagner[5] and references therein). Thus, increased leakage of these solutes does not separate leakage across the two membranes either. Unfortunately, the technique of compartmental analysis[49] cannot be used to separate leakage across the two membranes because permeability of the tonoplast must be increased before any significant increase in $^{86}Rb^+$ leakage is observed.[50] Thus, the situation is similar to betacyanin leakage. In all the systems, the permeability of the plasma membrane had to be increased or else the solute would not have leaked out of the cell.

The extent of leakage across the tonoplast can be inferred from only a few of the studies. In the presence of alachlor or barban, oat protoplasts lost about 80% of the ^{14}C-solutes produced during 1 h of photosynthesis in $^{14}CO_2$.[29] Oryzalin caused the leakage of 63% of ^{14}C-solutes from cotton mesophyll cells[40] and dinitramine caused all $^{86}Rb^+$ to leak from soybean roots.[39] The large extent of leakage in these three instances strongly suggests that

the solutes leaked from the vacuole as well as the cytoplasm. Although diclofop-methyl increased the leakage of amino compounds from maize root tips, at most 3% of the total amino compounds diffused out of the tissue in 12 h. This may indicate that permeability of the plasma membrane but not the tonoplast was increased. Definitive experiments to measure leakage across these two membranes separately are now possible. One technique is to measure leakage of $^{86}Rb^+$ from tissue or cells (the latter are preferable) loaded with $^{86}Rb^+$ for short periods of time (less than 30 min) so that radiolabel is primarily in the cytoplasm and not the vacuole.[49] In this system, increased leakage in the presence of a herbicide would indicate that permeability of the plasma membrane has been increased. Alternatively, now that intact vacuoles[6] as well as protoplasts[6] can be isolated, the influence of herbicides on the permeabilities of tonoplast and plasma membrane can be addressed directly.

Although these experiments have shown that herbicides can increase membrane permeability, in no instance do they prove that increased leakage is a primary effect. In most instances 1 h or longer was the assay time (Table 1). In this period of time many intracellular metabolic processes were most likely inhibited (see Chapters 1, 2, and 4). One result of inhibited metabolism would be the loss of membrane integrity[51] in a manner similar to what happens during natural senescence of plant tissues.[52] Such disorganization of membranes by herbicides has been described in ultrastructural studies and reviewed by Anderson and Thomson[53] and Linck[54] (also see Chapter 3). Speed is often used as a criterion for the direct effect of a compound on the plasma membrane. However, metabolism can be affected very rapidly also, especially in isolated cells or protoplasts where diffusion of the herbicide into the cytoplasm can be very rapid.[55]

In addition to increasing membrane permeability, at least two herbicides have been shown to decrease leakage of solutes. Magalhães and Ashton[56] reported that dicamba decreased electrolyte leakage from *Cyperus rotundus* L. leaves. Recently Blein[46] reported that the substituted urea benzthiazuron decreased fluorescein leakage from *Acer pseudoplatanus* cells. Also, there are reports that some of the herbicides in Table 1 did not increase membrane permeability in certain systems (e.g., atrazine, metolachlor, paraquat, and nitrofen in suspension culture cells of *Nicotiana tabacum*[33]). These contradictory findings may have resulted from different herbicide concentrations, times of exposure, plant species, or plant systems being used in the experiments.

B. Inhibition of Transmembrane Transport

Transport of solutes across the membrane barrier is the second major function of both the plasma membrane and the tonoplast (see Figure 2). In fact, it is because membranes are barriers to hydrophilic solutes, that mechanisms must exist for the transport of selected solutes across the barrier. Transmembrane transport is essential for maintaining the proper concentrations of mineral ions and organic solutes in the cytoplasm. Thus, transport of organic metabolites and hormones is as important as the transport of inorganic ions. Herbicides can inhibit transmembrane transport of both inorganic and organic moieties.

1. Transport of Mineral Ions

Alteration in the mineral content of plants treated with herbicides was the first indication that mineral ion transport can be affected by herbicides. This subject was reviewed in 1976 by Ashton and Bayer[57] and will be discussed only briefly here. Although numerous herbicides can affect the mineral content of plants, few conclusive statements can be made regarding the mechanisms involved because there are many possible causes for the phenomenon. Usually changes in mineral content are measured many hours or even days following herbicide application. Thus, changes in mineral content may represent secondary or tertiary effects of a herbicide on the senescing plant. Alterations in mineral content can be caused by herbicides affecting mineral absorption by roots, transpiration and thus movement of minerals through-

out the plant, and rate of growth. The last point especially relates to mineral content because a change in growth rate in concert with no change in total uptake of minerals will result in a change in the concentration of minerals in the plant.[57] Thus, it is really impossible to conclude that herbicide-induced changes in the mineral content of plants result solely from effects on the mineral absorption process in plant cells. Nonetheless, changes in mineral content have been reported after treatment of plants for long periods of time (greater than 2 h) with MCPA, fenuron, alachlor, and chlorpropham;[58] metobromuron and chlorbufam;[59] and napropamide[60] in intact plants[57,58] and tissue cultures.[59] In addition, diclofop-methyl[61] and trifluralin[62,63] decreased the mineral content of plants by inhibiting root growth.

Absorption of minerals over short time periods (minutes to a few hours) is much more revealing in regard to the effects of herbicides on mineral absorption. If inhibition of absorption is a primary mechanism of action of herbicides, absorption should be inhibited very quickly by the chemical because it contacts the plasma membrane very quickly (especially in roots, which lack a cuticle), and the plasma membrane is the site of active mineral transport into cells. In Table 2 are compiled the herbicides that inhibit mineral absorption in 4 h or less. Absorption was measured for 1 h or less in most of these studies. Although short time-course studies do not prove that mineral absorption is inhibited directly, such studies eliminate some of the interpretational problems associated with changes occurring after long periods of herbicide treatment.

Almost all the major classes of herbicides inhibit mineral absorption in some plant species (Table 2). Most of the herbicides that increased membrane permeability (Table 1) inhibited mineral absorption (Table 2). Notable absences are benzonitriles, bypyridiliums, carbamates, and dinitroanilines. However, because these herbicides increased membrane permeability at concentrations of 100 μM or less and times of 1 h or less, they will probably also inhibit mineral absorption rapidly, because a cell must maintain membrane semipermeability to accumulate minerals. Because of the wide diversity of experimental conditions, it is difficult to conclude that particular classes of herbicides are more inhibitory than other classes. Experiments with many classes of herbicides need to be performed in one system to answer the question of relative inhibitory capacity among herbicides.

Although a wide range of monocot and dicot plant species were used for these studies, most of the research has been done with excised roots as the experimental system. This system is very good for these studies because roots are the plant organs that naturally absorb minerals from the soil environment. In addition, the use of excised tissue allows elimination of translocation from the absorption process. Translocation of minerals out of intact roots is a major interpretational obstacle for absorption studies with intact plants because the translocation influences the retention of absorbed minerals by the root. In addition to excised roots; leaf discs, suspension culture cells, and isolated mesophyll cells have been used to measure mineral absorption in the absence of translocation.

Herbicides inhibited the absorption of many different mineral ions (Table 2). Absorption of monovalent cations (K^+, Rb^+) was inhibited by most of the herbicide classes tested. Absorption of divalent cations (Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}) was not reported to be affected in most instances. However, it is questionable whether plant cells actively absorb divalent cations.[16] Herbicides inhibited the absorption of mono-, di-, and trivalent anions. On thermodynamic grounds, anion transport is against the electrochemical gradient in plant cells. Therefore, it is the active absorption of anions that is inhibited by herbicides. Herbicides inhibit absorption of both ions that are (SO_4^{2-}, PO_4^{3-}, NO_3^-, NH_4^+) and are not (K^+, Cl^-) incorporated into organic molecules in cells. Therefore, one can conclude that at least for some of the ions, their absorption and not their utilization is inhibited by herbicides.

Only a few studies have dealt with the relative inhibition of the absorption of particular ions in a single system. Alachlor inhibited Cl^- absorption more than K^+(Rb^+) absorption by excised oat roots.[67] However, the related amide propanil inhibited K^+(Rb^+) and PO_4^{3-}

Table 2
INHIBITION OF MINERAL ION ABSORPTION BY HERBICIDES

HERBICIDE FAMILY Chemical	Plant			Herbicide treatment		
	Species[a]	System[b]	Mineral ion	Conc.[c]	Time[d]	Ref.
ALIPHATICS						
Dalapon	ZM	ER[f]	PO_4^{3-}	5 m*M*	12/3 h	64
	BR,HV,OS,PV	ER	SO_4^{2-}	100 μ*M*	20 min	65
AMIDES						
Alachlor	AS	ER	K^+	100 μ*M*	"Brief"	66
	AS	ER	$K^+(Rb^+)$, Cl	100 μ*M*	1 h	67
				1 μ*M*	6 or 12/1 h	
Propanil						
(Synpran N[®])[e]	OS	ER	$K^+(Rb^+)$, PO_4^{3-}	100 μ*M*	10 min	68
	BR,HV,OS,PV	ER	SO_4^{2-}	100 μ*M*	20 min	65
DINITROPHENOLS						
Dinoseb	LE	LD	PO_4^{3-}	1 μ*M*	30 min	69
DIPHENOXY-CARBOXYLIC ACIDS						
Diclofop-methyl[e]	ZM	ER	$K^+(Rb^+)$	1 p*M*[g]	15/45 min	70
				100 μ*M*	15/45 min	
DIPHENYL ETHERS						
Nitrofen	AP	SCC	$K^+(Rb^+)$	100 μ*M*	1 h	34
PHENOXYS						
2,4-D	TA	ER	$K^+(Rb^+)$, PO_4^{3-}, NO_3^-, NH_4^+	10 μ*M*	1 h	71, 72
	OS	ER	$K^+(Rb^+)$	2 μ*M*	1-72/1 h	73
	OS	ER	$K^+(Rb^+)$, NH_4^+, NO_3^-	10 μ*M*	1 h	74, 75
	ZM	ER	K^+, Cl	10 μ*M*	75/40 min	76
MCPA	VF	ERT	PO_4^{3-}	2 m*M*	1 h	77
				200 μ*M*	3 h	
	OS	ER	Rb^+	100 μ*M*	15 min	74
THIOCARBAMATES						
Molinate	BR,HV,OS,PV	ER	SO_4^{2-}	100 μ*M*	20 min	65
TRIAZINES						
Atrazine	HV	RIP	K^+	33 μ*M*	1 h	78
	HV,TA,ZM	ER	SO_4^{2-}	130 μ*M*	1 h	79
	BR,HV,OS,PV	ER	SO_4^{2-}	100 μ*M*	20 min	65
	AS,PS,ZM	ER	K^+	140 μ*M*	2 h	80
COMBINATIONS						
Propanil + 2,4,5-T	OS	ER	$K^+(Rb^+)$, PO_4^{3-}	79 + 21 μ*M*	60 or 80 min	81
(Synpran 111[®])[e]			NO_3^-, NH_4^+			
UNCLASSIFIED						
Chlorfenpropmethyl[e]	AF	RIP	PO_4^{3-}	5.4 m*M*[h]	2/4 h	82
Glyphosate	PV	MC	$K^+(Rb^+)$, PO_4^{3-}	1 m*M*	90 min	83

[a] Abbreviations of plant species: AP, *Acer pseudoplatanus;* AS, *Avena sativa* L.; AF, *Avena fatua* L.; BR, *Brassica rapa* L.; HV, *Hordeum vulgare* L.; LE, *Lycopersicon esculentum* Mill.; OS, *Oryza sativa* L.; PS, *Pisum sativum* L.; PV, *Phaseolus vulgaris* L.; TA, *Triticum aestivum* L.; VF, *Vicia faba* L.; ZM, *Zea mays* L.

[b] Abbreviations of plant systems: ER, excised roots; ERT, excised root tips; LD, leaf discs; MC, mesophyll cells; RIP, roots of intact plants; SCC, suspension culture cells.

[c] Herbicide concentration causing effect at time stated.

[d] Pretreatment/absorption times. If only one time is given, there was no pretreatment. Abbreviations: h, hour; min, minute.

[e] Formulated product used.

[f] Roots excised after pretreatment of roots on intact plant.

[g] Absorption stimulated.

[h] Foliar application.

absorption to the same extent in excised rice roots.[68] Glyphosate inhibited K^+ (Rb^+) and PO_4^{3-} absorption equally in isolated bean mesophyll cells.[83] A mixture of propanil and 2,4,5-T (Synpran III®) inhibited absorption in excised rice roots in the order K^+ (Rb^+) > NO_3^- > NH_4^+ = PO_4^{3-}, and the ranking of inhibition by 2,4-D in excised wheat roots was NO_3^- > K^+ (Rb^+) > NH_4^+ > PO_4^{3-}.[72]

As in all mechanism-of-action research, interaction of herbicide concentration and absorption time is important when investigating the inhibition of mineral absorption by herbicides. The studies in Table 2 usually used herbicide concentrations less than or equal to 100 μM. In a few instances, very low concentrations of herbicides stimulated instead of inhibited mineral absorption.[34,70,73] Millimolar concentrations were tested in a few instances and those studies must be questioned because it is questionable if the herbicide concentration ever reaches these levels in cells in the field. On-the-other-hand, millimolar concentrations might be reached in particular cellular organelles if a herbicide were selectively accumulated by that organelle. As mentioned previously, the 1-h absorption time was most common (Table 2). However, several papers looked at time courses from 0 through several hours.[67-69] Time course measurements are valuable because they show how rapidly inhibition takes place and if the degree of inhibition changes with time of absorption. Also, comparisons of various herbicide concentrations with time courses are advantageous. For example, using this approach, the I_{50} for $^{32}PO_4^{3-}$ accumulation by tomato leaf discs decreased with increasing absorption time.[69]

A few studies determined the relationship between inhibition of absorption and phytotoxicity for tolerant and susceptible plants. This correlation was very good for dalapon, molinate, propanil, atrazine, and chlorfenprop-methyl.[65,82] Relative inhibition of SO_4^{2-} absorption among tolerant and susceptible species and genotypes correlated with susceptibility to toxicity[65] as did inhibition of PO_4^{3-} absorption between a tolerant and a susceptible species.[82] In other studies this relationship did not hold for K^+ (Rb^+)[79,80] absorption among species. However, in these studies the sensitivity of mineral absorption by maize genotypes was demonstrated. The possibility of determining the sensitivity of various genotypes to herbicidal phytoxicity by measuring the ability of the herbicide to inhibit mineral absorption deserves attention.

The most detailed information regarding inhibition of mineral absorption by herbicides is available for the phenoxys. Prior to 1976, phenoxys were the only group that had been investigated seriously in this regard. Reviews of various aspects of the early work on this subject are available.[84-87] Research prior to 1976 established that phenoxy herbicides (2,4-D in particular) would affect the absorption of several mineral ions. At low concentrations (less than 10 μM), 2,4-D stimulated mineral absorption, but at higher concentrations (up to 1 mM), 2,4-D inhibited absorption. The length of time elapsed between herbicide treatment and measurement of uptake was important. For example, when 2,4-D was applied to the foliage of bean plants, there was an initial large increase in mineral uptake from the soil; however, after 24 h, uptake was inhibited. Evidence was obtained that 2,4-D did not inhibit absorption of any one particular ion. Which minerals were affected depended upon the plant species and the combination of minerals present. For example, in wheat plants the order of inhibition was K^+ > Na^+ > Ca^{2+}.[88] Furthermore, in the absence of K^+, 2,4-D and 1-naphthalene acetic acid (1-NAA) inhibited Na^+ uptake more; and in the presence of no K^+ and low Na^+, 1-NAA inhibited Mg^{2+} uptake more. Collectively, these results were interpreted as resulting from effects of phenoxy herbicides on cell permeability and respiration.

Research since 1976 (Table 2) has verified previous research and extended findings further. Both 2,4-D and MCPA inhibit absorption of several different mineral ions,[72,74-77] and the inhibition is more pronounced at acidic than alkaline pH. Greater leakage of ions from tissue also occurs at acidic pH.[71,74,76] When 2,4-D (2 μM) was administered to roots of intact rice plants in the presence of 0.5 mM Ca^{2+}, subsequent K^+ absorption by the excised roots was

stimulated for 1 to 6 h; but by 24 to 48 h, K^+ absorption was inhibited similarly to roots pretreated in 2,4-D without Ca^{2+}.[73] A major difference between 2,4-D and 3,5-D was reported. The former, but not the latter, inhibited K^+ and Cl^- absorption in response to elevated KCl concentrations in the solution bathing excised maize roots.[76] Also, only 2,4-D inhibited H^+ efflux from the roots in response to K^+ absorption. Thus, it appears that pH, Ca^{2+}, and the location of chlorine on the phenol ring of phenoxys are important parameters affecting the inhibition of mineral absorption by these herbicides.

Recently, effects of atrazine on mineral absorption have been characterized, especially by the Italian groups of Nardi and Ferrari[65,79] and Giardina et al.[80] The fact that atrazine is active on plant roots is interesting in light of the primary action of triazines on photosynthetic electron transport (see Chapters 1 and 7). However, the minimum atrazine concentration that inhibited absorption was about 33 μM[78] and more commonly 100 to 150 μM.[65,80] These concentrations are 20 to 100 times the concentrations at which inhibition of electron transport occurs (about 0.25 to 4.5 μM gives 50% inhibition, see Chapters 1 and 7). The unanswered question is whether the concentration of triazines reaches these levels in the root under situations where their concentration in the chloroplasts is at or below the I_{50} for photosynthesis. Because triazines are soil-applied, lipophilic, and absorbed by plant roots, it may be possible for the triazine concentration to be considerably higher in the root than shoot, especially under conditions favoring low transpiration.

Atrazine inhibited the absorption of both cations (K^+)[78,80] and anions (SO_4^{2-}),[79] but at 65 μM PO_4^{3-} uptake was not affected.[78] Also, humic substances extracted from soil protected excised barley roots from inhibition of SO_4^{2-} uptake by atrazine.[89] Atrazine could be washed from barley[78] and maize[79] roots so that K^+ and SO_4^{2-} absorption recovered, respectively. The kinetics of inhibition also differed among the grasses. In maize, SO_4^{2-} and atrazine interacted noncompetitively, whereas in wheat and barley, partially competitive inhibition was observed.[79]

One intriguing finding is that different genotypes of barley,[78] maize,[79] and *Brassica rapa* L.[65] responded differently to atrazine. This phenomenon was studied most thoroughly in *Brassica* where a susceptible and a tolerant genotype were compared. Sensitivity of SO_4^{2-} absorption by roots correlated with sensitivity of the Hill reaction in isolated chloroplasts, and these sensitivities correlated with the amount of atrazine binding to roots and chloroplasts, respectively. The authors concluded that mineral transport in the roots might be used to select genotypes with greater tolerance to herbicides. Further studies are needed to verify and extend this hypothesis.

The rapid influence of herbicides on absorption by excised tissues strongly suggests that the mineral absorption process in plant cells can be inhibited directly by herbicides. However, these experiments still do not clarify the mechanism by which the inhibition takes place. The inhibition could be at the level of plasma membrane transport. This possibility is supported by the rapid inhibition of absorption in several instances, which suggests, but does not prove, that the site of inhibition is at the plasma membrane. In addition, absorption might be inhibited indirectly by a reduction in cellular ATP, which is required for mineral absorption and for maintenance of membrane integrity. These possibilities are discussed in Section IV.

2. *Transport of Organic Molecules*

Little is known about herbicidal alteration of organic-molecule transport across membranes. This is primarily due to the absence of studies that have dealt with the question, as well as our limited knowledge of the mechanisms for such absorption (see Section II.B). However, because the proposed mechanism of transport of organic solutes is similar to inorganic ions, it is very likely that herbicides affect organic molecule transport.

Listed in Table 3 are herbicides shown to inhibit absorption of the amino acids leucine

Table 3
INHIBITION OF AMINO ACID ABSORPTION BY HERBICIDES

HERBICIDE FAMILY Chemical	Plant			Herbicide treatment		
	Species[a]	System[b]	Amino acid[c]	Conc.	Time[d]	Ref.
AMIDES						
CDAA	HV; SE	EC; EH	α-ABA	23 μ*M*	1/1 h	90
Alachlor	AS	ERT	Leu, α-ABA	100 μ*M*	15 min	66
Metolachlor	CS	ER	Leu	10 μ*M*	24/4 h	91
BENZONITRILES						
Ioxynil	HV; SE	EC; EH	α-ABA	2 μ*M*	1/1 h	90
CARBAMATES						
Chlorpropham	HV; SE	EC; EH	α-ABA	23 μ*M*	1/1 h	90
DIPHENYL ETHERS						
Nitrofen	AC	SCC	Leu	100 μ*M*	10 min	34
PHENOLS						
PCP	HV; SE	EC; EH	α-ABA	33 μ*M*	1/1 h	90
PYRIDAZINONES						
Pyrazon	HV; SE	EC; EH	α-ABA	25 μ*M*	1/1 h	90
TRIAZINES						
Atrazine	HV	ER	Leu	130 μ*M*	90 min	79
Prometryne	CS	CoD	Leu	100 μ*M*	24/4 h	92
URACILS						
Lenacil, Bromacil	AC	SCC	Leu	100 μ*M*	10 min	34
UNCLASSIFIED						
Endothall	HV; SE	EC; EH	α-ABA	27 μ*M*	1/1 h	90

[a] Abbreviations of plant species: AC, *Acer pseudoplatanus* AS, *Avena sativa* L.; CS, *Cucumis sativus* L.; HV, *Hordeum vulgare* L.; SB, *Sorghum bicolor* (L.) Moench; SE, *Sesbania exaltata*.

[b] Abbreviations of plant systems: CoD, cotyledon discs; EC, excised coleoptiles; EH, excised hypocotyls; ER, excised roots; ERT, excised root tips; SCC, suspension culture cells.

[c] Abbreviations of amino acids: α-ABA,DL-α-amino-*n*-butyric acid; Leu, leucine.

[d] Pretreatment/absorption time. If only one time is given, there was no pretreatment. Abbreviations: h, hour; min, minute.

[e] Pretreatment in the dark, absorption in the dark or light.

and α-aminoisobutyric acid by plants. Because the absorption of any solute is influenced by its utilization inside cells, incorporation of leucine into protein increased the total amount of leucine absorbed by these tissues. Because a herbicide might affect leucine incorporation independently of leucine absorption, several authors[79,91-93] determined both the amount of amino acid absorbed and the amount incorporated into protein, and based conclusions on absorption and incorporation relative to each other. However, it is still difficult to interpret these experiments unless the rates of all the reactions that utilize or produce the amino acid in the cell are known, and those are determined rarely. Another approach is to measure absorption of a nonmetabolized analogue of an amino acid. Use of α-aminoisobutyric acid, an amino acid which is not incorporated into protein, has an advantage over leucine in this regard, although leucine is better than other amino acids (e.g., glutamine) which participate in many reactions in cells. Still another approach is to do very short time courses for both leucine absorption and leucine incorporation, and compare the inhibition of each relative to controls with time. If absorption is inhibited prior to incorporation, inhibition (as a percentage of the control) of leucine absorption will be greater than inhibition of leucine incorporation into protein. Several researchers[66,92,93] took this approach and showed that amino acid absorption was more sensitive to several herbicides than was incorporation into protein.

Several different classes of herbicides were reported to inhibit the absorption of leucine or α-aminoisobutyric acid (Table 3). However for CDAA,[90] metolachlor,[91] chlorpropham,[90] and PCP,[90] incorporation of leucine into protein was inhibited more than leucine or α-aminoisobutyric acid absorption. The strongest cases can be made for inhibition of amino acid absorption by alachlor[66] and ioxynil.[90] Thus, the evidence for inhibition of amino acid absorption by herbicides is minimal at this time but deserves more attention.

Absorption of several other organic solutes was affected by herbicides. Alachlor rapidly inhibited the absorption of 3-*O*-methylglucose,[66] a glucose analogue that is not metabolized by cells. Glyphosate (1 m*M*) inhibited uracil absorption by mesophyll cells of *Phaseolus vulgaris* L. treated 3 h before cell isolation, but uracil absorption was not inhibited by glyphosate in mesophyll cells treated only after their isolation from leaves.[83] Alachlor (500 μ*M*) inhibited the absorption of lysine and glutamic acid, but not mannitol, by excised oat roots at 1 h.[94] Because mannitol moves through membranes by passive diffusion, absence of an effect of alachlor on mannitol absorption suggests that the inhibition of leucine, lysine, and glutamic acid movement through the plasma membrane is by some mechanism other than alteration of membrane permeability.

One herbicide has been shown to alter the transport of a hormone into plant cells. Naptalam (NPA) stimulated the accumulation of IAA by maize coleoptile segments.[95] This greater accumulation resulted from inhibition of the efflux of IAA anions out of the cells and is proposed to explain the inhibition of polar auxin transport by naptalam.[96] Perhaps the movement of other plant hormones across the plasma membrane and tonoplast are affected by herbicides.

C. Alteration of Enzymatic Activities

In spite of the probability that the activity of membrane-associated enzymes will be affected by herbicide molecules binding to or partitioning into membranes, little research has investigated this possibility. This is primarily due to the fact that only a few enzymes have been located on the plasma membrane and tonoplast to date (see Section II.B). Herbicides have been found to affect activities of ATPase, glucan synthetase, and cellulose biosynthetic enzymes located on the plasma membrane.

The most work has been done with plasma membrane-associated ATPase, but even here the results are contradictory. Alachlor has been reported to inhibit the $Mg^{2+} + K^{+}$-ATPase activity in a plasma membrane-enriched vesicle preparation from oat roots.[29] At 1.0 m*M* this herbicide inhibited the enzyme 25%. Balke[97] reported that alachlor (500 μ*M*) stimulated the oat plasma membrane $Mg^{2+} + K^{+}$-ATPase 25%, but did not effect the Mg^{2+}-ATPase activity. After treatment with 0.05% Triton X-100 to make the vesicles permeable, both Mg^{2+}- and $Mg^{2+} + K^{+}$-ATPase were inhibited less than or equal to 20% by alachlor. Another report[98] showed no effect of alachlor on oat ATPases, whereas in *Neurospora crassa*, 1.0 m*M* alachlor inhibited Mg^{2+}-ATPase 30%[99] Quite possibly variable purity of the membrane preparations or tightness of the vesicles caused these contradictory results with alachlor.

The phenoxy herbicide 2,4-D inhibited or stimulated ATPase activity depending upon concentration. A microsomal preparation from soybean roots that contained plasma membrane vesicles, also contained Mg^{2+}-ATPase activity that was stimulated 30% by 1 n*M* 2,4-*D* at pH 5.5.[100] The stimulation decreased with increasing pH. A more heterogeneous microsomal preparation from wheat roots contained $Ca^{2+} + K^{+}$-ATPase activity that was inhibited less than or equal to 20% by 10 μ*M* 2,4-D; as pH increased from 3.0 to 7.5 the inhibition increased.[71] $Ca^{2+} + K^{+}$-ATPase in a similar membrane preparation from rice roots was stimulated slightly by 0.1 and 10 n*M* 2,4-D, but 50 n*M* inhibited the enzyme 33%.[73] In vivo treatment of rice roots with 0.1 or 10 n*M* 2,4-D also produced a slight stimulation of the ATPase. The stimulations seen at low 2,4-D concentrations probably reflect action of the compound as an auxin hormone, whereas the inhibition at higher concentrations may be related to its action as a herbicide.

Several other herbicides have affected ATPase activity in plasma membrane-enriched preparations from oat,[29,101] soybean,[39] and wheat[102] roots. At 1.0 m*M* barban and dicamba were inhibitory,[29] at 20 to 200 μ*M* chlorpropham, oryzalin, and dinoseb were inhibitory,[102] and at 155 μ*M* dinitramine was inhibitory.[39] However, glyphosate (1.0 m*M*)[29] and diclofop (100 μ*M*)[101] stimulated ATPase activities.

Glucan synthetase associated with vesicle preparations containing plasma membranes was stimulated by 5 μ*M* 2,4-D in onion.[103] The effect was detected in both in vivo and in vitro treatments. However, 0.62 m*M* dinitramine inhibited glucan synthetase activity isolated from soybean roots 2 h after herbicide treatment.[39] This glucan synthetase is believed to make callose.[2] Cellulose synthesis has been shown to be inhibited by the herbicide dichlobenil.[104] dichlobenil.[104]

D. Effects of Herbicides on Hormonal and Environmental Regulation of Membrane Functions

If we assume that hormonal and environmental stimuli are detected by the plasma (and perhaps tonoplast) (Section II.B), then the interaction of herbicides with these membranes could possibly alter the reception of the stimuli or the response of the membrane to the stimuli. Little research has been done with herbicides in this regard, but a few studies indicate possible effects.

The best evidence to support the idea that herbicides disrupt membrane-mediated responses to hormones is with compounds that affect auxin in some way. At low concentrations 2,4-D possesses auxin activity itself. However, at higher concentrations it is phytotoxic. Thus, in order to address the mechanism of herbicidal action of 2,4-D, the question of what 2,4-D concentration to test arises. The real question is what concentration of 2,4-D is at the active site in the cell at the time of onset of toxicity. This is very difficult to answer because the concentration will probably change with time as the herbicide is first absorbed by the cells and then possibly metabolized or translocated either to or from the cells. Thus, auxin-like effects may be expected, as well as phytotoxic effects. Whether the two responses are the same or different is not known.

Most of the research with 2,4-D that relates to membranes has studied changes in cell enlargement, membrane electrical potential and resistance, and H^+/K^+ fluxes. Cell enlargement can be stimulated or inhibited depending upon the concentration of 2,4-D, the pH, and the tissue. In excised maize roots at pH 3.5, 10 μ*M* 2,4-D or 3,5-D produced an immediate slight hyperpolarization followed by a dramatic depolarization and increased resistance.[105] However, at pH 5.4, the compounds were not active unless the concentration was raised to 1 m*M*. Addition of $CaCl_2$ to the media containing 1 m*M* 2,4-D at pH 5.0 to 5.5 produced a transient hyperpolarization followed by depolarization. Bennett and Rideal[106] reported similar responses of membrane resistance to 2,4-D at neutral and acidic pHs in *Nitella* cells. Mineral absorption (including K^+) can be inhibited by 2,4-D, also (see Table 2). Kennedy and Stewart[76,105] reported that at pH 3.6, 2,4-D or 3,5-D (10 μ*M*) inhibited K^+ uptake and caused K^+ leakage from excised maize roots. Also, membrane permeability to H^+ increased so that net efflux of H^+ decreased. They concluded that both herbicides inhibited H^+/K^+ exchanges in maize roots. Because 3,5-D does not have auxin-like activity, these effects of 2,4-D and 3,5-D on PD, resistance, and H^+/K^+ exchange may be related to their phytotoxic rather than growth-promoting action.

Auxin antagonists, which are neither auxins nor anti-auxins themselves,[107] also possess herbicidal activity. Many of the diphenoxy-carboxylic acid herbicides (e.g., diclofop-methyl and chlorfenprop-methyl) are auxin antagonists. These herbicides are formulated as esters but are believed to be activated by esterases in plants.[107]

Both diclofop-methyl and chlorfenprop-methyl inhibit membrane-mediated responses of plants to auxins. IAA-stimulated cell elongation in oats and maize coleoptile was inhibited

by 50 μM chlorfenprop-methyl.[108] Similarly 10 μM diclofop-methyl inhibited coleoptile growth in wild oats; wheat (resistant) was not inhibited as much.[109] In both studies, the free acid was less active than the methyl ester. H^+ excretion from the coleoptiles was inhibited by chlorfenprop-methyl[108] and by both diclofop acid and methyl ester.[110] Chlorfenprop-methyl (10 μM) also inhibited auxin (NAA) uptake and polar transport.[108] Naptalam, another auxin antagonist, inhibited polar auxin (IAA) transport[95] but at submicromolar concentrations (0.01 to 1.0 μM) it stimulated IAA absorption. The greater cellular accumulation was postulated to result from decreased efflux of IAA anion from cells. Naptalam is known to bind to membrane sites in plant cells;[19] these sites may be on the plasma membrane.[111] Lastly, the electrogenic component of the membrane PD of internodal *Chara corallina*[112] and oat coleoptiles[101] was depolarized by 100 μM diclofop. Oat coleoptiles were more sensitive to diclofop than diclofop-methyl, and wheat was resistant to the methyl ester but not the free acid. In *Chara*, at pH 5.7 diclofop decreased the membrane resistance and produced a slow depolarization that was interrupted by a transient repolarization. At pH 7.0, the depolarization was slower and the membrane resistance increased. At pH 8.0, there was a hyperpolarization. ATP concentration of the tissue was decreased more at pH 5.7 than at 7.0, whereas at pH 8.2 ATP increased slightly.[112] The authors suggested that diclofop is a proton ionophore at pH 5.7 and 7.0 and thus shuttles H^+ across the plasma membrane into the cells resulting in collapse of the H^+ gradient, PD, and mitochondrial production of ATP. At pH 8.2, ATP was not affected, and thus it was suggested that the plasma membrane ATPase or OH^- efflux systems of *Chara* were affected.

In summary, both auxin and auxin antagonist herbicides affect membrane functions that are associated with the action of IAA in plant cells. Because gibberellic acid, cytokinins, and abscissic acid are postulated to act at membranes,[18] it is quite possible that herbicides might disrupt the action of these hormones at membranes. This possibility is worthy of investigation.

Herbicides can be expected to alter the response of membranes to environmental stimuli, also. Although no one has reported direct action of a herbicide on a temperature-regulated membrane activity,[113] temperature affects the activity of herbicides. This phenomenon is usually explained as resulting from increased transpiration, herbicide absorption, or metabolism of the herbicide. However, it may be the result of a direct herbicidal effect on a membrane that is regulated by temperature. For example, partitioning of a herbicide into a membrane results in herbicide molecules disrupting the associations among lipid and protein molecules. Similar changes take place when temperature changes alter membrane fluidity and membrane-associated enzyme activities.[26] Thus, addition of herbicide molecules to a membrane might alter the response of the membrane to temperature changes. The altered response might be reflected in membrane permeability, release of a secondary signal, or membrane transport. Therefore, many possibilities exist for the herbicidal alteration of temperature-induced membrane responses.

Alteration of light-induced membrane responses by herbicides has some experimental evidence. Several herbicides affected phytochrome-regulated Ca^{2+} transport by a plasma membrane-enriched vesicle fraction from maize and zucchini.[114] Trifluralin stimulated the Ca^{2+} transport, whereas ioxynil and dinoseb inhibited it slightly, and diclofop-methyl and oryzalin inhibited it the most. Diclofop-methyl inhibited transport 50% at 20 μM. Mitochondrial Ca^{2+} transport was inhibited more than transport by the membrane vesicle preparation. Thus, these herbicides might alter Ca^{2+} transport in response to light activation of phytochrome. Resultant elevated Ca^{2+} concentrations in the cytoplasm could disrupt numerous physiological processes.

The blue light-induced absorbance change (LIAC) is another light-regulated response that is modified by herbicides. The diphenyl ether acifluorfen enhanced the blue LIAC of a crude membrane vesicle preparation from oat coleoptiles.[115] This LIAC is known to be associated

with the plasma membrane,[24] which contains the flavin-cytochrome complex that absorbs blue light. Oxyfluorfen, nitrofen and bifenox enhanced the blue LIAC in a similar but smaller manner. Acifluorfen also sensitized dark-grown oats so that 2/3 less blue-light fluence induced phototropism as compared to plants grown in the absence of the herbicide. Thus, these diphenyl ethers modified the membrane response to blue light and again showed that herbicides can alter environmentally stimulated membrane functions.

IV. BIOCHEMICAL AND BIOPHYSICAL MECHANISMS OF HERBICIDE-INDUCED MEMBRANE DYSFUNCTION

On several occasions in this chapter, I have stated that a given physiological effect on the plasma membrane and tonoplast might result from either direct or indirect action of a herbicide on the membrane. Thus, herbicide-induced dysfunctions of membranes can be primary, secondary, or even tertiary effects of the phytotoxic chemicals. In this section, I will discuss several biochemical and biophysical mechanisms that may be responsible for membrane dysfunction produced by either indirect or direct action of herbicides.

A. Indirect Effects

Herbicides can indirectly affect the plasma membrane or tonoplast in at least three ways. These include interruption of the synthesis of membrane constituents, reduction in energy supply for maintenance of membranes, and production of secondary agents that disrupt membranes.

1. Synthesis of Membrane Constituents

As described in Section II.A, lipids, proteins, and carbohydrates are the major constituents of the plasma membrane and tonoplast. Thus, inhibition of the synthesis of any of these membrane components will lead to dysfunction of membranes. Herbicides are known to disrupt protein synthesis,[51,90] glycolipid and phospholipid synthesis,[116-119] and sterol synthesis[120] (see Chapter 4 for additional discussion and references). Synthesis of these membrane constituents and their precursors occur in the cytosol, endoplasmic reticulum, and Golgi apparatus, and they ultimately move into membrane vesicles, the plasma membrane, or the tonoplast. Synthesis of these chemical constituents are as important for mature cells as for developing cells because membrane components continuously turn over during the life of the cell.[121,122] Disruption of the synthesis of these components or their assembly into membranes will lead to alteration of the structure and function of membranes and, perhaps, phytotoxicity.

2. ATP Levels

ATP is required for both synthesis of membrane components (see Section IV.A.1) and the stabilization of membranes.[51] The way in which the latter occurs is not known, but ATP-requiring maintenance of electrical potential differences or H^+ gradients across membranes may be involved (see Section II.B). The primary sources of ATP in plant cells are mitochondrial oxidative phosphorylation and chloroplastic photophosphorylation. Electron transport coupled to these processes provides the driving force to phosphorylate ADP to ATP. Thus, disruption of electron transport, uncoupling of electron transport from phosphorylation, or inhibition of phosphorylation will result in decreased ATP levels in cells and thus the dysfunction of the plasma membrane and the tonoplast. These topics are discussed in detail in Chapters 1, 2, and 3.

3. Production of Secondary, Disruptive Agents

A herbicide molecule applied to a plant might not itself be disruptive to membranes but

might be converted to a disruptive agent or cause the production of a disruptive agent. An example of the former is the esterified diphenoxycarboxylic acids (e.g., diclofop-methyl) which are thought to be deesterified prior to becoming active. This deesterification produces the free acid which can disrupt membrane functions either directly or indirectly (see Section III.D). Of more interest in this section are those herbicides that cause the production of secondary agents. These herbicides require light to be active.

a. Electron Acceptors

The current model for the mechanism of action of the bipyridilium herbicides (e.g., paraquat and diquat) is that these herbicides accept electrons from the photosynthetic electron transport chain at ferrodoxin and become reduced to free radicals.[123] These free radicals reduce oxygen to form superoxide (O_2^-) and are thereby reoxidized. The large amount of O_2^- produced overcomes the capability of superoxide dismutase to remove the free radical from the cell. This superoxide free radical is very reactive and produces H_2O_2. Bipyridilium free radicals can react with H_2O_2 to produce hydroxyl-free radicals (OH^-). OH^- is extremely reactive and can initiate either polymerization or lipid peroxidation of unsaturated fatty acids in membrane lipids. Lipid peroxidation leads to a chain reaction resulting in massive destruction of membrane lipids. In either instance, the loss of membrane function and structure results. Furthermore, when the tonoplast permeability increases, acids and hydrolytic enzymes are released into the cytoplasm. Quickly cellular compartmentation is lost and the cell dies.

b. Photosynthetic Electron-Transport Inhibitors

Many classes of herbicides including the triazines, ureas, uracils, benzonitriles, and pyridazinones inhibit the flow of electrons among the intermediates of the electron transport chain located on the thylakoids of chloroplasts. The resultant build-up of triplet-state chlorophyll is believed to yield lipid-free radicals by abstracting electrons or hydrogen from unsaturated fatty acids.[124] The lipid-free radicals could lead to general peroxidation of membrane lipids and membrane destruction as occurs with the bipyridiliums.

c. Other Mechanisms

The diphenylether herbicides (e.g., acifluorfen) also require light to express phytotoxicity, but carotenoids rather than chlorophyll are involved in the action of these herbicides.[125] Also, it appears that production of diphenyl ether free radicals does not occur for these herbicides.[126,127] However, they do cause lipid peroxidation and release malonyldialdehyde and short chain hydrocarbon gases similarly to the electron acceptors (Section IV.A.3.a) and the electron-transport inhibitors (Section IV.A.3.b). Whether or not O_2^- and OH^-, in addition to lipid-free radicals, are involved is an unanswered question. Thus, the diphenyl ethers are similar to other light-activated herbicides in disrupting membranes, but the details of the mechanism are different.

B. Direct Effects

In order for a herbicide to directly affect the plasma membrane or tonoplast, the molecules must interact with the membrane constituents (see Figures 1 and 2) in some way. This interaction might be mediated through binding of the herbicide to a particular constituent or more general partitioning (dissolving) of the herbicide into the membrane. By necessity the discussion in this section must be speculative because few studies have shown convincingly that herbicides can disrupt the plasma membrane or tonoplast directly. Thus, I will discuss possible mechanisms for the direct action of herbicides on membranes.

Herbicides might bind to proteins, lipids, or carbohydrates that form the membrane (see Figure 1). Toxic agents are usually thought to act by binding to particular proteins (enzymes)

and thus inhibiting their activity.[128] Such binding has been verified for certain herbicides[51] (also see Chapters 1, 4, and 7). However, because lipids and carbohydrates are very important for the functioning of membranes, binding of herbicides to these membrane constituents may be as important as binding to proteins. Herbicide binding might take place at the surface of membranes or internally. Binding at either place could lead to membrane dysfunction.

Herbicide molecules may also partition into the lipid matrix of membranes by loosely associating with lipid molecules. This partitioning results from herbicides being partially nonpolar and thus being more soluble in the region of the hydrocarbon tails of phospholipids in the membrane. Herbicide molecules might partition between lipid and protein molecules and thus disrupt protein structure and cause dysfunction of the protein.

In the process of partitioning into membranes, herbicides might solubilize certain membrane proteins or lipids out of the membrane. Thus, herbicides might act like detergents or surfactants to remove protein or lipid molecules from membranes. Because many herbicides are amphoteric like detergents and surfactants (see Chapter 6), the likelihood is greater that some herbicides could dissolve membrane constituents out of the membrane and thus alter the membrane. They might similarly cause premature release of secondary hormonal messengers from membranes.

Several herbicide molecules might collectively partition into a membrane to produce a channel through the membrane. Metribuzin (formulated) increased the conductance of artificial lipid bilayers.[129] This increased conductance was interpreted as resulting from the production of ion channels in the membrane due to complex formation by the herbicide molecules.

The influences of herbicides on the major membrane functions of solute transport and retention, enzymatic activities, and regulation by hormonal and environmental stimuli (see Section III) are potentially explainable by direct effects of herbicides on the plasma membrane and tonoplast. Membrane permeability could be altered by the partitioning of herbicide molecules into the membrane and thus disrupting lipid/lipid or lipid/protein interactions, producing channels, or solubilizing membrane components. Solute transport is directly dependent upon ATPase activity to generate the proton motive force necessary for absorption. ATPase activity can be altered by herbicides binding directly to the ATPase or disrupting phospholipid/ATPase interactions necessary for ATPase activity. Lastly, alteration of membrane respones to hormonal and environmental stimuli might be mediated by herbicides binding to membrane components or partitioning into the membrane to alter membrane fluidity. Although all of these physiological actions of herbicides on membranes are potentially explainable by direct effects of herbicides on membranes, future studies will determine if herbicides do in fact act in this manner.

V. CONCLUSIONS

This chapter has presented both hypotheses and experimental evidence regarding disruption of the major functions of the plasma membrane and tonoplast of plant cells by herbicides. Two major questions must be answered before it can be stated unequivocally that disruption of membrane functions is a primary mechanism of action for any herbicide. First, can herbicides directly affect membranes, or are they only active on membranes in a secondary or tertiary manner? Second, are the concentrations of herbicides in (or on) membranes at the time of initiation of phytoxicity of sufficient magnitude to disrupt membrane functions? The second question is difficult to answer because herbicides are active at low concentrations and thus a very sensitive assay is needed. Techniques to localize these small quantities of herbicides at the subcellular level still need to be developed and proven reliable.

The first question is difficult, also, but can be addressed with currently available techniques. The only way to convincingly prove that herbicides can directly affect the plasma

membrane or tonoplast is to isolate each membrane separately from plant cells and then test the action of herbicides on some function of that in vitro membrane system. Now that vesicles of plasma membrane[5] and tonoplast[6] can be isolated from plant cells, it is possible to perform such tests. The key is to isolate membrane vesicles that are both sufficiently homogeneous and function as the membrane does *in situ*. One membrane vesicle system that displays properties associated with transmembrane transport has been isolated from oat roots.[130] The oat root vesicles had ATPase activity and generated both a H^+ gradient and an electrical potential. Unfortunately, this microsomal preparation is probably a mixture of plasma membrane and tonoplast vesicles. However, this system proved that membrane vesicles can be isolated that will function like membranes in cells. It may be easier to isolate homogeneous tonoplast vesicles that function as in vivo tonoplast[131] because vacuoles can be purified from protoplasts more easily than homogeneous plasma membrane vesicles can be purified from plant tissues or protoplasts.

Availability of homogeneous plasma membrane and tonoplast vesicles will permit the use of probes[132] to assess the alteration of physical properties of these membranes by herbicides. Such probes have been used to show that herbicides can alter the fluidity of mitochondrial membranes[133] and should be readily adaptable to studies of herbicide action on plasma membrane and tonoplast vesicles.

Use of functionally intact membrane vesicles will aid in answering the questions of if and how herbicides directly alter membrane functions. The challenge is to utilize the sophisticated techniques of membranologists to address these questions.

REFERENCES

1. **Sitte, P.,** Functional organization of biomembranes, in *Lipids and Lipid Polymers in Higher Plants*, Tevini, M. and Lichtenthaler, H. K., Eds., Springer-Verlag, New York, 1977, chap. 1.
2. **Leonard, R. T. and Hodges, T. K.,** The plasma membrane, in *The Biochemistry of Plants: A Comprehensive Treatise*, Vol. 1, *The Plant Cell*, Tolbert, N. E., Ed., Academic Press, New York, 1980, chap. 4.
3. **Hall, J. L.,** Plasma membranes, in *Isolation of Membranes and Organelles from Plant Cells*, Hall, J. L. and Moore, A. L., Eds., Academic Press, New York, 1983, chap. 3.
4. **Marty, F., Branton, D., and Leigh, R. A.,** Plant vacuoles, in *The Biochemistry of Plants: A Comprehensive Treatise*, Vol. 1, *The Plant Cell*, Tolbert, N. E., Ed., Academic Press, New York, 1980, chap. 15.
5. **Wagner, G. F.,** Compartmentation in plant cells: the role of the vacuole, *Rec. Adv. Phytochem.*, 16, 1, 1982.
6. **Wagner, G.,** Higher plant vacuoles and tonoplasts, in *Isolation of Membranes and Organelles from Plant Cells*, Hall, J. L. and Moore, A. L., Eds., Academic Press, New York, 1983, chap. 4.
7. **Newcomb, E. H.,** The general cell, in *The Biochemistry of Plants: A Comprehensive Treatise*, Vol. 1, *The Plant Cell*, Tolbert, N. E., Ed., Academic Press, New York, 1980, chap. 1.
8. **Roland, J. C., Lembi, C. A., and Morré, D. J.,** Phosphotungstic acid-chromic acid as a selective electron-dense stain for plasma membranes of plant cells, *Stain Technol.*, 47, 195, 1972.
9. **Nagahashi, G., Leonard, R. T., and Thomson, W. W.,** Purification of plasma membranes from roots of barley. Specificity of the phosphotungstic acid-chromic acid stain, *Plant Physiol.*, 61, 993, 1978.
10. **Quail, P. H.,** Plant cell fractionation, *Annu. Rev. Plant Physiol.*, 30, 425, 1979.
11. **Marty, F.,** Isolation and freeze-fracture characterization of vacuole membrane fragments, in *Plasmalemma and Tonoplast: Their Functions in the Plant Cell*, Marmé, D., Marré, E., and Hertel, R., Eds., Elsevier, New York, 1982, 179.
12. **Larsson, C.,** Partition in aqueous polymer two-phase systems: a rapid method for separation of membrane particles according to their surface properties, in *Isolation of Membranes and Organelles from Plant Cells*, Hall, J. L. and Moore, A. L., Eds., Academic Press, New York, 1983, chap. 11.
13. **Widell, S., Lundborg, T., and Larsson, C.,** Plasma membranes from oats prepared by partition in an aqueous polymer two-phase system. On the use of light induced cytochrome *b* reduction as a marker for the plasma membrane, *Plant Physiol.*, 70, 1429, 1982.

14. **Hodges, T. K.,** ATPases associated with membranes from plant cells, in *Transport in Plants II, Part A, Cells, Encyclopedia of Plant Physiology, New Series,* Lüttge, U. and Pitman, M. G., Eds., Springer-Verlag, New York, 1976, 260.
15. **Wagner, G. J. and Mulready, P.,** Characterization and solubilization of nucleotide-specific, Mg^{2+}-ATPase and Mg^{2+}-pyrophosphatase of tonoplast, *Biochim. Biophys. Acta,*728, 267, 1983.
16. **Leonard, R. T.,** Membrane-associated ATPases and nutrient absorption by roots, in *Advances in Plant Nutrition,* Vol. 1, Tinker, P. B. and Läuchli, A., Eds., Praeger Scientific, New York, 1984, in press.
17. **Jung, K-D. and Lüttge., U.,** Mechanisms of amino acid transport in *Lemna gibba* L., in *Plasmalemma and Tonoplast: Their Functions in the Plant Cell,* Marmé, D., Marré, E., and Hertel, R., Eds., Elsevier, New York, 1982, 21.
18. **Moore, T. C.,** *Biochemistry and Physiology of Plant Hormones,* Springer-Verlag, New York, 1979.
19. **Rubery, P. H.,** Auxin receptors, *Annu. Rev. Plant Physiol.,* 32, 569, 1981.
20. **Ohlrogge, J. B., Garcia-Martinez, J. L., Adams, D., and Rappaport, L.,** Uptake and subcellular compartmentation of gibberellin A_1 applied to leaves of barley and cowpea, *Plant Physiol.,* 66, 422, 1980.
21. **Raven, J. A.,** Transport of indoleacetic acid in plant cells in relation to pH and electrical potential gradients and its significance for polar IAA transport, *New Phytol.,* 74, 163, 1980.
22. **Marmé, D.,** Phytochrome: membranes as possible sites of primary action, *Annu. Rev. Plant Physiol.,* 28, 173, 1977.
23. **Sundqvist, C. and Widell, S.,** Binding of phytochrome to plasma membranes in vitro, *Physiol. Plant.,* 59, 35, 1983.
24. **Leong, T-Y. and Briggs, W. R.,** Partial purification and characterization of a blue light-sensitive cytochrome-flavin complex from corn membranes, *Plant Physiol.,* 67, 1042, 1981.
25. **Caubergs, R. J., Widell, S., Larsson, C., and deGreef, J.,** Comparison of two methods for the preparation of a membrane fraction containing a blue light reducible *b*-type cytochrome, *Physiol. Plant.,* 57, 291, 1983.
26. **Raison, J. K.,** Membrane lipids: structure and function, in *The Biochemistry of Plants: A Comprehensive Treatise,* Vol. 4, *Lipids: Structure and Function,* Stumpf, P. K., Ed., Academic Press, New York, 1980, chap. 2.
27. **Singer, S. J. and Nicolson, G.,** The fluid mosaic model of the structure of cell membranes, *Science,* 175, 720, 1972.
28. **O'Brien, M. C. and Prendeville, G. N.,** Effect of herbicides on cell membrane permeability in *Lemna minor, Weed Res.,* 19, 331, 1979.
29. **Watson, M. C., Bartels, P. G., and Hamilton, K. C.,** Action of selected herbicides and Tween 20 on oat (*Avena sativa*) membranes, *Weed Sci.,* 28, 122, 1980.
30. **Mellis, J. M., Pillai, P., Davis, D. E., and Truelove, B.,** Metolachlor and alachlor effects on membrane permeability and lipid synthesis, *Weed Sci.,* 30, 399, 1982.
31. **Hofstra, G. and Switzer, C. M.,** The phytotoxicity of propanil, *Weed Sci.,* 16, 23, 1968.
32. **Price, H. C.,** unpublished data, 1969.
33. **Talbert, D. M. and Camper, N. D.,** Herbicide and inhibitor effects on cell leakage in suspension cultures of tobacco *(Nicotiana tobacum), Pestic. Biochem. Physiol.,* 19, 74, 1983.
34. **Blein, J-P.,** Action of some herbicides on growth, respiration, plasmalemma integrity, and proton extrusion of *Acer pseudoplatanus* cells, II. Amides, diphenyl ethers, nitriles, phenols, triazines, and uracils, *Pestic. Biochem. Physiol.,* 17, 156, 1982.
35. **Merkle, M. G., Leinweber, C. L., and Bovey, R. W.,** The influence of light, oxygen, and temperature on the herbicidal properties of paraquat, *Plant Physiol.,* 40, 832, 1965.
36. **Prendeville, G. N. and Warren, G. F.,** Effect of four herbicides and two oils on leaf-cell membrane permeability, *Weed Res.,* 30, 251, 1977.
37. **O'Brien, M. C. and Prendeville, G. N.,** Effect of herbicides on cell membrane permeability in *Lemna minor, Weed Res.,* 19, 331, 1979.
38. **Harris, N. and Dodge, A. D.,** The effect of paraquat on flax cotyledon leaves: physiological and biochemical changes, *Planta,* 104, 210, 1972.
39. **Travis, R. L. and Woods, W. G.,** Studies on the mechanism of action of dinitramine. Effect on soybean root plasma membrane, *Plant Physiol.,* 60, 54, 1977.
40. **Towne, C. A., Bartels, P. G., and Hilton, J. L.,** Interaction of surfactant and herbicide treatments on single cells of leaves, *Weed Sci.,* 26, 182, 1978.
41. **St. John, J. B. and Hilton, J. L.,** Lipid metabolism as a site of herbicide action, *Weed Sci.,* 21, 477, 1973.
42. **Crowley, J. and Prendeville, G. N.,** Effect of dichlofop methyl on leaf-cell membrane permeability in wild oat, barley and wheat, *Can. J. Plant Sci.,* 59, 275, 1979.
43. **Orr, G. L. and Hess, F. D.,** Characterization of herbicidal injury by acifluorfen-methyl in excised cucumber (*Cucumis sativus* L.) cotyledons, *Pestic. Biochem. Physiol.,* 16, 171, 1981.

44. **Bachelard, E. P. and Ayling, R. D.,** The effects of picloram and 2,4-D on plant cell membranes, *Weed Res.*, 11, 31, 1971.
45. **Wilkinson, R. E. and Smith, A. E.,** EPTC altered beet disc betacyanin efflux and fatty acid synthesis, *Weed Sci.*, 24, 235, 1976.
46. **Blein, J-P.,** Action of some herbicides on growth, respiration, plasmalemma integrity, and proton extrusion of *Acer pseudoplatanus* cells. I. Substituted ureas, *Pestic. Biochem. Physiol.*, 16, 179, 1981.
47. **Sikka, H. C., Saxena, J., and Zweig, G.,** Alteration in cell permeability as a mechanism of action of certain quinone pesticides, *Plant Physiol.*, 51, 363, 1973.
48. **Pallet, K. E.,** The contact activity of difenzoquat in two United Kingdom spring wheat cultivars, *Weed Res.*, 22, 329, 1982.
49. **MacRobbie, E. A. C.,** Fluxes and compartmentation in plant cells, *Annu. Rev. Plant Physiol.*, 22, 75, 1971.
50. **Balke, N. E. and Hodges, T. K.,** Effect of diethylstilbestrol on ion fluxes in oat roots, *Plant Physiol.*, 63, 42, 1979.
51. **Moreland, D. E.,** Mechanisms of action of herbicides, *Annu. Rev. Plant Physiol.*, 31, 597, 1980.
52. **Beevers, L.,** Senescence, in *Plant Biochemistry*, 2nd ed., Bonner, J. and Varner, J. E., Eds., Academic Press, New York, 1976, 771.
53. **Anderson, J. L. and Thomson, W. W.,** The effects of herbicides on the ultrastructure of plant cells, *Residue Rev.*, 47, 167, 1973.
54. **Linck, A. J.,** Effects on the cytology and fine structure of plant cells, in *Herbicides: Physiology, Biochemistry, and Ecology*, Vol. 1, 2nd ed., Audus, L. J., Ed., Academic Press, New York, 1976, chap. 3.
55. **Darmstadt, G. L., Balke, N. E., and Schrader, L. E.,** Use of corn root protoplasts in herbicide absorption studies, *Pestic. Biochem. Physiol.*, 19, 172, 1983.
56. **Magalhães, A. C. and Ashton, F. M.,** Effect of dicamba on oxygen uptake and cell membrane permeability in leaf tissue of *Cyperus rotundus* L., *Weed Res.*, 9, 48, 1969.
57. **Ashton, F. M. and Bayer, D. C.,** Effects on solute transport and plant constituents, in *Herbicides: Physiology, Biochemistry, and Ecology*, Vol. 1, 2nd ed., Audus, L. J., Ed., Academic Press, New York, 1976, chap. 7.
58. **Kranz, E. and Jacob, F.,** Zur Mineralstoffkonkurrenz zwischen *Linum* und *Camelina*. III. Aufnahme von ^{35}S-Sulfat unter einfluss von herbiziden, *Flora*, 167, 561, 1978.
59. **Zippel, K., Mäder, M., and Bopp, M.,** The effect of some herbicides on ion accumulation in tissue culture of *Anagallis arvensis*, *Angew. Bot.*, 51, 77, 1977.
60. **Devlin, R. M., Zbiec, I. I., and Karczmarczyk, S. J.,** Influence of napropamide on the uptake and translocation of mineral nutrients, *Weed Sci.*, 30, 503, 1982.
61. **Crowley, J., O'Donovan, J. T., and Prendeville, G. N.,** Phytotoxicity of soil-applied diclorfop methyl and its effect on uptake of ^{45}Ca in wild oats, barley and wheat, *Can. J. Plant Sci.*, 58, 395, 1978.
62. **O'Donovan, J. T. and Prendeville, G. N.,** Uptake patterns of soil-applied ^{45}Ca and ^{32}P in some legume species as influenced by differential trifluralin placement, *Weed Res.*, 17, 311, 1977.
63. **Cathey, G. W. and Sabbe, W. E.,** Effects of trifluralin on fertilizer phosphorus uptake patterns by cotton and soybean seedlings, *Agron. J.*, 64, 254, 1972.
64. **Ingle, M. and Rogers, B. J.,** Some physiological effects of 2,2-dichloropropionic acid, *Weeds*, 9, 264, 1961.
65. **Ferrari, G., Nardi, S., Cacco, G., and Dell'Agnola, G.,** Sulfate transport of excised roots as an index of genotype response to herbicides, *Physiol. Plant.*, 52, 29, 1981.
66. **Marsh, H. V., Bates, J., and Downs, S.,** Inhibition of metabolite uptake and transport by alachlor, *Plant Physiol.*, 57s, 61, 1976.
67. **Balke, N. E.,** Inhibition of ion absorption by acetanilide herbicides, *Proc. Weed Sci. Soc. Am.*, Weed Science Society of America, Champaign, Ill., 1979, 93.
68. **Zsoldos, F. and Mécs, P.,** Ion uptake and cell-membrane behaviour of Synpran N and Dacthal herbicide-treated rice plants, *Acta Biol. (Szeged)*, 20, 115, 1974.
69. **Wojtaszek, T., Cherry, J. H., and Warren, G. F.,** Effect of 4,6-dinitro-o-sec-butylphenol on phosphorus accumulation and incorporation in tomato leaf disks, *Plant Physiol.*, 41, 34, 1966.
70. **Albough, G. P., Regehr, D. L., and Frick, H.,** The effects of diclofop on $K^+/^{86}Rb^+$ uptake in maize, *Proc. Weed Sci. Soc. Am.*, Weed Science Society of America, Champaign, Ill., 1980, 99.
71. **Zsoldos, F., Karvaly, B., Toth, I., and Erdei, L.,** 2,4-D induced changes in the K^+ uptake of wheat roots at different pH values, *Physiol. Plant.*, 44, 395, 1978.
72. **Zsoldos, F. and Haunold, E.,** Effects of pH changes on ion and 2,4-D uptake of wheat roots, *Physiol. Plant.*, 47, 77, 1979.
73. **Erdei, L., Toth, I., and Zsoldos, F.,** Hormonal regulation of Ca^{2+}-stimulated K^+ influx and $Ca^{2+} + K^+$-ATPase in rice roots: *in vivo* and *in vitro* effects of auxins and reconstitution of the ATPase, *Physiol. Plant.*, 45, 448, 1979.

74. **Zsoldos, F. and Haunold, E.,** Potassium influx and efflux of 2,4-D- and MCPA-treated rice plants, *Plant and Soil*, 49, 219, 1978.
75. **Zsoldos, F. and Haunold, E.,** Influence of 2,4-D and low pH on potassium, ammonium and nitrate uptake by rice roots, *Physiol. Plant.*, 54, 63, 1982.
76. **Kennedy, C. D. and Stewart, R. A.,** The effects of 2,4-dichlorophenoxyacetic acid on ion uptake by maize roots, *J. Exp. Bot.*, 31, 135, 1980.
77. **Matlib, M. A. and Kirkwood, R. C.,** Effect of MCPA and MCPB on ^{32}P accumulation and its incorporation into ATP in *Vicia faba* L., *Pestic. Sci.*, 1, 193, 1970.
78. **Zhirmunskaya, N. M. and Ioffe, G. S.,** Effect of atrazine on potassium uptake by barley roots, *Sov. Plant Physiol.*, 20, 398, 1973.
79. **Renosto, F., Nardi, S., and Ferrari, G.,** Atrazine inhibition of ion uptake by plant roots, *Pestic. Biochem. Physiol.*, 11, 243, 1979.
80. **Giardina, M. C., DeAgazio, M., Giardi, M. T., and Buffone, R.,** Potassium uptake in atrazine-treated roots of sensitive and resistant plants, *Pestic. Biochem. Physiol.*, 19, 11, 1983.
81. **Zsoldos, F. and Haunold, E.,** Studies on herbicide sensitivity in the uptake of different ions by rice roots, *Acta Biol. Szeged.*, 23, 63, 1977.
82. **Bergmannova, E. and Taimr, L.,** The effect of chlorfenprop-methyl on ^{32}P uptake, transport and metabolism in *Avena fatua* L. and *Hordeum vulgare* L., *Weed Res.*, 20, 1, 1980.
83. **Brecke, B. J. and Duke, W. B.,** Effect of glyphosate on intact bean plant (*Phaseolus vulgaris* L.) and isolated cells, *Plant Physiol.*, 66, 656, 1980.
84. **Hilton, J. L., Jansen, L. L., and Hull, H. M.,** Mechanisms of herbicide action, *Annu. Rev. Plant Physiol.*, 14, 353, 1963.
85. **Brian, R. C.,** The effects of herbicides on biophysical processes in the plant, in *The Physiology and Biochemistry of Herbicides*, Audus, L. J., Ed., Academic Press, New York, 1964, chap. 12.
86. **Penner, D. and Ashton, F. M.,** Biochemical and metabolic changes in plants induced by chlorophenoxy herbicides, *Residue Rev.*, 14, 39, 1966.
87. **Morrod, R. S.,** Effects on plant cell membrane structure and function, in *Herbicides: Physiology, Biochemistry, Ecology*, Vol. 1, 2nd ed., Audus, L. J., Ed., Academic Press, New York, 1976, chap. 9.
88. **Swenson, G. and Burstrom, H.,** On the influence of auxins on salt and water uptake, *Physiol. Plant.*, 13, 846, 1960.
89. **Dell'Agnola, G., Ferrari, G., and Nardi, S.,** Antidote action of humic substances on atrazine inhibition of sulfate uptake in barley roots, *Pestic. Biochem. Physiol.*, 15, 101, 1981.
90. **Mann, J. D., Jordan, L. S., and Day, B. E.,** A survey of herbicides for their effect upon protein synthesis, *Plant Physiol.*, 40, 840, 1965.
91. **Pillai, P., Davis, D. E., and Truelove, B.,** Effects of metolachlor on germination, growth, leucine uptake, and protein synthesis, *Weed Sci.*, 27, 634, 1979.
92. **Truelove, B., Jones, L. R., and Davis, D. E.,** Light and prometryne effects on leucine uptake and incorporation, *Weed Sci.*, 21, 24, 1973.
93. **Ketchersid, M. L., Vietor, D. M., and Merkle, M. G.,** CGA-43089 effects on metolachlor uptake and membrane permeability in grain sorghum (*Sorghum bicolor*), *J. Plant Growth Regul.*, 1, 285, 1982.
94. **Balke, N. E.,** unpublished data, 1982.
95. **Sussman, M. R. and Goldsmith, M. H. M.,** Auxin uptake and action of N-1-naphthylphthalamic acid in corn coleoptiles, *Planta*, 150, 15, 1981.
96. **Goldsmith, M. H. M.,** The polar transport of auxin, *Annu. Rev. Plant Physiol.*, 28, 439, 1977.
97. **Balke, N. E.,** Mechanism of inhibition of ion absorption by alachlor, *Proc. Weed Sci. Soc. Am.*, Weed Science Society of America, Champaign, Ill., 1980, 84.
98. **Bates, J. R., Marsh, H. V., and Downs, S.,** Rapid inhibition of metabolite transport by alachlor, *Proc. Weed Sci. Soc. Am.*, Weed Science Society of America, Champaign, Ill., 1976, 1.
99. **Bowman, B. J., Mainzer, S. E., Allen, K. E., and Slayman, C. W.,** Effects of inhibitors on the plasma membrane and mitrochondrial adenosine triphosphatases of *Neurospora crassa*, *Biochim. Biophys. Acta*, 512, 13, 1978.
100. **Scherer, G. F. E. and Morré, D. J.,** *In vitro* stimulation by 2,4-dichlorophenoxyacetic acid of an ATPase and inhibition of phosphatidate phosphatase of plant membranes, *Biochem. Biophys. Res. Commun.*, 84, 238, 1978.
101. **Wright, J. P. and Shimabukuro, R. H.,** unpublished data, 1983.
102. **Edgecomb, D. W., Moreland, D. E., and Huber, S. C.,** Effects of selected herbicides on properties of plasma membranes, *Proc. Weed Sc. Soc. Am.*, Weed Science Society of America, Champaign, Ill., 1982, 87.
103. **Vanderwoude, W. J., Lembi, C. A., and Morré, D. J.,** Auxin (2,4-D) stimulation (*in vivo* and *in vitro*) of polysaccharide synthesis in plasma membrane fragments isolated from onion stems, *Biochem. Biophys. Res. Commun.*, 46, 245, 1972.

104. **Delmer, D. P.,** Biosynthesis of cellulose, *Adv. Carbohydr. Chem. Biochem.*, 41, 105, 1983.
105. **Kennedy, C. D. and Stewart, R. A.,** The action of 2,4-dichlorophenoxyacetic acid on maize root membranes, *Chem. Soc. (London) Spec. Publ.*, No. 29, 99, 1977.
106. **Bennett, M. C. and Rideal, F. R. S.,** Membrane behaviour in *Nitella*, *Proc. R. Soc. London Ser. B*, 124, 483, 1954.
107. **Fedtke, C.,** *Biochemistry and Physiology of Herbicide Action*, Springer-Verlag, New York, 1982, chap. K.
108. **Andreev, G. K. and Amrhein, N.,** Mechanism of action of the herbicide 2-chloro-3(4-chlorophenyl) propionate and its methyl ester: interaction with cell responses mediated by auxin, *Physiol. Plant.*, 37, 175, 1976.
109. **Shimabukuro, M. A., Shimabukuro, R. H., Nord, W. S., and Hoerauf, R. A.,** Physiological effects of methyl 2-[4(2,4-dichlorophenoxy)phenoxy] propanoate on oat, wild oat, and wheat, *Pestic. Biochem. Physiol.*, 8, 199, 1978.
110. **Shimabukuro, M. A., Shimabukuro, R. H., and Walsh, W. C.,** The antagonism of IAA-induced hydrogen ion extrusion and coleoptile growth by diclofop-methyl, *Physiol. Plant.*, 56, 444, 1982.
111. **Ray, P. M.,** Auxin-binding sites of maize coleoptiles are localized on membranes of the endoplasmic reticulum, *Plant Physiol.*, 59, 594, 1977.
112. **Lucas, W. J., Wilson, C., and Wright, J. P.,** Perturbation of *Chara* plasmalemma transport function by 2[4(2',4'-dichlorophenoxy)phenoxy]-propionic acid, *Plant Physiol.*, 74, 61, 1984.
113. **Rivera, C. M. and Penner, D.,** Effect of herbicides on plant cell membrane lipids, *Residue Rev.*, 70, 45, 1979.
114. **Hertel, C. and Marmé, D.,** Herbicides and fungicides inhibit Ca^{2+} uptake by plant mitochondria: a possible mechanism of action, *Pestic. Biochem. Physiol.*, 19, 282, 1983.
115. **Leong, T-Y. and Briggs, W. R.,** Evidence from studies with acifluorfen for participation of a flavin-cytochrome complex in blue light photoreception for phototropism of oat coleoptiles, *Plant Physiol.*, 70, 875, 1982.
116. **Mann, J. D. and Pu, M.,** Inhibition of lipid synthesis by certain herbicides, *Weed Sci.*, 16, 197, 1968.
117. **St. John, J. B.,** Effects of herbicides on the lipid composition of plant membranes, in *Biochemical Responses Induced by Herbicides*, Moreland, D. E., St. John, J. B., and Hess, F. D., Eds., American Chemical Society, Washington, D.C., 1982, Chap. 6.
118. **Sumida, S. and Ueda, M.,** Studies of pesticide effects on *Chlorella* metabolism. I. Effect of herbicides on complex lipid biosynthesis, *Plant Cell Physiol.*, 14, 781, 1973.
119. **Hatzios, K. K.,** Effects of sethoxydim on the metabolism of isolated leaf cells of soybean [*Glycine max* (L.) Merr.], *Plant Cell Reports*, 1, 87, 1982.
120. **Wilkinson, R. E.,** Alachlor influence on sorghum growth and gibberellin precursor synthesis, *Pestic. Biochem. Physiol.*, 17, 177, 1982.
121. **Morré, D. J.,** Membrane biogenesis, *Annu. Rev. Plant Physiol.*, 26, 441, 1975.
122. **Mazliak, P. and Kader, J. C.,** Phospholipid exchange systems, in *The Biochemistry of Plants: A Comprehensive Treatise*, Vol. 4, *Lipids: Structure and Function*, Stumpf, P. K., Ed., Academic Press, New York, 1980, chap. 10.
123. **Summers, L. A.,** *The Bipyridinium Herbicides*, Academic Press, New York, 1980, chap. 9.
124. **Dodge, A. D.,** The role of light and oxygen in the action of photosynthetic inhibitor herbicides, in *Biochemical Responses Induced by Herbicides*, Moreland, D. E., St. John, J. B., and Hess, F. D., Eds., American Chemical Society, Washington, D.C., 1982, chap. 4.
125. **Orr, G. L. and Hess, F. D.,** Proposed site(s) of action of new diphenyl ether herbicides, in *Biochemical Responses Induced by Herbicides*, Moreland, D. E., St. John, J. B., and Hess, F. D., Eds., American Chemical Society, Washington, D.C., 1982, chap. 8.
126. **Orr, G. L., Elliott, M. C., and Hogan, M. E.,** Activity *in vivo* and redox states *in vitro* of nitro- and chlorodiphenyl ether herbicide analogs, *Plant Physiol.*, 73, 939, 1983.
127. **Ensminger, M. P. and Hess, F. D.,** Nitro free radical formation of diphenyl ether herbicides is not related to their mechanism of action, *Pestic. Biochem. Physiol.*, 1984, in press.
128. **Albert, A.,** *Selective Toxicity, The Physico-Chemical Basis of Therapy*, 6th ed., Chapman and Hall with Methuen, New York, 1981, 295.
129. **Ziegler, W.,** Ion channels in planar lipid bilayers, formed by the herbicide SENCOR 70 WP, *Biologia (Bratislava)*, 37, 1071, 1982.
130. **Churchill, K. A. and Sze, H.,** Anion-sensitive, H^+-pumping ATPase in membrane vesicles from oat roots, *Plant Physiol.*, 71, 610, 1983.
131. **Marin, B., Marin-Lanza, M., and Komor, E.,** The protonmotive potential differences across the vacuolysosomal membrane of *Hevea brasiliensis* (rubber tree) and its modification by a membrane-bound adenosine triphosphatase, *Biochem. J.*, 198, 365, 1981.

132. **Andersen, H. C.,** Probes of membrane structure, *Annu. Rev. Biochem.*, 47, 359, 1978.
133. **Siedow, J. N., Huber, S. C., and Moreland, D. E.,** Effects of dibromothymoquinone on mung bean mitochondrial electron transfer and membrane fluidity, *Biochim. Biophys. Acta*, 547, 282, 1979.

Chapter 6

THE PHYSIOLOGICAL EFFECTS OF ADJUVANTS ON PLANTS

C. G. McWhorter

TABLE OF CONTENTS

I. INTRODUCTION: ADJUVANTS USED IN WEED CONTROL

Herbicide applications with which an adjuvant is not included in the spray solution are rare. One or more adjuvants may be included in the herbicide formulation and additional adjuvants may be included in the herbicide formulation and additional adjuvants may be added to the spray mixture by the farmer. They are used for many different purposes, including the surfactants that are used as wetting agents, penetrants, or spreaders, co-solvents used as coupling agents, deposit builders or hydroscopic agents used as stickers, stabilizing agents used as emulsifiers or dispersants, and activators used to increase herbicide toxicity.

As discussed by Foy and Smith,[1] the types of action mentioned above "may sometimes be real, sometimes imaginary, but they are not necessarily mutually exclusive". The terminology of herbicidal additives is often confusing because: (1) it is often erroneously assumed that many of the terms listed above are synonymous; (2) the exact role and function of adjuvants is poorly understood; and (3) it is too often assumed that any material that lowers the surface tension of water or increases wettability of the spray solution serves adequately as an adjuvant for herbicides. The vast array of compounds often used in a single adjuvant and the interchangeable but incorrect use of adjuvant terms perpetuate the confusion.

Surfactant is a name derived from the term surface-active agent. In general, these are chemicals that reduce the surface tension of water or increase its wettability. Surfactants are defined by the Weed Science Society of America (WSSA) as materials "that facilitate and accentuate the emulsifying, dispersing, spreading, wetting, or other surface-modifying properties of liquids".[2] The WSSA defines wetting agents as compounds that, "when added to a spray solution, causes it to contact plant surfaces more thoroughly". Adjuvants are materials that facilitate action of a herbicide or that facilitate or modify characteristics of herbicide formulations or spray solutions. There are many adjuvants such as anti-foam agents, buffering agents, compatibility agents, and liquid fertilizer-herbicide mixtures that have little, if any, effect on herbicide activity. Even so, they are valuable aids in dispensing herbicides. All surfactants and wetting agents are adjuvants, but many adjuvants are neither surfactants nor wetting agents.

The most commonly used classification of adjuvants subdivides them into groups according to the type of action: (1), activator adjuvants, which include surfactants, other wetting agents, penetrants, and oils; (2), spray-modifier adjuvants, which include the stickers, film formers, spreaders, spreader-stickers, deposit builders, thickening agents, and foams; and (3), utility modifiers, which include emulsifiers, dispersants, stabilizing agents, coupling agents, co-solvents, compatibility agents, buffering agents, and anti-foam agents. The exact physiological role of most of these is poorly understood although the use of adjuvants in all three categories is increasing. This discussion will be limited primarily to the activator adjuvants, especially the surfactants and oils.

A chemical qualifies as a surfactant "if it tends to concentrate at the surface of a liquid in which it is dissolved".[2] For this to occur in an aqueous system, the surfactant molecule should be comprised of two components, (1) a polar hydrophilic segment that is readily soluble in water; and (2) a water insoluble (lipophilic) portion that is comparatively nonpolar. Surfactants are usually classified on the basis of the polar, hydrophilic portion of the molecule, i.e., anionic, cationic, nonionic, or ampholytic.

Commonly used anions with anionic surfactants include the sulfates, phosphates, and carboxylates. The primary type of cation in cationic surfactants is a quaternary nitrogen compound. Cationic surfactants are rarely used with herbicides as are the ampholytic surfactants. The latter contain hydrophilic segments with both acidic and basic segments.

The most common surfactants used with the herbicides are nonionic. Although they do not have ionizable polar end groups, nonionic surfactants contain both hydrophilic and

Table 1
HYDROPHILE-LIPOPHILE BALANCE VALUES AND SURFACTANT USEFULNESS

HLB range of surfactants	Area of general surfactant usage
4 to 6	Water in oil emulsifiers
7 to 9	Wetting agents
8 to 18	Oil in water emulsifiers
13 to 15	Detergents
10 to 18	Solubilizers

lipophilic segments. Various molecular weights of ethylene oxide most commonly represent the hydrophilic group in nonionic surfactants. The hydrophilic nature of the molecule usually occurs because of the presence of sulfur or oxygen linkages. These may be amides, esters, or ether groups. The water solubility of commonly used nonionic surfactants such as octylphenol attached to ethylene oxide generally increases as the level of ethylene oxide attached increases.

Hydrophobic-lipophobic-balance (HLB) numbers are an index used to suggest the applicability of surfactants as emulsifiers, detergents, and, increasingly, as activator adjuvants. The HLB system was originally introduced by Griffin[3] in the late 1940s as an aid in emulsifier selection. Nonionic surfactants are assigned numbers on a 1 to 20 scale; however, some ionic surfactants may have HLB values up to 40. HLB numbers reflect the hydrophile-lipophile balance of the polar-nonpolar groups. HLB values are used in a practical manner to match compatible oil phases and surfactants of mixtures by selecting components with the same HLB number for trial emulsions. HLB values have been used with increasing frequency in recent years in attempts to estimate and select desired increases in activity of herbicides obtained from the use of activator adjuvants. HLB values also have other purposes as summarized in Tables 1 and 2.

Until the mid 1970s, the principal activator adjuvants used in conjunction with herbicides were surfactants. Since that time, blends of phytobland oils and surfactant have become increasingly popular as adjuvants with herbicides applied postemergence. Phytobland oils are paraffinic petroleum distillates that are nontoxic to plants. Originally phytobland oils were marketed with 1 to 2% surfactant, which served as the emulsifying agent, but the primary products marketed in recent years contain 80 to 93% phytobland oil and 7 to 20% surfactant. The base oil is a highly refined paraffinic hydrocarbon.[2] There are dozens of the oil-surfactant concentrates sold as adjuvants throughout the U.S., but the identity of the surfactant or mixture of surfactants in these concentrates is rarely known. The lack of defined chemistry in these products has almost certainly discouraged basic studies of their physiological effects and on their mechanisms of action.

II. EFFECTS OF ADJUVANTS ON HERBICIDE PENETRATION

A. The Plant Surface

Components of the leaf surface that influence the retention and ultimate penetration of an herbicide into the plants include the cuticle, the epidermal wall, stomata, and leaf hairs. The cuticle, composed of epidermal wax and cutin, presents the most effective barrier to penetration into the leaf. This barrier is not completely effective because it is known that water and solutes are able to move across the cuticle.[4] Also, cuticular transpiration is a

Table 2
HYDROPHILE-LIPOPHILE BALANCE VALUES AND SURFACTANT DISPERSIBILITY OR SOLUBILITY IN WATER

HLB range	Dispersibility or solubility of surfactant in water
1 to 3	No dispersibility in water
3 to 6	Poor dispersion
6 to 8	Milky dspersion after vigorous agitation
8 to 10	Stable milky dispersion
10 to 13	Translucent to clear dispersion
>13	Clear solution

commonly known phenomena, as is the cuticular penetration of pesticides that have been applied to the leaf surface.

The plant cuticle is composed of both surface and subsurface wax, with the subsurface wax often associated with cutin. A typical cross-section of a leaf surface in schematic form is shown in Figure 3, Chapter 8.

All plants have an amorphous wax film.[5] The wax film may be covered with a large array of microscopic wax structures that are in the form of ribbons, rods, tubes, plates, dendrites, or filaments. According to Baker[5] the surface waxes are made up of many different sterols, flavonoids, long-chain aliphatics, and pentacyclic triterpenoids. The primary alcohols, hydrocarbons, secondary alcohols, β-diketones, and ursolic acid do not commonly occur as major components. The embedded waxes that often occur in association with cutin are primarily C_{16}:C_{18} fatty acids. Baker has proposed that the surface waxes are transported through the cuticular membrane in solution in a volatile solvent.[5] The morphology of surface wax is determined by the chemical composition of the wax exudates, but these may vary as a result of crystallization rates, solution concentration, and environmental conditions under which plants are growing.

Holloway has presented a detailed review of plant cutins including chemistry, occurrence, structure, and factors affecting cutin content and composition.[6] He states that the entire process of synthesis and development of cutin is under the control of both specific and localized enzymes and that the cutin monomers appear to be derived primarily from the biotransformation of two commonly occurring fatty acids, hexadecanoic, and 9-octadecenoic. Basically, the cutins are insoluble, high molecular weight lipid polyesters that are composed of different long-chain substituted aliphatic acids. The primary functional group of the monomers is hydroxyl, although many other groups may be present, including aldehyde, ketone, epoxide, and unsaturated groups. Cutins in higher plants are in three broad categories, based on chain lengths of the major monomers, including a C_{16} type, a C_{18} type, and a mixed C_{16} and C_{18} type.[6]

The cellulose wall of epidermal cells is not considered to be a major barrier to the penetration of adjuvants and pesticides into plants.[7] Similarly, neither the layer of pectin often found between the cuticular layers and the walls of cells nor the pectin associated with the cellulose wall are considered to be major barriers to penetration. There has been little research reported to demonstrate the relative importance of pectin or cell walls in herbicide or adjuvant penetration.

Trichomes or leaf hairs occur in a wide variety of shapes and sizes.[7-9] The morphology of these varies between genera and even between plant species. Trichomes often play an

important role in spray retention on leaf surfaces, but there has been little research reported on the specific role of these regarding adjuvant penetration into leaves.

Stomata and accompanying guard cells are significant components of leaf surfaces, but their involvement in the overall efficiency of adjuvant activity has not been reported. It has been shown that stomata may be penetrated by herbicides, fluorescent dyes, and other materials,[7,10-12] but the role of these, with regard to adjuvant penetration and with regard to adjuvants increasing herbicidal penetration, is essentially unknown.

B. Effect of Adjuvants on Herbicide Efficacy and on Spray Properties

1. Effect of Adjuvants on Herbicidal Efficacy

It has been known for more than 30 years that surfactants increased the activity of sprays of herbicides.[13] Many subsequent reports have shown that this occurs with many different types of herbicides under many different environmental conditions.[14-22] Their use often increases the level of weed control obtained by 10 to 50%. Perhaps surfactants enhance herbicidal action by their combined polar and nonpolar properties in the same molecule, providing compatible aqueous and lipoidal phases.[1] This theory is supported by Becher and Becher who showed a relationship between HLB of the surfactant used and the increase in herbicide activity.[23]

The surfactants most commonly used as activator adjuvants in North American agriculture are octoxynol {α[*p*-1,1,3,3-tetramethyl butyl phenyl]-ω-hydroxypoly(oxyethylene)}, dooxynol {α(*p*-dodecylphenyl-ω-hydroxypoly(oxyethylene)}, nonoxynol {α-(*p*-nonylphenyl)-ω-hydroxypoly(oxyethylene)}, oxysorbic, or ethoxylated alcohols.[2] These nonionic surfactants are added to spray mixtures to provide concentrations of 0.1 to 0.5% (v/v) surfactant. They have been used most often in mixtures with substituted urea, triazine, arsenical, and phenoxy herbicides to control weeds in all major crops.[2]

Surfactants used as activators are marketed as liquids, usually containing 50 to 90% active ingredient. They generally have HLB values of 10 to 13. Activator surfactants that are commonly applied with growth regulators, desiccants, chemical thinners, and pruners, have HLB values of 15 to 16. These are usually applied in the same concentrations as the surfactants used with herbicides.

The oil-surfactant concentrates are usually used at a rate of about 2.4 ℓ/ha, regardless of the volume of diluent. As a result, the concentration of adjuvant that would be applied in the spray mixture will vary from about 0.62 to 2.5% when herbicide applications are in water at 90 to 360 ℓ/ha. Because the oil-surfactant concentrates contain only 7 to 20% surfactant, the amount of surfactant applied per hectare is lower than when activator surfactants are used.

2. Effects of Adjuvants on Spray Properties

Many different theories have been proposed in the attempt to explain why adjuvants enhance herbicide penetration and effectiveness of foliar-applied herbicides. Those offered by Parr and Norman[24] include: (1) lowering of surface tension; (b) improved coverage; (c) removal of air films between spray and leaf surface; (d) reduced interfacial tension between relatively polar and nonpolar submicroscopic regions of the leaf cuticle; (e) induced stomatal entry; (f) increased permeability of the leaf cuticle and plasma membrane; (g) facilitated cell-wall movement in the region of the wall-cytoplasm interface; (h) action as a co-solvent; (i) reaction or interaction directly with the herbicide in some manner to allow a more ready penetration of electrically charged cuticles and membranes; and (j) action as a humectant. Each of these may be important, but little definitive evidence has been reported to support any of these theories.

Adjuvants cause a drastic reduction in the surface tension of spray solutions.[25] Reduction of surface tension reduces droplet size, and it is generally agreed that reduced droplet size

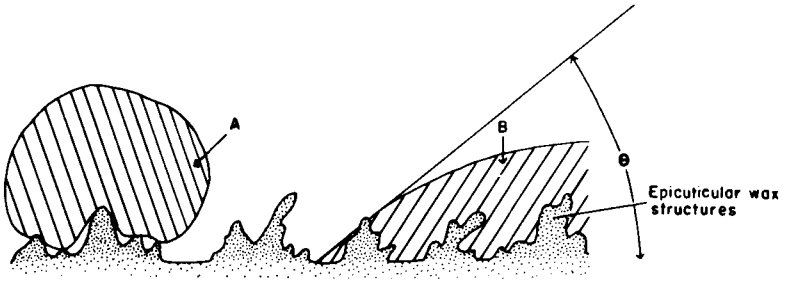

FIGURE 1. Diagrammatic representation of spray droplets on a leaf surface as seen in cross section. Surface wax structures prevent a spray droplet without surfactant (A) from contacting the cuticle proper. A droplet with proper surfactant (B) has a greatly reduced contact angle (θ), enabling it to effectively contact the leaf surface. (Adapted from Hull, H. M., Davis, D. G., and Stolzenburg, G. E., in *Adjuvants for Herbicides*, Weed Science Society of America, Champaign, Ill., 1982. With permission.)

results in increased herbicide penetration. A number of earlier reviews are available on pesticide absorption and translocation that relate to this subject.[7,19,24,26-29] These will not be discussed here because they do not relate directly to the effect of adjuvants on plants.

The reduced surface tension of spray solutions caused by the addition of adjuvants reduces the contact angle of the impinging droplet as illustrated in Figure 1. This results in better initial coverage of the spray droplet and increased secondary wetting. Details of increased wetting have been discussed by Johnstone[30] and the kinetics of increased wetting were reviewed by Freed and Witt.[31] It is not possible to generalize in claiming that adjuvants always increase herbicide deposition on leaves. Reduced surface tension may result in increased spray retention up to a point, but may then reduce spray retention because of increased runoff.[22,32,33] A given surfactant may enhance retention and penetration in some species, but not in others, altering selectivity.[22]

A large number of herbicides have been shown to alter the synthesis of surface wax. Several references on this effect are available in a recent review,[7] and this subject is discussed further in Chapters 4 and 8. However, there is only one report available that shows that a specific surfactant altered the structure of surface wax. Takeno and Foy[34] showed that a lipophilic polysorbate surfactant, with an HLB of 4.3, caused a reticulate pattern on the surface of cotton (*Gossypium hirsutum* L.) leaves. Surfactants with higher HLB numbers (12 to 15) had a lower affinity for the surface waxes. Based on specific interactions between plant-surfactant-pesticide activity, Price concludes that surfactants do more than improve adhesion and droplet behavior on the leaf surface.[35] He also suggested that surfactants might, by modifying the hydrophilic-lipophilic interface within the cuticle, increase the size and accessibility of aqueous routes. In general, the influence of surfactants on wax viscosity is unknown.[19] While it is known that surfactants penetrate and may even cross the cuticle, Price[19] suggests that it may be possible for them to change viscosity locally by mixing or co-micellizing with amphipathic acids and alcohols of the wax.

Another important property of surfactants in herbicide sprays is that they retain water and act as humectants, especially those with higher HLBs.[35] Also, they maintain solution activity, facilitating solute partition into wax. This action results from a supply of water from surfactant micelles to maintain a high concentration of solute.[35]

III. FATE OF ADJUVANTS IN PLANTS

The extent of research on the quantitative uptake of adjuvants is surprisingly limited,

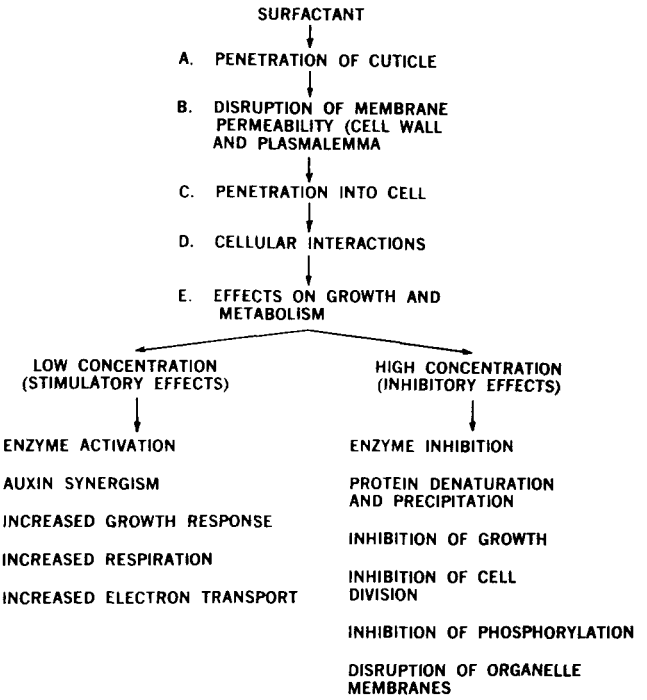

FIGURE 2. Probable pathway and possible sequence of events involving surfactants in plant systems. (Adapted from Parr, J. F., in *Adjuvants for Herbicides*, Weed Science Society of America, Champaign, Ill., 1982, 93. With permission.)

especially when viewed from the perspective of the extensive annual use of adjuvants. A large number of reports that are based on overall gross plant responses strongly suggest that adjuvants enter the plant, but this information is empirical and does not provide useful information on the amount of adjuvant that enters the plant or on its distribution. This type of information is summarized in a recent review.[29] Most of the reported research has been conducted with commercially available samples that are poorly defined chemically.[36] The chemical structures provided have often been general in nature, and the formulations tested may have contained mixtures of surfactants rather than specific compounds. Ethoxylated-nonionic surfactants are usually manufactured in such a manner that they are mixtures of compounds with numbers of side chain or side groups distributed around a Poisson distribution curve. The distribution of chemicals within the mixture may vary between different batches or lots although the "average" composition remains constant. This could be of critical importance in physiological research, so caution needs to be exercised in reviewing the literature. A summary of pathways and events that could be involved in the effect of surfactants on plants is shown in Figure 2.

A. Uptake and Translocation of Adjuvants

Research on uptake has been conducted with only about seven radiolabelled surfactants. Foy et al.[11] reported that ^{35}S-labeled sodium lauryl sulfate penetrated cotton and barley (*Hordeum vulgare* L.) leaves rapidly, but they did not provide quantitative levels of entry. Movement of the surfactant following uptake was apoplastic. Although the surfactant was not metabolized after 24 h, it had apparently been metabolized after 7 days and the ^{35}S was widely distributed throughout the plants. Foy et al.[11] also reported that ^{14}C-T-1947 (po-

lyoxyethylene polyoxypropylene polyol) entered cotton and barley leaves much slower than radiolabeled sodium lauryl sulfate, but that subsequent translocation was similar to that obtained with sodium lauryl sulfate. They also reported that the ^{14}C-T-1947 tended to accumulate in the lysigenous glands of cotton, an effect not found with radiolabeled sodium lauryl sulfate.

It was concluded that low levels of ^{14}C-Pluronic L-62 (condensate of ethylene oxide with hydrophobic bases formed by condensing propylene oxide with propylene glycol) entered the leaves of bean (*Phaseolus vulgaris* L.) plants.[37] Less than 20% of the radiolabeled applied remained on the leaf after washing the leaf with ethanol-water, but it was not determined if the level that absorbed translocated from the cuticle.

Smith and Foy[38] reported on the uptake and distribution of the radiolabel from ^{14}C-Tween® 20 (polyoxyethylene sorbitan monolaurate), utilizing samples radiolabeled in both the fatty acid and the oxyethylene. They cautioned that Tween® 20 is normally a complex mixture of ethoxylated sorbitol and its anhydrides esterified with lauric acid. In its commercial synthesis, Tween® 20 could possibly include free polyols, polyoxyethylene polyols, poloxyethylated sorbitol, polyoxyethylated sorbitan, polyoxyethylated sorbide, and the mono- and diesters of the above polyols. They acknowledged that the chemical composition of the radiolabeled samples used in their studies may have varied from that of commercial Tween® 20. They recovered above 99% of the total ^{14}C-activity applied 4 days after foliar treatment when they used ^{14}C-fatty acid-labeled Tween® 20 and nearly 98% of the ^{14}C-activity when the surfactant was oxyethylene radiolabeled. Only very low levels of the ^{14}C-fatty acid Tween® 20 translocated out of the treated leaf, although ^{14}C-activity was detected in other plant parts including roots and stems of beans. About 2.5% of the ^{14}C-oxyethylene Tween® 20 translocated away from the treated spot into the distal portion of bean leaves, but very low concentrations were found in other plant parts. Their data suggested that there was some evidence of metabolism of the ^{14}C-fatty acid material. The ^{14}C-fatty acid Tween® 20 primarily was phloem mobile while the oxyethylene label was primarily xylem mobile.[38]

Norris studied the penetration of astomatous pear (*Pyrus communis* L.) leaf cuticle by ^{14}C-labeled Tween® 20, Span® 20 (sorbitan monolaurate), and Span 80 (sorbitan monooleate).[39] The ^{14}C-Span® 20 penetrated slightly more rapidly than the other surfactants, but, nevertheless, barely detectable levels of radioactivity penetrated the cuticle. The largest amount of penetration found 48 h after treatment with Span® 20 was only 2% of the total applied.

About 90% of the applied ^{14}C-labeled fatty acid portion of Tween® 80 (polyoxyethylene sorbitan monooleate) remained on the leaf surface or in treated leaves of johnsongrass [*Sorghum halepense* (L.) Pers.] and soybeans [*Glycine max* (L.) Merr.] 3 days after treatment.[40] Translocation of ^{14}C from the treated spot was basipetal, but ^{14}C was detectable in all plant parts. ^{14}C was also found in the nutrient solution in which plants were growing (0.3 to 0.4% of that applied in johnsongrass and soybeans, respectively) and as $^{14}CO_2$ in the enclosed systems in which plants were growing. Only 0.8% of the applied radiolabel was recovered as $^{14}CO_2$ in containers in which johnsongrass was growing, but 9.1% of the total radioactivity applied to soybeans was recovered as $^{14}CO_2$ 3 days after application. More than 97% of the radioactivity remained on leaf surfaces and in treated leaves of johnsongrass and soybeans when Tween® 80 was labeled in the polyoxyethylene portion of the molecule. Only trace amounts of the ^{14}C-polyoxyethylene was found in untreated portions of the plants or in the nutrient solution in which plants were growing.

Stolzenberg et al.[41] found that ^{14}C-Triton® X-100 (isoctyl phenyl polyethoxy ethanol) was absorbed from the surface of barley and that water or methanol rinses removed less than 30% of the ^{14}C-activity 1 day after treatment and less than 20% up to 8 days after treatment. Most of the ^{14}C-activity remained in the treated portion of the leaf, about 10% was translocated acropetally, and there was no appreciably basipetal translocation. They also found

that excised barley leaves took up more than 70% of the ^{14}C-Triton® X-100 within 5 h after treatment. The ^{14}C-activity was uniformally distributed throughout the leaf blade within 5 h and greatest accumulation occurred in the cut ends of leaves in the end tissues adjacent to the midvein and the four main veins in each leaf.

Other research has been conducted concerning the penetration of adjuvants into the plants, but these used less direct methods than those discussed above and will not be discussed because they are summarized in a recent review.[29] Thus, in summary, there is increasing evidence that surfactants enter the plant through foliar absorption, but the mechanisms and mode of entry are unknown.

Roots also absorb surfactants of different types, although the amounts absorbed and translocated are usually less than with foliar applications. Even less is known about root uptake of surfactants than is known about foliar uptake. This literature has been recently reviewed.[29]

B. Metabolism of Adjuvants

There has been comparatively little research to describe the metabolism of surfactants in plants. The most definitive research reported is that of Stolzenberg et al.[41] who studied the metabolism of ^{14}C-Triton® X-100 in barley plants. They found that about 50% and less than 20% of the ^{14}C in methanol extracts from surface-rinsed leaves was the parent surfactant 1 and 6 days after treatment, respectively. They found greater quantities of nonpolar metabolites when treatments were applied via the cut ends of excised leaves than when the surfactant was applied to the leaf surface. Several nonpolar metabolites, including lower molecular weight ethoxylates were present, but there were higher concentrations of the polar ^{14}C-labeled products. Acid hydrolysis of the polar products released about 30% of the ^{14}C-label as the parent surfactant. Their results indicated that up to 20% of the surfactant taken up by plants was oxygenated in the *tert*-octyl moiety and the resulting metabolites occurred primarily in polar conjugates.[41]

Smith and Foy's[38] results suggest that Tween® 20 was at least partially metabolized 4 days after application. The identity of the metabolites was unknown, but they suggested the possibility that the ester, whether formed between the lauric acid portion of the molecule and the sorbitan ring or as a polyoxyethylene chain, might be cleaved and that the fatty acid portion of the molecule might be metabolized by β-oxidation.

Results similar to those of Smith and Foy[38] have been obtained using ^{14}C-Tween® 80.[40] Paper chromatography was used for comparative separation of the original Tween® 80 sample (labeled in both the fatty acid and the polyoxyethylene), leaf washes, and extracts from treated leaves 4 days after surface applications. The results strongly suggested that the surfactant molecule was cleaved on the surface of the leaf and that there was further cleavage after penetration. Results were confirmed by spraying the paper chromatograms with reagents to distinguish spots of nonionic surfactants from those of the polyethylene glycols. Further evidence that ^{14}C-Tween® 80 is metabolized in soybeans and johnsongrass is the presence of ^{14}C-CO_2 from plants treated on leaf surfaces with ^{14}C-Tween® 80. The $^{14}CO_2$ was found only after use of Tween® 80 radiolabeled in the fatty acid, but not when the radiolabel was in the polyoxyethylene.[40]

Stolzenberg and Olson[42] reported that Triton® X-100 was not extensively de-ethoxylated by rice (*Oryza sativa* L.) leaf tissue. Frear et al.[43] reported that plant cells metabolized TOP-6EOH and TOP-9EOH in vitro with formation of both glucose and fatty acid conjugates.

IV. EFFECTS OF ADJUVANTS ON PHYSIOLOGY OF THE PLANT

A. Gross Effects on Growth and Development

A wide variety of surfactants have been shown to stimulate or inhibit plant growth. Most

of the reported research concerns the effect of surfactants on crop plants, but similar responses have been obtained on weeds. Phytotoxic and inhibitory effects include epinasty, discoloration, dessication, a water-soaked appearance on leaves, and plant death. The number of reports showing effects of surfactants on plants is so great that this review will include only selected references. More extensive reviews are available.[14,29,36,44]

According to Dobozy and Bartha,[44] the use of surfactants to increase crop yields is first mentioned in U.S. patent 2,350,709 in 1942. Relatively few reports have been published that verify increased crop yields following the soil applications of surfactants. These include Nadasy and Dobozy,[45] who increased the growth of corn (*Zea mays* L.), beans, wheat (*Triticum aestivum* L.), and tomato (*Lycopersicon esculentum* L.) with octadecaglycol monooleate, Seymour,[46] who increased the yield of carrots (*Daucus carota* L.) with Ultrawet® PR 51 (alkyl aryl sulfonate), Dobozy,[47] who increased the growth and yield of wheat and potatoes (*Solanum tuberosum* L.) with Evagros surfactants (chemistry not provided), and Beal et al.,[48] who increased the dry weight of *Datura tatula* L. plants with Tween® 20. There are many different reports which either indicate no growth or yield effect from surfactants or which indicate that higher concentrations of surfactants inhibit growth.[49]

Several reports on the effect of surfactants on root growth exist. In general, these reports show that roots are very sensitive to the presence of surfactants in the growth medium and that the level of inhibition or growth retardation is related to surfactant concentration. Some of the more extensive reports are by Parr and Norman,[24] Luxmoore et al.,[49] MacDowall,[50] Endo et al.,[51] and Buchanan.[52]

One of the most extensive reports on the effect of surfactants on plants, both in scope and the number of surfactants evaluated, is that of Buchanan.[52] The effects of about 100 surfactants from four ionogenic groups were studied on seed germination, elongation of corn seedling radicles, growth of soybean seedlings following applications to foliage or roots, and on loss of permeability of cells of beet (*Beta vulgaris* L.) root tissue. He found that the toxicity of materials in most surfactant families decreased with increasing content of polyoxyethylene per mole of hydrophobe. The hydrophobe was often influential in determining toxicity of individual surfactants. Buchanan felt that it was not possible to classify the toxicity of surfactants on the basis of ionogenic groupings. He did find, however, that a large number of surfactants are phytotoxic to plants and that, in general, root elongation was more sensitive to surfactants than the other bioassay methods. It has been suggested that root surfaces may be more sensitive to surfactants than leaves or stems because of the absence of a cuticle.[36]

There is considerable evidence that the Tween® surfactants may inhibit or stimulate plant growth.[53-58] Mendez et al.[54] and Vieitez et al.[58] reported that Tween® 80 increased cell elongation in *Avena* coleoptile section studies at concentrations as low as 0.01% in some experiments, but not in others. Tween® 20 at 0.01% inhibited elongation of coleoptiles, but the results that these workers obtained with Tween® 40 (polyoxyethylene sorbitan monopalmitate) were inconclusive. Stowe[56,57] found that low concentrations (3 to 30 mg/ℓ) of both Tween® 20 and Tween® 80 stimulated pea (*Pisum sativum* L.) stem section elongation in studies with indoleacetic acid and gibberellic acid, but that Tween® 80 at 100 mg/ℓ continued to cause stimulation while Tween® 20 at the same concentration was inhibitory. In research with *Amaranthus caudatus* L., Knypl[53] found that Tween® 20 increased hypocotyl expansion, although he also found inhibition of internode elongation. He also found that different lots of Tween® 20 significantly varied in their effect on plants. Knypl also reported a remarkably high level of activity when Tween® 60 and Tween® 65 (polyoxyethylene sorbitan triesterate) were applied to *Amaranthus*. As a result of studies showing biological activity of the Tween® compounds, several investigators such as Vieitez et al.[58] abandoned the use of the Tween® compounds in biological research with growth regulators.

Davis et al.[59] found that ethoxylated 1-dodecanol of three chain lengths would either

stimulate or inhibit growth of soybean cell suspensions. Stimulated growth occurred at concentrations of 55 μM or less, depending on the degree of ethoxylation, but growth inhibition occurred at concentrations ranging from 11 to 120 μM depending on the compound. Growth inhibition was related to the degree of ethoxylation.

Surfactants may inhibit mitoses.[49,60-62] Nethery[60] founded more than half of 22 surfactants evaluated caused inhibition of mitosis when applied at 0.1% in an assay on pea root meristem mitosis. Several of the surfactants inhibited mitosis more than 80%. There are no reports that explain the mechanism of the surfactant-inhibited mitosis.

In general, surfactants are more toxic to fungi and bacteria than to higher plants. In fact, so many specific surfactants are toxic to these organisms that they are effective as bactericides and fungicides. These effects are well documented and a number of reviews are available.[24,29,36,63-65]

The cationic surfactants, often quaternary ammonium compounds, are among the most effective as bactericides.[36] They are apparently toxic because they cause membrane leakage. Other reasons given for a high level of toxicity to bacteria include denaturation and precipitation of enzymes and other proteins and formation of various surfactant-protein complexes.[36]

In general, fungi are not as sensitive to surfactant treatment as bacteria, but many surfactants are highly toxic to fungi. Cationic surfactants are frequently cited as being more toxic than other ionic groups.[36] Germination and growth of germ tubes of spores are particularly sensitive to surfactants. Damage to the membrane is often cited as one of the primary reasons for surfactants having fungicidal properties. Many other physiological activities are disturbed by surfactants, including inhibition of nucleic acid and protein synthesis and, in a few examples, either increased or decreased enzyme activity.

B. Effects on Membranes

According to Miller and St. John,[66] surfactants are well known for their ability to disrupt the normal permeability characteristics of plant cells. Membrane alterations caused by surfactants can result in varied effects including leakage from cells, enhanced growth rates, and inhibition of photophosphorylation. These membrane-associated effects can contribute to phytotoxicity.

Norris[39] states that the effect of surfactants on membranes results from both surface tension-modifying effects and from the inherent HLB of the surfactant. When the surfactant concentration is below the critical micelle concentration (CMC), the effects may result from altering partitioning of other substances either into or through biological membrane. Norris concluded in his review that surfactants employed in concentrations above the CMC could solubilize either the lipid components of membranes or waxes of the cuticle.

Surfactants may exert an effect on membranes, either in true solution or as complex micelles, when the concentration is above the CMC. The formation of micelles results in the incorporation of normally insoluble substances into the micelle, causing solubilization. Surfactants that are in true solution may move freely through membranes while the micelles are almost completely excluded.[67-69] Even so, the toxicities of diuron, atrazine, and ametryne are greatly increased with surfactant concentrations above the CMC,[70] indicating increased membrane penetration of the herbicides. Also, Thomson and Moeller[71] have shown that Tween® 20 at 1%, well above the CMC, disrupts granal thylakoids of isolated spinach (*Spinacea oleracea* L.) chloroplasts. They suggest that Tween® 20, by preferentially disrupting granal thylakoids along their lateral margins, can serve as a new approach in probing the structure and function of photosynthetic membranes.

Quantin-Martenot et al.[72] found that the plasma membrane-bound uridine-dephosphate-glucose-sterol-β-D-glucosyltransferase of etiolated corn coleoptiles was activated by concentrations of Triton® X-100 that were less than the CMC. They also found that treatment of plasma membrane vesicles with hydroxyl ions or with phospholipase A_2 caused stimulation

of enzymatic activity and eliminated the capacity of the enzyme to be activated by Triton® X-100.

Haapala[73] studied the effect of Triton® X-100 on protoplasmic streaming, on plasmolysis, and on leakage of solutes from cells using tissues from several different plants. She found that surfactant concentrations were critical in causing detrimental effects in cells and that these concentrations were related to the CMC. At concentrations above the critical range at which the CMC occurred (0.007 to 0.01%), protoplasmic streaming ceased within a brief time, and in concurrent tests with beet tissues definite leakage of sugars occurred. Below the critical range, there was an enhanced retention of solutes in beet discs.

Several other investigators have shown that surfactants have a profound effect on the integrity of plant cell membranes, including the plasmalemma and membranes within the cell.[73-80] These studies include the effect of surfactant on the loss of radiolabeled carbon from isolated cells, betacyanin efflux from beet tissue, the movement of dyes into the lipid micelles, and the inhibition of protoplasmic streaming in *Nitella*. These results strongly suggest that surfactants have far-reaching effects on plant membranes.

Helenius and Simons[75] have provided an extensive review on the solubilization of membranes by surfactants. The primary interest of their review was on the effect of surfactants on bacteria, but they presented a vast amount of information, including coverage of: (1) effects of surfactants on lipids; (2) binding of surfactants to water-soluble proteins; (3) binding of surfactants to membrane proteins; (4) the usefulness of surfactants in the solubilization of membranes into lipoproteins, proteins, and mixed micelles; separation of lipids and proteins; and (5) selective solubilization of membrane components.

C. Effects on Photosynthesis

There are several reports which show that surfactants and phytobland oil-surfactant combination interfere with photosynthesis. This was demonstrated by St. John et al.[78] using nonionic, anionic, and cationic surfactants. They used Tween® 20 and Daxad® 21 (monocalcium salt of polymerized aryl alkyl sulfonic acids) as examples of surfactants with low inherent phytotoxicity and Sterox® SK (polyoxyethylene thioether) and AHCO® DD 50 (alkylbenzyl quaternary ammonium halide) as examples of surfactants with high inherent phytotoxicity. All four surfactants at a concentration of 0.01% decreased the rate of photosynthetic $^{14}CO_2$ fixation by more than 50% in wild onion (*Allium canadense* L.), but only Sterox® SK and AHCO® DD 50 reduced $^{14}CO_2$ fixation in soybeans, 16 and 4%, respectively. They hypothesized that: (1) the phytotoxic surfactants had a direct interaction with membranes, rendering them incapable of maintaining normal permeability characteristics; and (2) surfactants promote herbicide uptake and movement through a surfactant-effected reduction of permeability barriers in treated leaves.[78]

Coats and Foy[81] reported that foliar applications of a phytobland oil containing 1% Triton® X-207 (alkylaryl polyether alcohol with nonionic solubilizer) reduced $^{14}CO_2$ fixation in corn. The degree of inhibition of photosynthesis was dosage-dependent within the application rate range of 9.35 to 99.2 ℓ/ha. Inhibition was about 50% when the oil was applied at 18.7 ℓ/ha.

Several reports show that Triton® X-100 can adversely affect photosynthesis and chloroplast organization. Some of these effects include inhibition of chlorophyll biosynthesis,[82] disorganization of the grana of chloroplast,[83] reduced chloroplast volume,[84] reduced photophosphorylation,[85,86] and altered donor-acceptor reactions.[86] Apparently there is only one report of increased photosynthesis from use of surfactants.[87]

As mentioned earlier, Thomson and Moeller[71] showed that Tween® 20 preferentially disrupts granal membranes from spinach. They used Tween® 20 at 1% (v/v), but this concentration would be unlikely to occur within plant cells following applications to the leaf surface, based on the limited absorption and translocation discussed earlier. Even so, there

is an increasing amount of evidence which indicates that surfactants can be detrimental to the photosynthetic process.

D. Effects on Translocation of Herbicides

There are numerous reports showing that surfactants increase the translocation of herbicides. The degree to which translocation of herbicides is increased may be dependent on many different variables including the species of plant under investigation, age of the plant, portion of the plant treated, the volume of diluent in which the treatment is applied, surfactant concentration, herbicide concentration, the use of other herbicides or other pesticides in the treatment mixture being applied, and the environmental conditions preceding and following the application of the herbicide.

There are so many reports available that demonstrate the efficiency of surfactants in increasing herbicide translocation that only three examples from the author's research will be briefly discussed.

Sterox® NJ (ethoxylated nonylphenol) at 0.5% increased the amount of ^{14}C-glyphosate that absorbed into soybean leaves 72 h after treatment from 25 to 55%.[88] About 50% more ^{14}C was either in the treated leaf or had translocated out of the treated leaf 72 h after treatment when the surfactant was included in the treatment mixture than when glyphosate was applied alone.

The amount of ^{14}C-dalapon translocated out of johnsongrass leaves 24 h after treatment with 0.5% (v/v) Sterox® NJ was increased from 29 to 34% with an air temperature of 21°C and 35% relative humidity (RH); translocation increased from 42 to 55% at 21°C and 100% RH.[89] At 38°C the surfactant increased translocation of the ^{14}C-dalapon from 23 to 30% at 35% RH, and from 8 to 15% at 100% RH. These increases may not seem large, but increases of this type may result in a much higher level of weed control under field conditions than if surfactant had not been included in the mixtures.

Both Sterox® NJ and Ethomeen® 0/15 (ethylene oxide condensate of oleyl amine) at 0.5% increased the absorption and translocation of ^{14}C-mefluidide in johnsongrass, soybeans, and cocklebur (*Xanthium pensylvanicum* Wallr.).[90] At 22°C and 100% RH, the Ethomeen® 0/15 was more effective in increasing translocation of the ^{14}C-label out of the treated area than Sterox® NJ, but at 32°C in 100% RH the reverse was true. This work also showed that the surfactant that provided the greatest increase in absorption was not always best in increasing translocation out of the treated area. There has been no in-depth research on this variable, but perhaps the most efficient surfactants in increasing herbicide penetration into the plant are not necessarily the best to increase subsequent translocation throughout the plant. The overall picture may even be more complicated because those surfactants that are most efficient in increasing absorption and/or translocation under one environmental condition may not be best under other conditions. A further complication is that while there is considerable research reported on surfactant-caused increased absorption and translocation of ^{14}C-herbicides there are no reports that show the molecular form of the herbicide after translocation from the treated area. This, too, could be affected by the presence of surfactant and, conceivably, could be altered by the type and concentration of surfactant used.

Most of the limited work done on the effect of surfactants on ion absorption and translocation has been with phosphorus and potassium. Some surfactants may have little or no effect on absorption and translocation of these elements, but there is an increasing amount of evidence that some surfactants inhibit uptake and translocation. Both Parr and Norman[24] and Cairns[91] have shown that surfactants reduced uptake of K^+ by barley. Parr and Norman found that Tween® 20 and Tween® 80 at 0.1% (v/v) reduced uptake in excised barley roots. Cairns obtained similar results with Aerosol OT® (sodium dioctyl sulfosuccinate) and Sorapon® S-96 (sodium dodecylbenzene sulfonate) at 0.6 and 1.2%, respectively. Cairns also found that Aerosol OT® reduced Mg^{2+} uptake, but that Sorapon® S-96 did not. Four reports

indicate that surfactants reduce absorption and translocation of phosphorus in beans.[92-95] Swanson and Whitney[92] reported reduced absorption and translocation of phosphorus by 90% following foliar application of Tween® 80. Koontz and Biddulph[93] found reduced translocation of phosphorus from sprayed leaves when solutions contained Tergitol®-7 (sodium sulfate derivative of 3,9 diethyl tridecanol-6) and Vatsol® OTB (dioctyl ester of sodium sulfosuccinic acid) at 0.05%. They also found, however, that several other surfactants had little effect on absorption and translocation of phosphorus. Teubner et al.[94] reduced the amount of phosphorus that translocated from sprayed leaves when a 1% solution of several different surfactants were used. Vlazyuk and Prikaodko[95] reported reductions in phosphorus uptake from solutions that contained sodium dodecylsulfate and Triton® X-100. Other research has also suggested that surfactants applied in the root zone may alter ion content in plants.[96] It would not appear, however, that the surfactant content under field conditions would be as high as those used in most of the reported research. This conclusion has also been reached by others.[91]

E. Other Physiological Effects

Surfactants will precipitate and denature proteins that are in solution and Glassman[63] provided an extensive review on the subject as early as 1948. These effects occur because of the charge on the surfactant and may be dependent on factors such as the degree of ethoxylation, the pH of the solution, the mass ratio of surfactant to protein, temperature, and surfactant concentration.

Although surfactants have been used as a means of selectively extracting enzymes they can be detrimental to enzyme activity. Care should be taken when using surfactants in enzyme extractions to determine possible effects of surfactants on enzyme activities due to surfactant-protein complexes, as has been suggested.[97] Frear et al.[98] reported that several surfactants at 0.1 to 1.0% partially or completely inhibited *N*-demethylase activity in cotton. Other surfactants may have been without effect or could have even stimulated enzyme activity. Other examples of enzymatic deactivation include α-amylase in wheat flour by sodium dodecylsulfate,[97] RNAase activity by both sodium dodecylsulfate and dodecylamine-HCl,[99] and proteases by sodium lauryl sulfate or sodium dioctylsulfosuccinate.[100] Parr and Norman[24] also noted decreased activity of urease, phosphatase, and tyrosinase in their review.

Sugiura et al.[100] did not find any effect from Span® 20, Span® 60 (sorbitan monostearate), Tween® 20, Tween® 60 (polyoxyethylene sorbitan monostearate), or Tween® 80 on proteases. The addition of Tween® 80 and Tween® 40 at 0.1% greatly increased cellulase yields in cultures of *Trichoderma viride*.[101] This was attributed to the oleate portion of the molecule.

The research conducted to determine the effect of surfactants on enzymes extracted from higher plants may have little relationship to the effects that might be obtained following foliar application of surfactants. Surfactant concentrations applied with herbicides would normally be less than 0.5% and almost certainly only minute amounts of this would be translocated within the plant. Limited evidence to date suggests that the surfactant molecule may not be intact by the time that significant translocation occurs so that the effect of surfactants *in situ* remains to be determined.

V. SUMMARY

Surfactants and other adjuvants are highly important in increasing herbicide efficacy. They are being used in increasing quantities by farmers, but little is known about either how they increase the entry of herbicides into plants or how the surfactants themselves enter and translocate throughout the plant. The limited evidence available suggests that relatively small levels of the applied surfactant translocates from the site of application and that the surfactant molecule may not be intact when this occurs. Even so, the use of surfactants with herbicides

introduces biologically active materials into the plant and these materials, either the intact molecule or its breakdown products, could have significant effects on a large number of physiological processes.

Surfactants are useful biological tools for protein separations, research on membranes, and photosynthetic research. It will be important in future research in these areas to use surfactants of the highest possible purity and most exactly known and reliable chemical characterization. Research with formulations that contain mixtures of different individual surfactants will possibly only add confusion to a field of research that is already complex. A vast amount of research is needed to establish the effects of residual levels of surfactants within plants on the many biochemical processes before the true role of adjuvants is established in increasing herbicide penetration, translocation, and activity.

DISCLAIMER

Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply their approval to the exclusion of other products that may also be suitable.

REFERENCES

1. **Foy, C. L. and Smith, L. W.,** The role of surfactants in modifying the activity of herbicidal sprays, in *Pesticidal Formulations Research,* Van Valkenburg, J. W., Ed., ACS Adv. Chem. Ser. 86, American Chemical Society, Washington, D.C., 1969, 55.
2. **McWhorter, C. G.,** The use of adjuvants, in *Adjuvants for Herbicides*, Weed Science Society of America, Champaign, Ill., 1982, chap. 2.
3. **Griffin, W. C.,** Calculation of HLB of non-ionic surfactants, *J. Soc. Cosmet. Chem.*, 5, 249, 1954.
4. **Tukey, H. B.,** The leaching of substances of plants, *Annu. Rev. Plant Physiol.*, 21, 305, 1970.
5. **Baker, E. A.,** Chemistry and morphology of plant epicuticular waxes, in *The Plant Cuticle*, Cutler, D. F., Alvin, K. L., and Price, C. E., Eds., Academic Press, New York, 1982, 139.
6. **Holloway, P. J.,** The chemical constitution of plant cutins, in *The Plant Cuticle*, Cutler, D. F., Alvin, K. L., and Price, C. E., Eds., Academic Press, New York, 1982, 45.
7. **Hull, H. M., Davis, D. G., and Stolzenberg, G. E.,** Action of adjuvants on plant surfaces, in *Adjuvants for Herbicides*, Weed Science Society of America, Champaign, Ill., 1982, chap. 3.
8. **Esau, K.,** *Plant Anatomy*, 2nd ed., John Wiley & Sons, New York, 1965, 767.
9. **Martin, J. T. and Juniper, B. E.,** *The Cuticles of Plants*, St. Martin's Press, New York, 1970, 347.
10. **Dybing, C. D. and Currier, H. B.,** Foliar penetration by chemicals, *Plant Physiol.*, 36, 169, 1961.
11. **Foy, C. L., Whitworth, J. W., Muzik, T. J., and Currier, H. B.,** The penetration, absorption, and translocation of herbicides, *Oreg. Agric. Exp. Stn. Tech. Bull.*, 100, 3, 1967.
12. **Gustafson, F. G.,** Comparative absorption of cobalt-60 by upper and lower epidermis of leaves, *Plant Physiol.*, 32, 141, 1957.
13. **Zimmerman, P. W. and Hitchcock, A. E.,** Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity, *Contrib. Boyce Thompson Inst.*, 12, 321, 1942.
14. **Ennis, W. B., Jr., Williamson, R. E., and Dorschner, K. P.,** Studies on spray retention by leaves of different plants, *Weeds*, 1, 274, 1952.
15. **Hull, H. M.,** Studies on herbicidal absorption and translocation in velvet mesquite seedlings, *Weeds*, 4, 22, 1956.
16. **Freed, V. H. and Montgomery, M.,** The effect of surfactants in foliar absorption of 3-amino-1,2,4-triazole, *Weeds*, 6, 386, 1958.
17. **Foy, C. L. and Smith, L. W.,** The role of surfactants in modifying the activity of herbicidal sprays, in *Pesticidal Formulations Research. Physical and Colloidal Chemical Aspects,* Van Valkenburg, J. W., Ed., ACS Adv. Chem. Ser. 86, American Chemical Society, Washington, D.C., 1969, 55.

18. **Norris, R. F.,** Cuticular 2,4-D penetration enhancement by surfactants and isopropyl alcohol, *Weed Sci. Soc. Amer. Abstr.*, 46, 1972.
19. **Price, C. E.,** A review of the factors influencing the penetration of pesticides through plant leaves, in *The Plant Cuticle*, Cutler, D. F., Alvin, K. L., and Price, C. E., Eds., Academic Press, New York, 1982, 237.
20. **Kirkwood, R. C., McKay, I., and Livingstone, R.,** The use of model systems to study the cuticular-penetration of ^{14}C-MCPA and ^{14}C-MCPB, in *The Plant Cuticle*, Cutler, D. F., Alvin, K. L., and Price, C. E., Eds., Academic Press, New York, 1982, 253.
21. **Jansen, L. L.,** Relation of structure of ethylene oxide ether-type nonionic surfactants to herbicidal activity of water-soluble herbicides, *J. Agric. Food Chem.*, 12, 223, 1964.
22. **Jansen, L. L., Gentner, W. A., and Shaw, W. C.,** Effects of surfactants on the herbicidal activity of several herbicides in aqueous spray systems, *Weeds*, 9, 381, 1961.
23. **Becher, P. and Becher, D.,** The effect of hydrophile-lipophile balance on contact angle of solutions of nonionic surface-active agents, *Adv. Chem. Ser.*, 86, 15, 1969.
24. **Parr, J. F. and Norman, A. G.,** Effect of nonionic surfactants on root growth and cation uptake, *Plant Physiol.*, 39, 502, 1964.
25. **McWhorter, C. G.,** Effects of surfactants on the herbicidal activity of foliar sprays of diuron, *Weeds*, 11, 265, 1963.
26. **Hammerton, J. L.,** Environmental factors and susceptibility to herbicides, *Weeds*, 15, 330, 1967.
27. **Holly, K.,** Herbicide selectivity in relation to formulation and application methods, in *The Physiology and Biochemistry of Herbicides*, Audus, L. J., Ed., Academic Press, New York, 1964, 423.
28. **Evans, E.,** *Plant Diseases and Their Chemical Control*, Blackwell Scientific, Oxford, 1968, 288.
29. **Norris, R. F.,** Action and fate of adjuvants in plants, in *Adjuvants for Herbicides*, Weed Science Society of America, Champaign, Ill., 1982, chap. 4.
30. **Johnstone, D. R.,** Spreading and retention of agricultural sprays on foliage, in *Pesticide Formulations*, Van Valkenburg, J. W., Ed., Marcel Dekker, New York, 1973, 343.
31. **Freed, V. H. and Witt, J. M.,** Physicochemical principles in formulating pesticides relating to biological activity, in *Pesticidal Formulations Research. Physical and Colloidal Chemical Aspects*, Van Valkenburg, J. W., Ed., ACS Adv. Chem. Ser. 86, American Chemical Society, Washington, D.C., 1969, 70.
32. **Blackman, C. E., Bruce, R. S., and Holly, K.,** Studies in the principles of phytotoxicity. V. Interrelationships between specific differences in spray retention and selective toxicity, *J. Exp. Bot.*, 9, 175, 1958.
33. **Thomas, W. D. E. and Potter, L.,** The influence of surface active compounds on initial deposits of zineb on leaf surfaces, in *Proc. 4th Intern. Congr. Surface Active Substances, Brussels, 1964*, Gordon & Breach, New York, 3, 475, 1967.
34. **Takeno,T. and Foy, C. L.,** Effect of polysorbate surfactants on the ultrastructure of leaf surfaces, *Weed Sci. Soc. Amer. Abstr.*, Weed Science Society of America, Champaign, Ill., 1974, 92.
35. **Price, C. E.,** Penetration and translocation of herbicides and fungicides in plants, in *Herbicides and Fungicides — Factors Affecting Their Activity*, McFarlane, N. R., Ed., Chemical Society Special Publication 29, The Chemical Society, London, 1976, 42.
36. **Parr, J. F.,** Toxicology of adjuvants, in *Adjuvants for Herbicides*, Weed Science Society of America, Champaign, Ill., 1982, 93.
37. **Norris, L. A. and Freed, V. H.,** Movement of C^{14}-surfactant and 2,4,5-T in bean leaves, in *Res. Prog. Rept. West. Weed Control Conf.*, Weed Science Society of America, Champaign, Ill., 1962, 92.
38. **Smith, L. W. and Foy, C. L.,** Penetration and distribution studies in bean, cotton, and barley from foliar and root applications of Tween 20-C^{14}, fatty acid and oxyethylene labeled, *J. Agric. Food Chem.*, 14, 117, 1966.
39. **Norris, R. F.,** Surfactants and cuticular penetration, *Weed Sci. Soc. Am. Abstr.*, 11, 1971.
40. **McWhorter, C. G.,** unpublished data.
41. **Stolzenberg, G. E., Olson, P. A., Zaylskie, R. G., and Mansager, E. R.,** Behavior and fate of ethoxylated alkylphenol nonionic surfactant in barley plants, *J. Agric. Food Chem.*, 30, 637, 1982.
42. **Stolzenberg, G. E. and Olson, P. A.,** Nonionic surfactant behavior and fate in rice plants: hexaethoxylated octylphenol, in *175th National Meeting of the America Chemical Society, Abstr., Pesticide Division*, American Chemical Society, Washington, D.C., 1978, 67.
43. **Frear, D. S., Swanson, H. R., and Stolzenberg, G. E.,** Polyethoxylated alkyl phenol detergents: *In vitro* plant metabolism studies, in *174th Annual Meeting of the Americal Chemical Society, Abstr., Pesticide Division*, American Chemical Society, Washington, D.C., 1977, 16.
44. **Dobozy, O. K. and Bartha, B.,** Non-polluting surfactants stimulating the growth of plants, *Tenside Deterg.*, 13, 139, 1976.
45. **Nadasy, M. and Dobozy, O. K.,** (Surfactant application in farming), *Chem. Phys. Chem. Anwendungstech. Grenzflachenaktiven Stoffe, Ber. Int. Kongr.*, 6th Sept., 1973 (German).

46. **Seymour, J. E.,** Status of research related to the use and effect of surfactants on various crops and soils, *J. Am. Oil Chem. Soc.*, 34, 215, 1957.
47. **Dobozy, O. K.,** Cationic surfactants as plant growth regulators, in *9th Int. Conf. Plant Growth Subst. Coll. Abst. Paper Demon.*, Pilet, P. E., Ed., 1976, 75.
48. **Beal, J. L., Christensen, B. V., and Colby, A. B.,** The effect of selected chemicals on alkaloid yield of *Datura tatula* Linne, in *J. Am. Pharm. Assoc., Sci. Ed.*, 43, 282, 1954.
49. **Luxmoore, R. J., Valoras, N., and Letey, J.,** Nonionic surfactant effects on growth and porosity of barley roots, *Agron. J.*, 66, 673, 1974.
50. **MacDowall, F. D. H.,** Effects of nonionic surfactants on tobacco roots, *Can. J. Bot.*, 41, 1281, 1963.
51. **Endo, R. M., Letey, J., Valoras, N., and Osborn, J. F.,** Effects of nonionic surfactants on monocots, *Agron. J.*, 61, 850, 1969.
52. **Buchanan, G. A.,** Patterns of Surfactant Toxicity to Plant Tissues, Ph.D. Thesis, Iowa State University (LC Card No. Mic. 65-7599); available from University Microfilms, Ann Arbor, Mich., 1965.
53. **Knypl, J. S.,** Tween surfactants stimulate growth of *Amaranthus* seedlings, *Z. Pflanzenphysiol.*, 81, 147, 1977.
54. **Mendez, J., Vasquez, A., Mato, M. C., and Vieitez, E.,** Direct and synergistic influence of Tweens on *Avena* coleoptile section elongation, *Physiol. Plant.*, 20, 437, 1967.
55. **Schonherr, J. and Bukovac, M. J.,** Modification of succinic acid 2,2-dimethyl-hydrazide growth inhibition of bean plants by surfactants, *J. Am. Soc. Hortic. Sci.*, 97, 384, 1972.
56. **Stowe, B. B.,** Growth promotion in pea stem sections I. Stimulation of auxin and gibberellin action by alkyl lipids, *Plant Physiol.*, 35, 262, 1960.
57. **Stowe, B. B.,** The stimulation of auxin action by lipides, in *Plant Growth Regulation*, Iowa State University Press, Ames, 1961, 419.
58. **Vieitez, E., Mendez, J., Mato, C., and Vasquez, A.,** Effect of Tweens 80, 40, and 20 on growth of *Avena* coleoptile sections, *Physiol. Plant.*, 18, 1143, 1965.
59. **Davis, D. G., Stolzenberg, R. L., and Stolzenberg, G. E.,** Phytotoxicity of selected non-ionic surfactants to soybean *Glycine max* cell suspensions, *Environ. Pollut.*, 27, 197, 1982.
60. **Nethery, A. A.,** Inhibition of mitosis by surfactants, *Cytologia*, 31, 321, 1967.
61. **Rotini, O. T. and Caloppinin, C.,** Les detergents synthetiques, le sol et les cultures, in *Proc. 4th Int. Cong. Surf. Act. Subst.*, III, Paris, 1964, 451.
62. **Rotini, O. T., Guerrucci, N., and Maffei, L.,** (Antimitotic effect of synthetic detergents on plants), *Ricerca Sci., Rend.*, 1, 30, 1961 (Italian).
63. **Glassman, H. N.,** Surface active agents and their application in bacteriology, *Bacteriol. Rev.*, 12, 105, 1948.
64. **Mitchell, P.,** Toxicity of surface tension depressants, in *Bacterial Physiology*, Werkman, C. H. and Wilson, P. W., Eds., Academic Press, New York, 1951, 149.
65. **Wyss, O.,** The surface active agents, in *Bacterial Physiology*, Werkman, C. H. and Wilson, P. W., Eds., Academic Press, New York, 1951, 204.
66. **Miller, G. M. and St. John, J. B.,** Membrane-surfactant interactions in lipid micelles labelled with 1-anilo-8-naphthalenesulfonate, *Plant Physiol.*, 54, 527, 1974.
67. **Harrap, B. S. and O'Donnell, I. J.,** On the permeability of cellophane membranes to sodium dodecyl sulfate solutions, *J. Phys. Chem.*, 58, 1097, 1954.
68. **Klevens, H. B. and Carr, C. W.,** Equilibrium dialysis of soap and detergent solutions, *J. Phys. Chem.*, 60, 1245, 1956.
69. **Ruinen, J.,** Nitrogen fixation in the phyllosphere, *Front. Biol.*, 33, 121, 1974.
70. **Temple, R. E. and Hilton, H. W.,** The effect of surfactants on the water solubility of herbicides, and the foliar phytotoxicity of surfactants, *Weeds*, 11, 297, 1963.
71. **Thomson, W. W. and Moeller, C. H.,** Effects of Tween-20, polyoxyethylene sorbitan monolaurate on the ultrastructure and organization of chloroplast membranes, *Protoplasma*, 114, 173, 1983.
72. **Quantin-Martenot, E., Benveniste, P., Hartmann, M. A., and Bouvier-Nave, P.,** Activation of etiolated maize coleoptiles plasma membrane-bound uridine-diphosphate-glucose-sterol-β-D-glucosyltransferase by Triton X-100, hydroxyl ions and phospholipase A_2, *Plant Sci. Lett.*, 29, 305, 1983.
73. **Haapala, E.,** The effect of a non-ionic detergent on some plant cells, *Physiol. Plant.*, 23, 187, 1970.
74. **Haydon, D. A. and Meyers, V. B.,** Surface charge, surface dipoles and membrane conductance, *Biochim. Biophys. Acta*, 307, 429, 1973.
75. **Helenius, A. and Simons, K.,** Solubilization of membranes by detergents, *Biochim. Biophys. Acta*, 415, 29, 1975.
76. **Mazliak, P. and Decotte, A. M.,** Effects of four detergents on the oxidative and phosphorylating capacities of potato mitochondria, *J. Exp. Bot.*, 27, 769, 1976.
77. **Newmann, P. M. and Prinz, P.,** Evaluation of surfactants for use in the spray treatment of iron chlorosis in *Citrus* trees, *J. Sci. Food Agric.*, 25, 231, 1974.

78. **St. John, J. B., Bartels, P. G., and Hilton, J. L.,** Surfactant effects on isolated plant cells, *Weed Sci.*, 22, 233, 1974.
79. **Sutton, D. L. and Foy, C. L.,** Effect of diquat and several surfactants on membrane permeability in red beet tissue, *Bot. Gaz.*, 132, 299, 1971.
80. **Watson, M. C., Bartels, P. G., and Hamilton, K. C.,** Action of selected herbicides and Tween 20 on oat (*Avena sativa*) membranes, *Weed Sci.*, 28, 122, 1980.
81. **Coats, G. E. and Foy, C. L.,** Effect of petroleum oils on the uptake of atrazine-^{14}C by corn, *Weed Sci.*, 22, 220, 1974.
82. **Gaponeka, V. I., Nikolaeva, G. M., Shevchuk, S. N., and Lositskaya, T. V.,** (Accumulation of chlorophylls a and b in post-etiolated corn sprouts infiltrated by detergents), *Vestsi Akad. Navuk. BSSR. Ser. Biyal., Navuk*, 6, 59, 1971.
83. **Moshkov, D. A., Moshkova, Z. G., and Shutilova, N. I.,** Effect of treatment of chloroplast membranes with detergents, *Biofiz. Zhivoi. Kletki.*, 3, 44, 1974.
84. **Deamer, D. W. and Crofts, A.,** Action of Triton X-100 on chloroplast membranes. Mechanisms of structural and functional disruption, *J. Cell Biol.*, 33, 395, 1967.
85. **Neumann, J. and Jagendorf, A.,** Uncoupling photophosphorylation by detergents, *Biochim. Biophys. Acta*, 109, 382, 1965.
86. **Vernon, L. P. and Shaw, E.,** Photochemical activities of spinach chloroplasts following treatment with the detergent Triton X-100, *Plant Physiol.*, 40, 1269, 1965.
87. **Batyuk, C. P.,** Effect of surface active agents on the quantum requireent for photosynthesis determined by a thermodynamic method, *Biol. Plant.*, 15, 161, 1973.
88. **McWhorter, C. G., Jordan, T. N., and Wills, G. D.,** Translocation of ^{14}C-glyphosate in soybeans (*Glycine max*) and johnsongrass (*Sorghum halepense*), *Weed Sci.*, 28, 113, 1980.
89. **McWhorter, C. G. and Jordan, T. N.,** Factors affecting dalapon absorption and translocation in johnsongrass, *Physiol. Plant.*, 38, 166, 1976.
90. **McWhorter, C. G. and Wills, G. D.,** Factors affecting the translocation of ^{14}C-mefluidide in soybeans (*Glycine max*), common cocklebur (*Xanthium pensylvanicum*), and johnsongrass (*Sorghum halepense*), *Weed Sci.*, 26, 382, 1978.
91. **Cairns, R. R.,** Effects of surfactants applied to samples of solonetz soil on water penetration and plant growth, *Can. J. Soil Sci.*, 52, 267, 1972.
92. **Swanson, C. A. and Whitney, J. B.,** Studies on the translocation of foliar-applied P^{32} and other radioisotopes in bean plant, *Am. J. Bot.*, 40, 816, 1953.
93. **Koontz, H. and Biddulph, O.,** Factors affecting absorption and translocation of foliar applied phosphorus, *Plant Physiol.*, 32, 463, 1957.
94. **Teubner, F. G., Wittwer, S. H., Long, W. G., and Tukey, H. B.,** Some factors affecting absorption and transport of foliar applied nutrients as revealed by radioactive isotopes, *Mich. Agric. Exp. Stn. Q. Bull.*, 39, 398, 1957.
95. **Vlazyuk, P. A. and Prikaodko, N. V.,** Possible regulation of ion uptake and transport in plants using surface active agents and chelates, *Dokl. Vses. Akad. Skh. Nauk.*, 1, 6, 1973.
96. **Luzzati, A.,** Effects of surfactants on plants. 1. Experiments with oats (*Avena sativa*), flax (*Linum usitatisimum*), and peas (*Pisum sativum*), *Boll. Lab. Chim. Prov.*, 25, 43, 1974.
97. **McDermott, E. E. and Elton, G. A. H.,** Effect of surfactants on the α-amylase activity of wheat flour, *J. Sci. Food Agric.*, 22, 131, 1971.
98. **Frear, D. S., Swanson, H. R., and Tanaka, F. S.,** *N*-demethylation of substituted 3-(phenyl)-1-methylureas: isolation and characterization of a microsomal mixed function oxidase from cotton, *Phytochemistry*, 8, 2157, 1969.
99. **Blinkhorn, C. and Jones, M. N.,** Effect of surfactant structure on ribonuclease A-surfactant interactions, *Biochem. J.*, 135, 547, 1973.
100. **Sugiura, M., Kato, S., and Tanaka, H.,** Pharmaceutical studies on enzyme preparations. VI. Influence of surface-active agents on protease activity, *Kakuz Igaku*, 24, 318, 1964.
101. **Reese, E. T. and Maguire, A.,** Increase in cellulase yields by addition of surfactants to cellobiose cultures of *Trichoderma veride*, *Dev. Ind. Microbiol.*, 12, 212, 1970.

Chapter 7

HERBICIDE TOLERANCE AND RESISTANCE: ALTERATION OF SITE OF ACTIVITY

J. Gressel

TABLE OF CONTENTS

I. INTRODUCTION: RESISTANCE — TOLERANCE — SUSCEPTIBILITY

The different levels of tolerance of crops and weeds to specific herbicides is the basis of herbicide selectivity; this is not the topic discussed in this chapter. Many weeds that were previously controlled by specific herbicides have evolved biotypes with increased levels of tolerance (Figure 1).[1-3] Some of these biotypes can have a seemingly absolute resistance; they can be treated with saturated solutions of herbicides that have little or no effect on the weed. Some resistant biotypes are controlled by concentrations of herbicides, but at application rates many times greater than the recommended agricultural dose of the herbicide. Biotypes with increased tolerance to herbicides are more common than resistant biotypes.[2-4] Tolerant biotypes are only partially affected by the herbicides: their yield is lowered but they complete a life cycle or there is only partial mortality.There can be many shapes of dose-response curve for tolerance (Figure 1) depending on the mode of tolerance and the number of sites of action and the proportion of tolerant individuals in the population. Unfortunately, the simple distinction between resistance and tolerance is often not understood and the literature is replete with references to resistance which are but minor increases in tolerance. True resistance has only appeared in the field to the *s*-triazines, to paraquat, to trifluralin, and to dichlofop-methyl. Selection for tolerance and resistance to many other herbicides has been possible in the laboratory, using algae[5-10] or higher plant tissue cultures.[11-13]

This chapter will deal only with those herbicides that interact with the Q-B-system of photosystem II (PS II). The two following chapters will deal with other modes of resistance and tolerance.

A. The Appearance of Herbicide Resistance

Herbicide resistance has appeared only when there were long periods where these herbicides were used, year after year with no other herbicide in rotation. Triazine-resistance has occurred in corn fields, orchards, vineyards, groves and tree plantations, and along roadsides and railroad rights-of-way. As of July 1983, there were confirmed reports of 38 species in 18 genera with triazine-resistant biotypes in 23 states in the U.S., 4 Canadian provinces, and 9 European and 1 Middle Eastern country.[1-3,14] Because of the large number of species and locations, new cases not involving new species are less often reported in the scientific literature. In the middle of the U.S. corn belt where the most triazines are used, there have been only a few reports of triazine resistance.[2] It is quite simple to delay the appearance of resistance by using the procedures followed by the corn belt farmers: rotate crops, rotate herbicides, and use herbicide mixtures. Theoretically this will not prevent eventual appearance of resistance; only considerably delay it.[4] The delay may be shortened by the spread of resistant weeds from nonagricultural weed control sites such as railroad rights-of-way[2] and roadsides[15] that have been heavily treated, yearly with *s*-triazines, which are now often populated with selected biotypes.

Tolerance[3,16] and resistance[3] to paraquat have appeared under no-till situations in groves and orchards where paraquat was used as the sole herbicide with muliple applications each year for a few years. The tolerant and resistant biotypes can detoxify the superoxide formed through the effect of paraquat on photosystem I[17] (see Chapter 9). Nothing is known about the mechanisms of the diclofop-methyl resistance that has appeared in *Lolium rigidum*.[18] A biotype of *Eleusine indica* (goosegrass) that is resistant to many dinitroaniline herbicides has been found in seven counties in northeastern South Carolina. It was first found in cotton fields treated ten successive years with trifluralin.[18a]

B. Genetic Nature of Tolerance and Resistance

1. Inheritance of Tolerance

Increased tolerance to most herbicides is generally inherited in a polygenic fashion; more

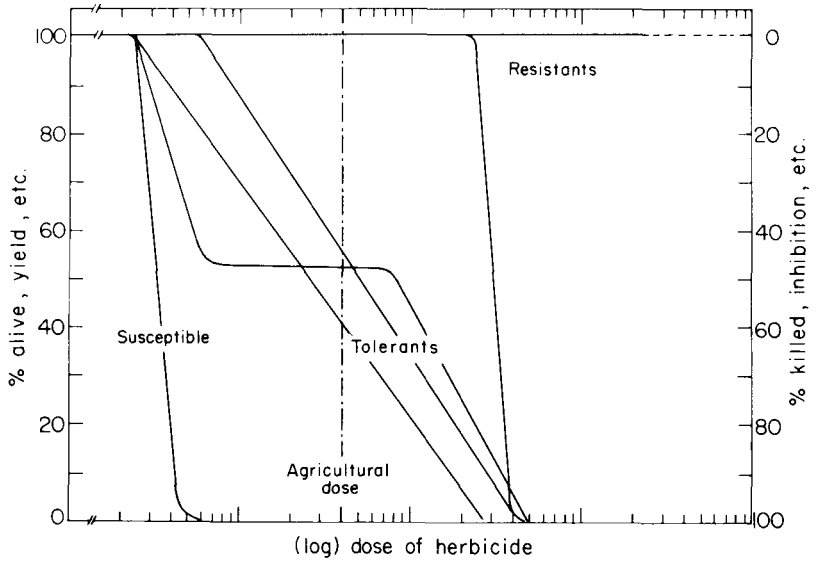

FIGURE 1. Schematic representation of susceptibility, tolerance, and resistance. Resistance is defined as having a normal yield at the recommended agricultural dose of the herbicide. When a weed is only partially affected, it is tolerant. Individuals and populations can both be called susceptible, tolerant, and resistant. (From Gressel, J., in *Origins and Development of Adaptation* (Ciba Symp. No. 102), Pitman, London, 1984, 73. With permission.)

than one allele on many genes give additive tolerance.[4,16] Some increases in tolerance of mammalian tissue to anticancer drugs (which are basically selective pesticides) are due to gene duplications. These give rise to higher levels of detoxifying enzymes.[19] Similar increases in insecticide tolerance may also be due to gene duplications of genes for phosphodiesterases that cleave and detoxify organophosphates.[20] Paraquat tolerance in *Lolium* is clearly polygenic and it seems evident that tolerance is polygenic in other cases in crops and weeds where this has been tested.[16]

2. Inheritance of Resistance

Resistance is usually inherited on one or at most two major nuclear genes in species where newly resistant biotypes or varieties have appeared. This is true for triazine resistance in corn,[16] picloram, phenmedipham, and bentazon resistances in tobacco,[11] metribuzin resistance in soybean,[16] and many other cases.[16] This has not yet been checked in the paraquat resistant *Conyza* and *Erigeron* spp. that have appeared due to repeated field selection.[3]

Atrazine and simazine are used mainly in corn and with developed trees. The mode of selectivity in corn is based on Mendelian genetic resistance[16] conferring the ability to detoxify the atrazine.[21] The binding of the herbicide to surface layers of soil above the roots is the basis for selectivity in woody species.

Inheritance of triazine resistance in weeds has proved to be a special case, because it is not inherited in a Mendelian fashion. The F_1 of any cross between a resistant and a sensitive biotype gives rise to offspring that are sensitive if the female parent was sensitive, and resistant if the female parent was resistant.[22] Because some of these species are hard to cross or had never been crossed before, it was not originally clear that the crosses were acually consummated; perhaps the plants had self-fertilized themselves despite the efforts of the geneticist. Semi-dominant nuclear markers were used to show that the inheritance of triazine-resistance followed the maternal line and that the F_1 plants were the results of the cross. In those cases, where this was done,[22-28] resistance was still found to be maternally inherited.

Maternal inheritance is not absolute; in the few cases where large numbers of crosses were made, there was 0.1 to 0.2% paternal inheritance as well,[23,24] which is typical with maternally inherited traits. Both mitochondria and plastids contain DNA, but it is presumed that the triazine resistance is inherited on chloroplast DNA. Plastids isolated from triazine-resistant weeds are resistant to the effects of the herbicide in vitro.[29,30] Additionally, interspecific protoplast fusions were made between potato and a triazine-resistant biotype of black-nightshade (*Solanum nigrum*).[31] Regenerated offspring that were resistant had *S. nigrum* plastid DNA; those that were sensitive had potato DNA.[32] Theoretically other cytoplasmic components such as mitochondria, plasmids, or unknown extranuclear DNA bodies could carry the trait and could have co-segregated with the plastids. It is not possible to say as yet whether one or more plastid genes are involved. Plastids have but one chromosome; all genes are linked and crossing-over has yet to be found in plastid systems.

Senecio vulgaris is a special case; both tolerant and resistant biotypes have been found in different locations in Britain.[3,33] The tolerant biotypes have a typical nuclear polygenic inheritance;[34] the one resistant type tested had maternal inheritance.[25] *Brachypodium distachyon* may be unusual; there is some evidence for increases in degradative tolerance in a biotype that has typical plastid resistance.[15]

Diuron resistance has not been found in field conditions, but it was selected for in various algae in the laboratory.[5-7,9,10] Metribuzin resistance was selected in *Chlamydomonas* as well.[8] In *Chlamydomonas* it was possible to make sexual crosses and show that in some mutants, diuron-resistance was also maternally inherited.[6] There is, at best, a small degree of cross tolerance between atrazine, metribuzin, and diuron in the resistant biotypes (Table 1). This means that atrazine and diuron resistance in *Chlamydomonas* are not inherited on the same allele. There are maybe two or more chloroplast alleles involved in atrazine resistance itself. There are also diuron-resistant mutants of *Chlamydomonas* where resistance is inherited on nuclear genes.[6] Different resistant biotypes of *Solanum nigrum* have clearly different responses to high levels of atrazine suggesting multiple alleles (Figure 2). Evidence that the same gene or gene products are involved in atrazine and diuron resistances will be discussed later.

II. PHENYLUREA AND TRIAZINE INHIBITION OF PHOTOSYSTEM II PHOTOSYNTHESIS

Diuron was found to inhibit the Hill reaction of photosynthesis, the splitting of water and evolution of oxygen (see Chapter 1) soon after its introduction as a herbicide. Atrazine had a similar effect. The effort to localize herbicide effects within photosystem II and to understand photosystem II itself eventually lead to considerably more information about photosynthesis and more about the mode action of triazines and phenyl-ureas than we know about any other groups of herbicides.

Our present understanding of PS II can briefly be summarized as follows: Light is intercepted by one of the many chlorophyll molecules of PS II embedded in the thylakoid membranes of the chloroplast. The energy from the many chlorophyll molecules which comprise an antenna is funneled to the chlorophyll *a* of the reaction center of PS II. This chlorophyll donates an electron to the intermediate acceptor 1 (Figure 3) which is transferred to the first "stable" acceptor Q, presumably a quinone. By accepting the electron, Q prevents the release of the energy from chlorophyll *a* as fluorescence; i.e., it is a "fluorescence quencher." It is presumed that there is only one Q per reaction center, thus it must transfer the electron on, or energy would be released as fluorescence, instead of ultimately providing the reductive capacity to synthesize ATP and $NADPH^+$. Q is a single electron carrier; but two electrons are needed to reduce plastoquinone (PQ). Thus, a secondary accumulating carrier B was proposed. After the energy of two quanta of light is accumulated, the electron

Table 1
COMPARATIVE EFFECTS OF VARIOUS PS II HERBICIDES ON PS II ACTIVITY IN THYLAKOIDS OF RESISTANT (VS. SENSITIVE) BIOTYPES OF VARIOUS SPECIES

Selector,[a] Genus, Species, and References

	Atrazine *Amaranthus*		Atrazine *Brassica*	Atrazine *Chenopodium album*[c]		Atrazine *Poa*	Metribuzin Diuron *Chlamydomonas reinhardii*			BNT[d] *Bumilleriopsis*	Diuron Aphanocapsa
	retroflexus	*hybridus*[b]	*campestris*			*annua*				*filiformis*[e]	6714[f]
	35	**36**	**10**	**30**	**10**	**37**	**8**	**9**	**6**	**10**	**10**
					$I_{50}R/I_{50}S$						
s-Triazine	251	1000	501	221	182	600	50	1.0	2.1	6	1
Atrazine											
as-triazines											
Metribuzin	1500	260	45	—	100	—	1740	—	—	398	68
Metamitron	40	—	—	—	—	—	—	—	—	—	—
Phenyl-ureas											
Linuron	3100	—	—	—	—	9	—	—	—	—	—
Chloroxuron	794	2.6	—	6.2	—	—	—	—	—	—	—
Diuron	4	1.4	0.7	—	1.2	2.5	14.5	15	17.2	23	79
Fluometuron	0.25	—	—	—	—	—	—	—	—	—	—
Uracils											
Bromacil	2000	2.0	—	24	—	—	106	—	—	—	—
Lenacil	50	—	132	—	56	—	—	—	—	25	1
Phenols											
Ioxynil	1.6	0.64	0.6	—	0.7	—	—	—	—	1.2	1
i-Dinoseb	0.5	—	—	—	—	—	0.7	—	—	—	—
Bromonitrothymol	2.0	—	0.2	—	1.3	—	—	—	—	10	1
Biscarbamate											
Phenisopham	3.2	—	13	—	5	—	—	—	—	4	—

Table 1 (continued)

COMPARATIVE EFFECTS OF VARIOUS PS II HERBICIDES ON PS II ACTIVITY IN THYLAKOIDS OF RESISTANT (VS. SENSITIVE) BIOTYPES OF VARIOUS SPECIES

[a] The compound used in the field or laboratory to select for resistance.
[b] Incorrectly classified as *A. retroflexus*.
[c] Estimated from data in Figure 4.
[d] BNT-bromonitrothymol.
[e] Alga from Xanthophycea.
[f] A cyanobacterium (Blue green alga), also called *Synechocystis 6714*.

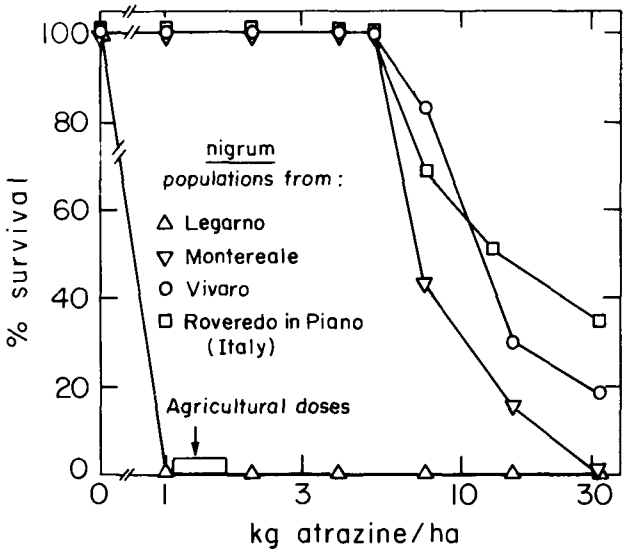

FIGURE 2. Variable response of atrazine-resistant *Solanum nigrum* accessions. Seeds of the resistant biotypes were gathered in northern Italy and assayed in pot tests. (Plotted by Gressel from the data of Zanin, G., Vecchio, B., and Gasquez, A. J., *Riv. Agron.*, 3—4, 196, 1981 in Gressel, J., in *Origins and Development of Adaptation* (Ciba Symp. No. 102), Pitman, London, 1984, 73. With permission.)

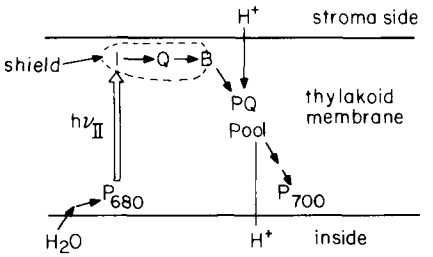

FIGURE 3. Flow of electrons in the PS II region of photosynthesis. P_{680}, the reaction center chlorophyll *a* of PS II; I, pheophytin, an intermediate electron acceptor; Q, bound quinone, the primary stable electron acceptor; B, the unknown plastoquinone binding protein; PQ, plastoquinone; P_{700}, the reaction center chlorophyll *a* of PS I. The part that must be shielded from protonation by the shield protein[35] hypothesized by Renger[39] is depicted by a dashed line.

pair is transferred to one of the plastoquinones from a soluble pool in the lipid matrix. The plastoquinones become protonated, therefore Q and B must not become protonated or the reaction will not continue. Renger[39] proposed that Q and B were insulated from protonation by the solutions in the "stroma," by a proteinaceous shield. The active sites of Q and B are presumed to be embedded in the lipophilic, unexposed part of this shield. Q, B, and the proteinaceous shield are referred to as the Q-B complex. Because many herbicides affect photosynthesis at photosystem II between the light reaction and before plastoquinone re-

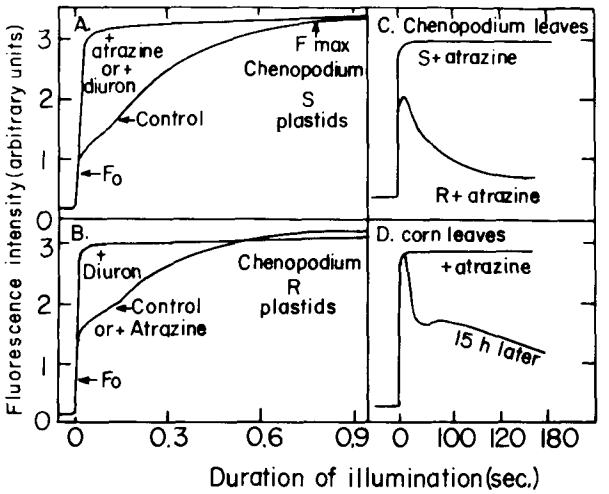

FIGURE 4. Increased chlorophyll fluorescence due to treatment with atrazine or diuron. (A and B) R and S plastids isolated from *Chenopodium album* biotypes were treated with 10 μ*M* atrazine or diuron after dark adaptation. (Redrawn from Reference 40.) (C) Whole leaves of *Chenopodium album* R and S biotypes were treated with 15 μ*M* atrazine 2 h before scanning. (Redrawn from Reference 41.) (D) Whole leaves of corn were treated with 15 μ*M* atrazine and scanned after 2 h and 15 h showing the effects of internal degradation of the atrazine. The untreated control was similar to 15 h after treatment. (Redrawn from Reference 41.) Note the differences in the time scales.

duction, it was presumed that they interact with this insulating shield protein, interrupting the electron flow, allowing the formation of toxic free radicals. The first immediate effect of these herbicides is that they increase the rate chlorophyll *a* fluorescence appearance (Figures 4A and B) and also increase the steady-state fluorescence level (Figure 4). This increase in fluorescence is not found in the chloroplasts of mutants that are triazine[30] or metribuzin[8] resistant, when treated with their respective herbicides, suggesting that some modification of the Q-B complex confers resistance.

Other compounds have similar effects on electron transfer through the Q-B complex. Many of the phenolic herbicides (e.g., dinitrophenols, ioxynil, nitrophenols, and possibly quinones) all seem to inhibit electron flow at the same low concentrations as diuron.[42] The presence of low levels of bicarbonate is also needed for transfer of electrons through the Q-B complex.[43] The interaction of all of these components, the evidence for their binding, and to what sites, and the peptide nature of the Q-B system are discussed more fully in Section IV.

III. PHYSIOLOGICAL REPERCUSSIONS OF RESISTANCE TO *S*-TRIAZINES AND PHENYL-UREAS

Haldane[44] has pointed out that there is usually a "cost" to any selection and that "cost" is in fitness; the ability of the new selected biotype to compete with the "wild" biotype of the same species under situations without the selector. What, therefore, are the effects of selection for triazine resistance on the newly appeared biotypes?

A. Modified Chlorophyll Fluorescence; Photosynthetic Efficiency

1. Fluorescence and Thermoluminescence

The first thing investigated when triazine-resistant weeds appeared[29] was whether they

had the same mechanism of detoxification as in corn (Chapter 9). The triazine-resistant weeds degraded atrazine at a much lower rate than corn. Moreover, isolated corn chloroplasts were sensitive to atrazine when photosystem II activity was measured; plastids from triazine-resistant weeds were not.[29]

In 1978, two groups reported that this resistance at the level of PS II could be measured by following chlorophyll fluorescence.[40,41] The chlorophyll of PS II (but not PS I) normally fluoresces at a low level when dark-adapted chloroplasts are illuminated, as some of the energy does not pass through the Q-B complex. This fluorescence is increased when the transfer of electrons from the PS II reaction center to the plastoquinone pool is inhibited. This enhanced fluorescence (f_{max}) is very rapid in PS II-inhibited plastids (Figure 4A); both in chloroplasts from the sensitive biotype, and in diuron-treated plastids of the atrazine-resistant biotype. In continuously illuminated leaves there is a subsequent return to a lower steady-state level of fluorescence (Figure 4C). Triazine-treated, susceptible-biotype leaves do not return to this lower steady-state level; but those of the resistant biotype do return to a lower steady-state fluorescence. This effect on leaves has been used by the Gasquez group to monitor European weeds for triazine resistance with simple equipment.[3,37,38] A similar system was used later by other groups in North America.[45,46]

Thermoluminescence of PS II has also been used to detect resistance/susceptibility differences with atrazine.[47] The detoxification of triazines can be measured indirectly by this means. The immediate effect of an atrazine application to corn and its effect 15 h later are shown in Figure 4D. Whole leaf fluorescence was also used to follow the detoxification (and lack of it) of a phenyl-urea herbicide, chlortoluron, in tolerant and susceptible wheat varieties.[48] Thus, there are many ways to measure resistance by fluorescence differences. In Figure 4 we see that this can be done by measuring the initial rise in 0.1 sec or by measuring total fluorescence after 60 sec. The initial rise F_0 is considered to be a function of either the antenna pigments or inactive reaction centers; the slow rise to F_{max} is the time it takes to fully reduce all the primary electron acceptors. The resistant biotypes also have an initial rapid increase, F_i, that is greater than in the wild biotypes; (Figures 4A and B).[36] This is considered to indicate a naturally slower rate of transfer from the reduced Q to B. This rate of transfer is easier to measure using nanosecond flashes of saturating laser light and measuring PS II fluorescence microseconds afterwards. There is a decay in fluorescence which is considered to monitor the kinetics of reoxidation of reduced Q by B. In untreated susceptible samples, the decay is half completed in 0.4 msec (Figure 5), but this decay is more than ten-fold slower in chloroplasts from resistant plants.

There is also a greater inherent fluorescence in phenyl-urea resistant *Euglena*[7] suggesting a similar problem within the reaction center of PS II. This increase in fluorescence was measured per unit chlorophyll, yet the resistant strain has 20% less chlorophyll, suggesting a considerable loss of efficiency.

Two triazine-resistant biotypes of *Poa annua* have been found. Both have the same chloroplast and seedling I_{50} resistance although one (M) has a minutely lower resistance to triazines in broken chloroplasts.[50] The other type (R) has the typical fluorescent pattern of resistant plastids, when treated with triazines. Strain M has an intermediate return to a higher steady state fluorescence than the R type.[50] This curve could be mimicked by mixing plastids of the susceptible and R type at a ratio 1:3.[50] This may be indicative of a mixed chloroplast population in the (M) type. This could be fortuitous if the (M) chloroplasts are stable through crosses and are inherited maternally.

2. Efficiency of Photosynthesis

There were differences in the F_i of the fluorescence curve of *Amaranthus*[36] and *Chenopodium* (Figure 4) and in the decay time of fluorescence induced by a flash of light (Figure 5).[49] These data were interpreted as meaning that the resistant biotypes have a slower transfer

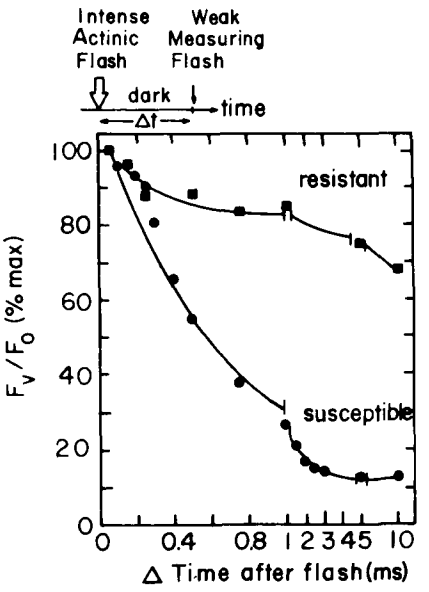

FIGURE 5. Decay of fluorescence following a single laser flash in susceptible and resistant *Amaranthus* chloroplasts. Chloroplasts were dark-adapted for 10 min, and fluorescence was detected at times between 50 μsec and 10 msec after a saturating laser flash. Ordinate values are variable fluorescence yield (F_v) divided by the F_0 level, normalized to the value at 50 μsec. F_0 values were measured with the non-actinic flash, without giving a laser flash. (Modified from Reference 49.)

of electrons along Q to B to plastoquinone.[30,36,39,40] What does such a situation mean to the plants; will the photosynthetic capacity be lowered? This question was attacked by Holt et al.[51,52] They followed PS II activity in isolated chloroplasts of *Senecio vulgaris* (Figure 6A). The activity of the resistant biotype was less than half of the wild sensitive type on a per chlorophyll basis at all light intensities measured. They used flashing light on isolated thylakoids and measured the five states of oxidation of water to O_2.[52] They were able to see that one of the four consecutive photoreaction transitions ($S_1 \rightarrow S_2$) is slower, and S_2 decay is faster in the R biotype. They hypothesize that the back-reactions from the reducing (acceptor) side of PS II to the oxidizing side occur at greater frequency in the R biotype because of altered Q → B-electron flow.[52] A series of synthetic quinones which are considered to replace plastoquinone on its binding site on B, were used to demonstrate that R plastids of *Amaranthus hybridus* have different affinities for quinones.[53] There was also a decrease of the midpoint redox potential of the B/B^- couple.[53]

These apparent inefficiencies may be of less importance to the plant; what is important is photosynthesis in the leaves. Holt et al.[51] measured photosynthesis (CO_2 fixation) per unit leaf area (Figure 6B). At low light intensities, where PS II reductive capacity should be limiting, the resistant biotypes were vastly ineffective compared to the susceptible type. At normal sunlight intensities, the differences between resistant and susceptible were minimal. Light is not the limiting factor for photosynthesis in many situations; CO_2 availability is. Still, as plants grow and leaves shade one another, a leaf absorbing light and not using it can be detrimental to the whole plant or to yield per unit area.

These studies should not be taken as the final word as they have only been made with a

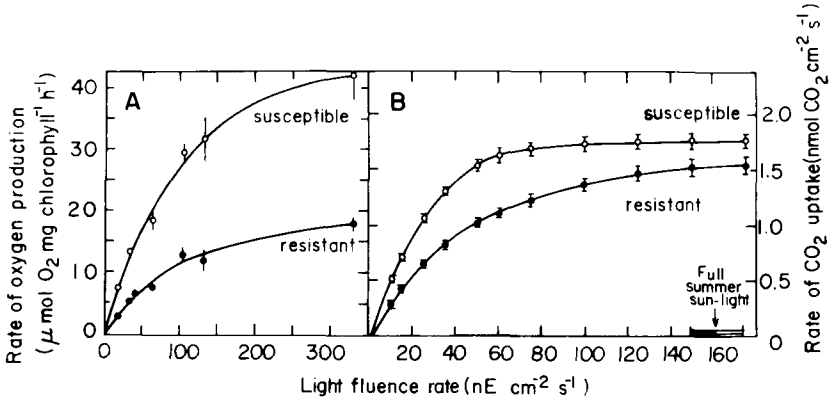

FIGURE 6. Decreased photosynthetic activity in the R biotype of *Senecio vulgaris*. (A) The rate of O_2 evolution as a function of light intensity in isolated chloroplasts measured in continuous light. Reduced light intensities were obtained with neutral density filters. (B) The light response of whole plant photosynthesis in the absence of herbicide. Vertical bars depict SE. (Modified from Reference 51.)

limited number of species. The material with slightly different responses to atrazine (Figure 2) should also be checked for differences in fluorescence and fluorescence decay as well as electron transport capacity to ascertain whether all alleles for triazine resistance are defective.

B. Pleiotropic Effects Accompanying Triazine Resistance

Many effects in different biochemical pathways often accompany a mutation. It is frequently hard to elucidate which is the primary effect of the mutation and which are secondary effects caused by a lack of product of a pathway, by a feedback mechanism, or by other tight coordinate controls between pathways. Albino plants are totally resistant to many herbicides that inhibit PS II; herbicide resistance is clearly a secondary pleiotropic effect. Some of the pleiotropic effects will seem secondary in the case of triazine resistance. Some that have seemed secondary could, formally at least, turn out to be primary.

All who have isolated plastids from resistant and sensitive biotypes have noticed that there is far less starch in the plastids of the resistant biotypes. This has also been seen by electron microscopy[54] (Figure 7) and is interpreted as the result of the less efficient photosynthesis in the resistants. Still, photosynthesis can be lowered by feedback in a system where the sink capacity is lowered by the lack of enzymes for starch biosynthesis. The plastids of triazine-resistant plants morphologically resemble those of "shade leaves" of plants, or leaves grown at low light intensity. They have more grana per stack and a lower chlorophyll *a/b* ratio. This would suggest that these plastids have larger PS II antennae.

A most intriguing pleiotropic effect reported independently by four groups is the differences in lipid composition in plastids between triazine-resistant and sensitive plants.[54-56] Triazine-resistant plastids had less total oleate in the polar lipids of *Chenopodium album* and *Brassica campestris*[54,55,56a] although the absolute levels of the polar lipids varied. There were higher levels of linolenate in the resistant plastids of *B. campestris*[54,55] and other significant differences betwen R and S biotypes, but not always the same differences between species and between groups.[54-56a] Similar changes can be achieved by growing *Lemna* in sublethal doses of atrazine[57] or by growing plants at low temperatures. Most of the differences are not of the magnitude of the diurnal variations within the same species.[58]

These changes in lipid composition are of interest because lipids are synthesized within the plastids in a light-driven process,[58] and may be coded for on the plastid genome. There is ample evidence that the lipid matrix in which the photosystems are embedded is not a

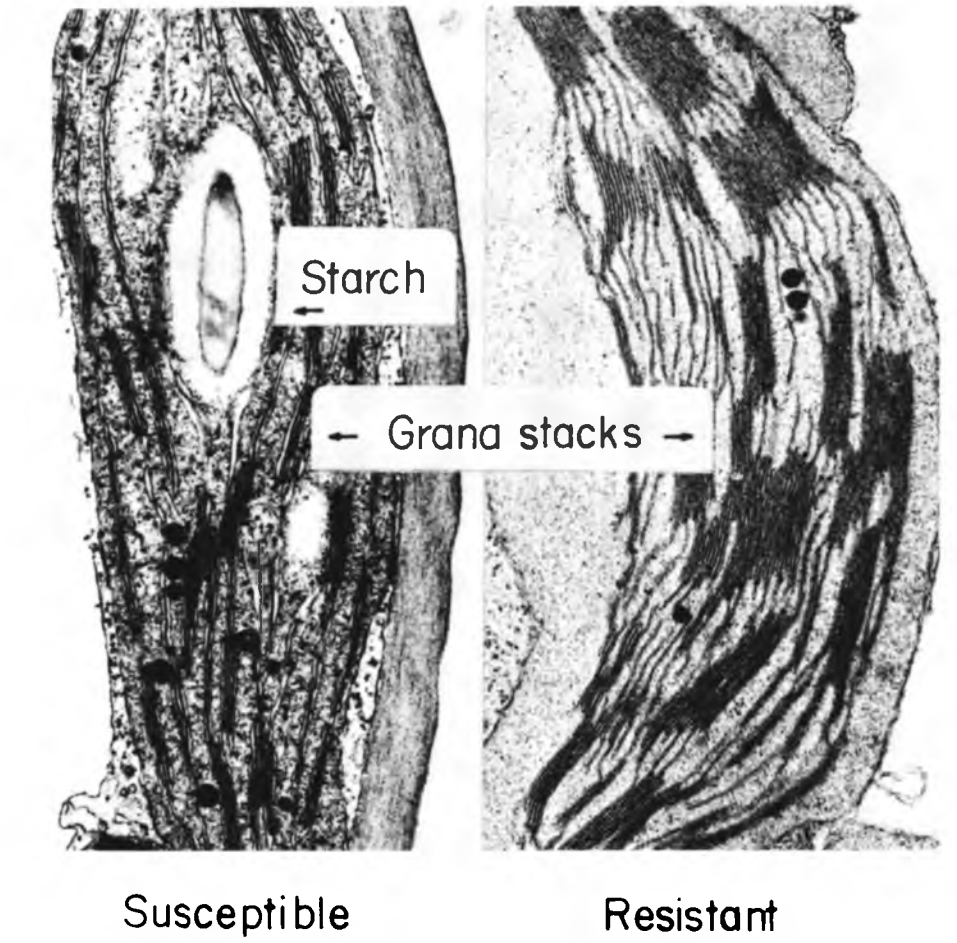

FIGURE 7. Electron micrographs of triazine-susceptible (A) and resistant (B) chloroplasts of *Brassica campestris*. Averages of five and eight thylakoids per stack were obtained for susceptible and resistant plastids, respectively. The starch grains were observed in triazine-susceptible plastids; they were smaller or absent in resistant plastids. (Modified from Reference 54 using micrographs kindly provided by Dr. J. J. Burke.)

random homogeneous mix of lipids. Lipids can affect the activity of reaction centers, which could include the accessibility of the herbicide binding sites to herbicides. The minor differences in fractionated total thylakoid lipids could easily represent major localized changes in the same lipids. Cold hardening of *Brassica napus* gives similar changes in total lipids[59] yet plastids from cold-hardened *B. napus* plants have no change in their susceptibility to atrazine.[60]

C. Fitness

The Haldanian fitness, i.e., the relative competitive ability of a biotype to compete with the wild type[44] is measured utilizing a procedure outlined by deWit.[61] In our particular case, R and S plants are grown in a marked matrix at fixed intervals and ratios. If there is a secondary morphological marker to differentiate between the biotypes, the seed could be randomly planted, i.e., not in a fixed matrix. By definition, fitness is measured without the selector.[44] It is clear that if atrazine were used, the (S) biotype would be completely unfit.

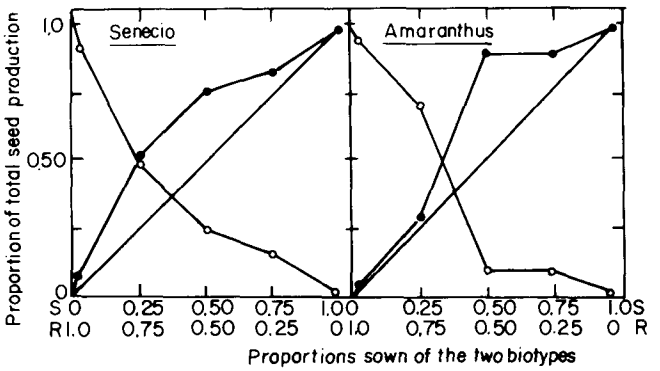

FIGURE 8. Fitness differences between R and S plants grown without atrazine. The proportion of total seed production (dry weight) of biotypes of *Senecio vulgaris* (a) and *Amaranthus* sp. (b) that are susceptible (●) or resistant (O) to atrazine. The biotypes were grown together in varying proportions but at constant overall density. (Modified from figures in Reference 62.)

Relative fitness can be estimated at any level of growth by measuring various parameters. The ultimate measure is reproductive fitness, the numbers of seed produced, because it is the integrated product of fitnesses at all levels; more fit germination, more fit establishment of seedlings to plants, more fit growth, and more fit seed production. Fitness of the triazine-resistant weeds was first estimated by Conard and Radosevich[62] (Figure 8). If there were no differences in fitness, an equal mixture of R and S *Senecio* seeds should have given rise to an equal mixture of seed at the end of the season; yet 65% of the seed were from the S type (Figure 8A). The fitness of the resistant is then calculated as 35/65 = 0.54. The actual field fitness may be much lower. In these experiments seedlings were pre-spaced because there were no secondary markers to tell them apart. "Establishment", the competition from the dense stand of seedlings giving rise to a few individuals, was not considered. Such decreases in fitnesses are typical for many selected properties, including "intelligence" in fruit flies[63] and heavy metal resistance in higher plants.[64] R (vs. S) *Amaranthus* was even less fit than R *Senecio* (Figure 8B). Similarly, R *Chenopodium album* had a fitness of less than 0.15 when compared to S biotype[65] and *Amaranthus hybridus* also had a very low fitness.[66] Still, resistant *Chenopodium strictum* did not have this decrease in fitness when compared to its wild type.[65] This again suggests that there are different alleles giving rise to resistance; each with different properties.

R and S biotypes should be tested under a variety of environmental conditions to truly judge fitness differences. The fitness differential might become larger or smaller depending on water, light, temperature, seeding density, etc. Indeed, one of these parameters was partially checked with *Chenopodium album*.[67] When R and S plants were grown separately at 25°C, the S plants produced more seed. At 30°C there was no significant difference in seed production between R and S.[67] Resistant chloroplasts of *Chenopodium album* also have the same ferricyanide reduction capacity at 10°C as plastids from the sensitive biotype, although they are inferior at 15°C.[68] Temperature optima differences were also found between R and S plastids from *Brassica campestris*.[56a,68]

Gasquez and co-workers have done pioneering population genetics studies of the newly appearing triazine resistance biotypes. They have shown by isozyme pattern analysis that the triazine-resistant biotype in any given area represents one of the many nuclear chemotypes of the area.[69] This nuclear chemotype background for the R type varies from area to area. The resistant biotype with its single nuclear genotype repesents a "founder" and spreads. They point out that a major drawback in all the studies on the pleiotropic effects and on the

fitness is that a single nuclear genotype (with special plastids) is compared with whole populations. The studies should be done with reciprocal F_1 crosses to get resistant and susceptible lines with the same (hybrid) nuclear background.[70] This criticism can also be made of the photosynthesis studies described below, as they were not done with "matched" nuclear material.

IV. HERBICIDE BINDING SITE(S) IN PS II

There has been much misunderstanding about photosystem II herbicide binding sites because of semantics; what is a site? Here we will narrowly define a site as the specific topological location where a herbicide binds, due to the inherent herbicidal structure and due to the complementary structure to which it is binding. Sites have been defined very broadly at times to include a much larger area that may bear many different sites, domains, or locales, which is confusing.

The binding of herbicides to PS II is clearly not covalent. If the binding were covalent, finding the specific binding site would be simpler. Instead, the binding is very loose, not too much stronger than diffusive forces. Herbicidal activity is stopped by washing thylakoids in buffer without herbicide or just by dilution. This poses innumerable problems in measuring the amounts of herbicide bound. The nature of PS II also adds to the problems. A very tightly linked structure is needed to transfer electrons. This is far from the random enzymatic processes occurring on spatially separated soluble enzymes in the stroma, such as CO_2 assimilation to carbohydrates. The electron-transfer proteins are partially embedded in a lipid matrix which insulates the electron-transport components from electron-conducting water. The partial hydrophobicity of the thylakoid proteins precludes their simple fractionation in buffer. Additionally, isolated membrane proteins should have different configurations out of the membrane than they have in, as they are no longer interacting with neighbor peptides or with the membrane lipids. Thus, we cannot expect isolated peptides to bind herbicides. This highly specific mechanism of collecting photons, splitting water, and reducing plastiquinones in the grana stacks is, however, subject to chemical and structural modulation by many influences. After a short time in low light, PS II proteins become phosphorylated. Part of the light harvesting system begins working for PS I, and the grana stacks seem to disappear. The same herbicides still inhibit PS II activity at the same I_{50} despite these fluid configurational changes. Configurational changes caused by heating clearly do affect the binding of diuron to membranes.[71] Still, the nucleus of PS II must remain much the same. Besides the ability to be phosphorylated and dephosphorylated, PS II activity is modulated by CO_2,[43] herbicides, and probably many other molecules. We must therefore envisage a large allosteric peptide or polypeptides that interact. Such interactions are well known with key soluble enzymes. Because of the close-packed nature of the electron transfer chain, it is easy to envisage that a modification of the configuration of one peptide can be propagated to become a modulation of other peptides down the chain. The literature must be evaluated with this overview in mind. Photosynthesis research, like other research is replete with dogmatic conclusions from good experiments; alternative conclusions compatible with the same excellent experiments are not always stated and thus, alternative hypotheses are not always tested.

A. The Measurements of Binding and of Competition

1. Binding Protocols

The experimental procedures to measure loosely bound regulators to membranes were first adapted to PS II herbicides by Tischer and Strotmann.[72] Plastids were isolated, osmotically ruptured, uncoupled from the photophosphorylation, and slightly "tightened" up by washing in a Mg^{++}-containing buffer. These plastid fragments were capable of carrying

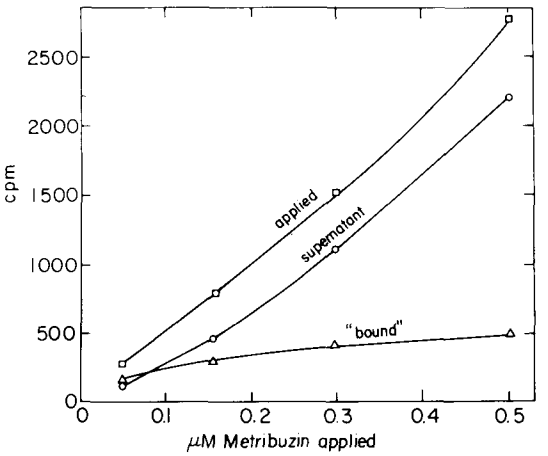

FIGURE 9. The measurement of herbicide binding. Chloroplasts were incubated with varying concentrations of radiolabeled metribuzin, centrifuged and the radioactivity of the supernatant was measured. The amount of radioactivity bound was calculated from the difference from the amount applied and the amount in the supernatant. Note that high affinity binding is only at the lowest concentrations where the differences are the least. (Plotted from a table in Tischer, W. and Strotmann, H., *Biochim. Biophys. Acta*, 460, 113, 1977.)

out PS II electron transport with ferricyanide as an electron acceptor. This electron transport was inhibited by a large number of PS II inhibiting herbicides. Various concentrations of radioactive herbicides were then added to a suspension of the chloroplast fragments and briefly equilibrated, and then centrifuged. As the binding was loose, one cannot wash the plastids a few times and count the radioactivity of the herbicides bound to the thylakoids. Despite the "rule" in enzymology to avoid measuring substrate disappearance, there was no choice but to measure the loss of counts from the supernatant. An example of such results is shown in Figure 9. Only a small proportion of the inhibitor is bound, especially at higher herbicide concentrations. By plotting the reciprocals of the amount of herbicide bound and the amount free (Figure 10A,B) one can extrapolate the linear portion of the curve to obtain the high affinity binding or specific binding of the herbicide. This is calculated from the *lowest* levels of herbicide with the lowest counts and the greatest error. The biphasic nature of the binding curves (Figure 9) implies a low number of high affinity binding sites as well. The specific binding constant (K) which is the herbicide concentration required to saturate 50% of the binding sites, and the concentration of binding sites can be calculated from the double reciprocal plots.

One prerequisite to show relevance of binding is to show that the same concentration range of herbicide inhibits electron transport as is specifically bound to the high affinity site. If this were not the case, we might be describing binding of a compound, but not to a binding site related to herbicidal activity. This again is plotted in a double reciprocal fashion. We see from the data in Figure 10 that the binding constant (K) and the inhibition constant (K_i) are the same and we may be dealing with a specific binding to an inhibitor binding site.

2. Competition Protocols

The standard methodology for studying specific vs. nonspecific binding is to either: (1) Measure binding of varying concentration of a radioactive herbicide in the presence and

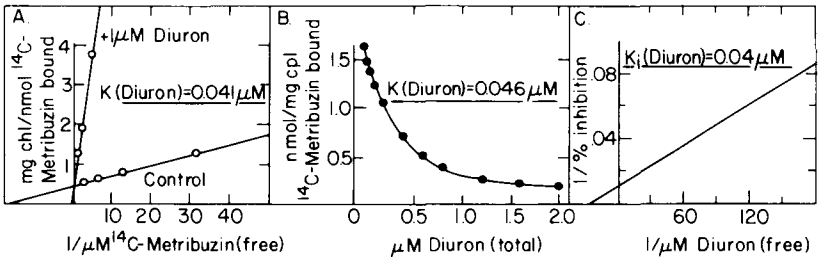

FIGURE 10. Competition for PS II herbicide binding: relation between the binding constant (K) and the inhibition contant (K_i). A, B binding of (^{14}C)-metribuzin as affected by unlabeled diuron. (A) The concentration of (^{14}C)-metribuzin was varied in the absence and presence of 1 μ*M* diuron. (B) The diuron concentration was varied at constant (^{14}C)-metribuzin concentration (0.125 μ*M*). The indicated K values were computed from the curves. (C) A double reciprocal plot showing the effect of diuron on PS II activity (uncoupled ferricyanide reduction). The K_i was calculated from the curve and is similar to the binding constant (K). (Redrawn from three figures in Reference 77.)

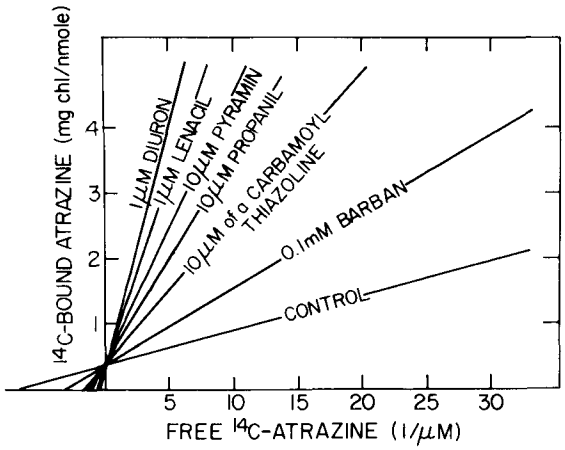

FIGURE 11. Variability of competitive binding ability of different PS II inhibiting herbicides against atrazine. Double reciprocal plot for binding of ^{14}C-atrazine in the presence of diuron, lenacil, pyramin, propanil, RU 21731 (a carbamoyl-thiazoline), and barban. (Modified from Reference 73.)

absence of a fixed concentration of another herbicide (Figure 10A). Binding is competitive if they have the same intercept on the Y axis. (2) Bind a fixed concentration of a radioactive herbicide in the presence of varied concentrations of the competitor (Figure 10B). A variety of different herbicides were found to compete with each other in this manner (Figure 11). Thus, atrazine or metribuzin could easily be competed off the thylakoids by diuron (a phenyl-urea), lenacil (a uracil derivative), propanil (an acylanilide), pyramin (a pyridazinone), and to lesser extents by other PS II inhibitors.[73] A common target or single herbicide binding site was easily surmised as they all similarly affected electron transport and fluorescence of the chloroplasts and their binding was typically competitive.[36,42,73,74]

3. Commonality of Sites

Do the data really prove a single common site? Indeed much evidence has been collected to show structure activity complementarity between phenolic, phenyl-urea, and triazine

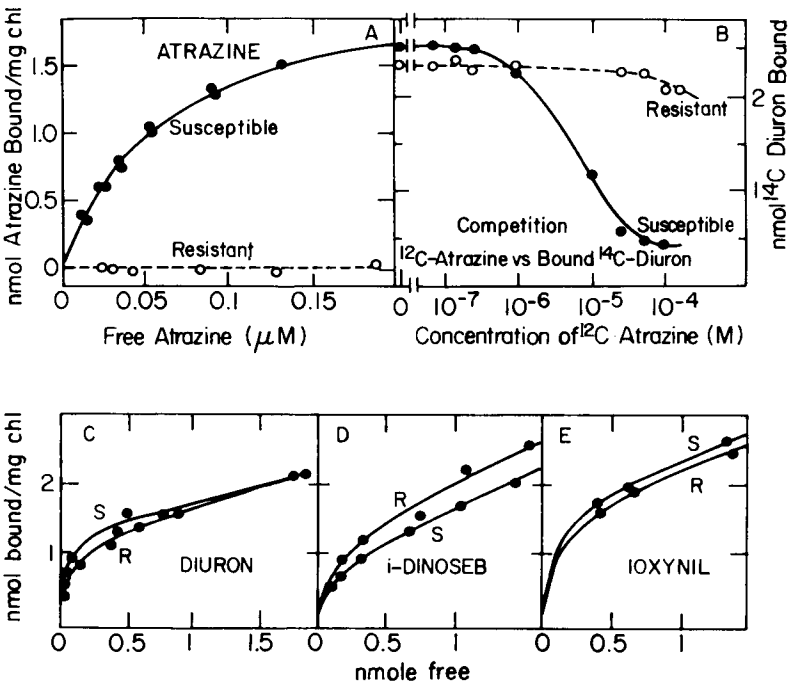

FIGURE 12. Specific loss of triazine-binding-site in thylakoids from triazine-resistant weeds. Herbicide binding to triazine R and S chloroplasts. (A) Binding of ^{14}C-atrazine to susceptible and resistant chloroplast membranes. Note that no atrazine binding to resistant chloroplasts was observed at these inhibitor concentrations. (Redrawn from Reference 36.) (B.) Competition between ^{14}C-diuron with atrazine in susceptible and resistant chloroplasts. Concentration of (^{14}C) diuron: 0.5 μM. (Redrawn from Reference 36.) (C,D,E) Binding of radioactively labeled diuron, dinoseb, and ioxynil to chloroplasts isolated from susceptible and atrazine-resistant *Amaranthus* sp. (Redrawn from Reference 35.)

herbicides, which also supports binding to the same target area.[36] Trebst[36] was the first to suggest a possible allosteric nature of competitive binding and proposed that these sites were all on the proteins shielding the Q-B complex.

Competition for binding between the two herbicides can be for two reasons: they bind to the same site or they bind to interacting sites of an allosteric system. These differences cannot be differentiated by physical binding experiments. Texts on enzyme kinetics usually contain the following warning about interpreting competition: "it is possible to visualize inhibition kinetics of the competitive type even when the inhibitor and the substrate do not compete for the same topological site, provided binding is mutually exclusive".[75] Many researchers still concluded from their results that the PS II-inhibiting herbicides tested (phenylureas, triazines, triazinones, phenmedipham) (1) bound to the same specific site of the thylakoid membrane and (2) binding competition could be generally used for the identification of a common binding site and mechanism of two different inhibitors.

The single site theory was weakened when it was found that plastids of the triazine-resistant weeds did not bind atrazine, but did bind other PS II-inhibiting herbicides purported to bind the same site. Atrazine could not compete with the bound diuron of thylakoids in resistant weed species (Figure 12B). Indeed, binding of diuron, dinoseb, and ioxynil were near normal in resistant weeds (Figures 12 C,D,E). A maternally inherited, diuron-resistant, triazine-sensitive mutant of *Chlamydomonas* did not lose affinity for atrazine but lacked the high affinity site for diuron.[9] Diuron competed for atrazine in the wild type, but the competition in the mutant was greatly reduced.[9] Thus, the genetic evidence that triazine-resistant

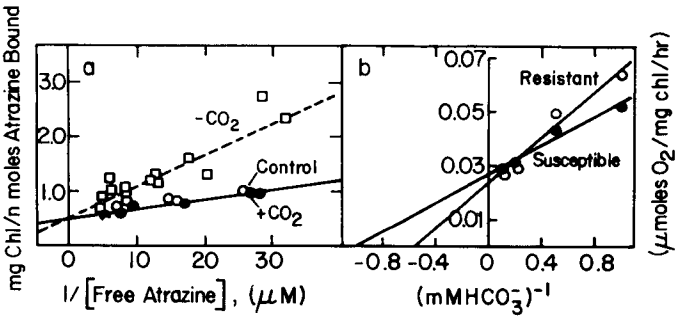

FIGURE 13. The effect of bicarbonate on triazine binding and effects in triazine susceptible pea chloroplasts. (A) The binding of (^{14}C) atrazine to control, CO_2-depleted and reconstituted (+20 m*M* $NaHCO_3$) thylakoids. Thylakoid membranes were incubated at 23°C with various concentrations of ^{14}C-labeled atrazine. The amount of bound inhibitor was calculated from the difference between the total radioactivity added to the thylakoid suspension and the amount of free inhibitor found in the supernatant after centrifugation. Double reciprocal plots are shown. (B) The effect of CO_2 on PS II activity in R and S chloroplasts. Double reciprocal plots of electron-transport rate (H_2O to ferricyanide) vs. bicarbonate concentrations in CO_2-depleted, atrazine-resistant, and susceptible *A. hybridus* chloroplasts are shown. (Modified from Reference 76.)

weeds and diuron-resistant *Chlamydomonas* are sensitive to many other PS II herbicides argues against an identical binding site for all. This is supported by the biochemical evidence that nontriazine herbicides continue to bind to thylakoids of triazine-resistant weeds.

4. Bicarbonate Binding

It has long been known that very low bicarbonate concentrations can modulate PS II electron transport activity in chloroplasts; CO_2 is necessary for PS II activity. Only cyclic photophosphorylation can operate in the absence of CO_2. It has been proposed, based on competitive binding studies, that the presence of bound bicarbonate increases the affinity of thylakoids for atrazine (Figure 13); i.e., there is a cooperative, not a competitive effect between the two.[76] Thus, a bicarbonate binding site must be added to the atrazine-binding protein complex. One of the properties of triazine-resistant weeds is that the affinity of their thylakoids for bicarbonate is decreased (Figure 13B). If this in turn decreases PS II activity,[77] then it might easily explain the lowered fitness of the triazine-resistant weeds.[51,52,62,65-67] Conversely, herbicides from the phenolic groups such as DNOC, which compete for atrazine, also compete with CO_2 for binding. Van Rensen[77] has proposed a scheme for all known binding sites (Figure 14) in which he attempted to account for all the allosteric data. Even this cannot be as clear-cut as desired; as we shall see below, there is evidence that some phenolic herbicides bind to a different peptide. Even the situation with the phenyl-ureas is not simple. Oettmeier et al.[35] tested the inhibition of PS II by 18 different phenyl-ureas on plastids of R and S *Amaranthus retroflexus*. Fluometuron was 4 times more effective on R than on S plastids. Some, including diuron were about equally inhibitory to both R and S plastids. Others, such as metoxuron had I_{50} ratios between R and S similar to atrazine (the I_{50} of the R more than 250 times that of the S). The extreme case was linuron, with a I_{50} R:S ratio of more than 3100, i.e., the plastids were more resistant to this phenyl-urea herbicide than to atrazine (Table 1).

It has recently been proposed that diuron and atrazine bind to the same site on the B protein as plastoquinone.[77a,77b,78] The proposal for atrazine was based on the finding that a

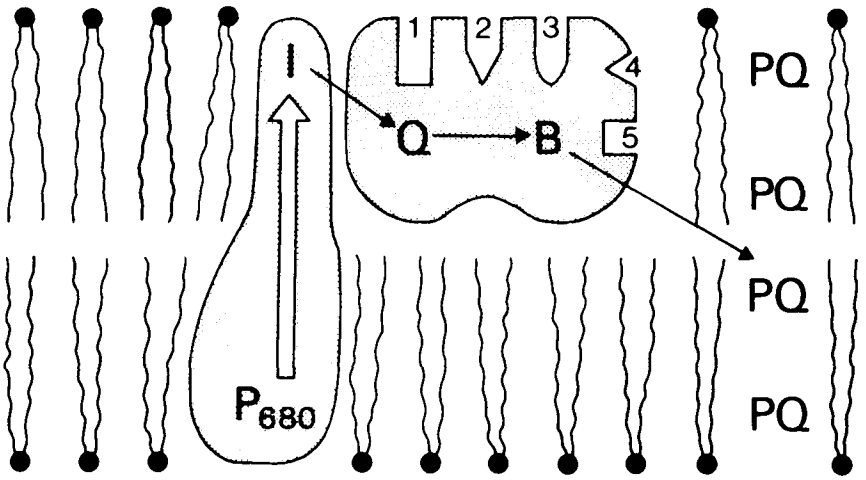

FIGURE 14. Allosteric visualization of the binding-sites of herbicides and bicarbonate to the Q-B protein complex. Urea herbicides bind to sites 1 and 2, the triazines bind to sites 2 and 3 phenolic herbicides bind to site 4, and bicarbonate to sites 4 and 5. Alas, some of the sites are probably on different peptides which make up the shaded area. (From Van Rensen, J. J. S., *Physiol. Plant.*, 54, 515, 1982. With permission.)

plastoquinone analogue (6-azido-5 decyl-2,3-dimethoxy-*p*-benzoquinone), which itself is not inhibitory to PS II, can compete with atrazine, but only when it is intercalated into thylakoids at a ratio of ten molecules per reaction center.[78] This synthetic quinone continued to partially compete with atrazine when it was covalently bound to the thylakoids at this ratio.[78] These data are hardly convincing that the sites are identical, considering the amounts of the quinone required and the incompleteness of the reaction. The number of compounds capable of competing ^{14}C-atrazine off of thylakoids increases; recently it was reported that the insecticides DDT, dieldrin, lindane, and others, will compete with atrazine.[79] It is possible that all these lipophilic compounds intercalate into membrane lipids and force a changed configuration on the binding site.

B. The Specific Site of Binding

1. Background

It is clear that triazines should bind somewhere to PS II which is made up of proteins and phospholipids in a tightly linked complex. An early approach to elucidate the triazine-binding protein was to determine whether there were any differences between the proteins of the R and S biotypes of a given species. Indeed, a slight change in electrophoretic mobility was reported for a peptide between 22 to 24 kD[80] in *Amaranthus hybridus* (incorrectly classified as *A. retroflexus*[81]). These authors, while continuing to study PS II-herbicide binding, have not reported further details about this difference.

It has been proposed that the triazine and phenyl-urea binding-sites are on the surface of the thylakoid, as a brief treatment with the protease trypsin prevents binding of both herbicide types but does not affect PS II activity.[39,82-85] The preferred explanation was that trypsin digested a surface binding-site, rendering PS II activity immune to the herbicide effects. There is an acceptable alternative explanation which is consistent with our knowledge of allosteric proteins: the trypsin treatment removed some surface protein, changing overall configuration of other particles, precluding herbicide binding. We have already seen how very small ions such as bicarbonate bind to the PS II system and change the affinity to triazines (Figure 13). One of the proteins partially digested by trypsin is a 32 kD protein.

A completely different explanation has been given to trypsinization studies: trypsin may have no direct effect whatsoever on herbicide binding. By removing surface peptide pieces, trypsin may allow the electron acceptor ferricyanide to penetrate the thylakoids and accept electrons before the position of herbicide blockage, "short circuiting" the system.[86] Trypsin acts by cleaving peptides next to arginine or lysine. As the 32 kD protein lacks lysine,[95] researchers treated thylakoids separately with arginine- and lysine-specific proteases. Only treatment with the lysine-specific protease prevented binding; the arginine-specific protease that should have acted if cleavage of the 32 kD protein was involved, was inactive.[87,88]

There is one useful clue to help understand the possible multiplicity of binding sites from the genetics of triazine resistance: resistance is maternally inherited.[22] Thus, the protein *controlling* triazine-binding is not inherited on the nuclear genome; it must be inherited either on plastid or mitochondrial DNA. It is hard to envisage that mitochondria will control binding of herbicides affecting plastids; thus we are left with the plastid genome. If we presume that a plastid gene product controls binding; this gene product may be (a) the binding peptide itself or (b) it may control binding to some other peptide which is the actual binding protein. With allostery in the PS II, both are viable hypotheses. There are not many known plastid-inherited peptides, but more than one are found in isolated PS II particles. There is considerable circumstantial evidence to believe that a 32,000 dalton (32 kD) protein binds all PS II-inhibiting herbicides.

2. Who Are You, 32?

In an elegant series of experiments, Eaglesham and Ellis[89] fed radioactive amino acids to isolated chloroplasts and showed that these plastids could synthesize proteins without ATP and exogenous mRNA, but only in the light. This light-driven synthesis could only be with plastid mRNA, and thus the peptides made with the plastids' own ATP must be coded on the plastid DNA. They fractionated these radioactive proteins on polyacrylamide gels and one of them ("peak D") had an apparent molecular weight near 32 kD. A few years later, a peculiar plastid mRNA of duckweed with a molecular weight of 500 kD was found, which was synthesized in much larger quantities in green and greening tissue than in dark-grown tissue.[90,91] It was later found that this mRNA was responsible for the synthesis of the 32 kD.[92] The 32 kD was also rapidly synthesized in corn where it was called the "photogene".[93] The term "photogene" is unfortunate because it is not a gene, but a gene product and it connotes that only one gene is turned on during greening, where as it is clear from the literature that many genes are turned on by light. The nomenclature committee of the Plant Molecular Biology Association has named this gene the psb A gene.

A few properties of this protein became quickly apparent, (1) it turns over rapidly; in duckweed it has a $t_{1/2}$ of 6 to 9 h in the light and much longer in the dark[85,94,95,98] (2) it is in the thylakoids in PS II, and part is on the surface and part extends internally[85,96-98] (3) it lacks lysine wherever checked (which helps differentiate between "the 32 kD" and other peptides of similar molecular mass),[98] and (4) it is synthesized as a larger peptide (33.5 kD)[95] which is processed by removing a piece of the carboxy terminal portion to produce the 32 kD size[99]

A considerable amount of plastid energy is used to manufacture the 32 kD peptide. The high-turnover of both the mRNA and the peptide requires about a third of the ATP used for internal syntheses. Its synthesis is under strict photocontrol and many compounds that prevent photosynthesis in the light bring about a stoppage of 32 kD synthesis. It is also under strong coordinate control with other pathways; nuclear inherited mutations causing lesions in photosynthetic pathways do not synthesize a 32 kD,[100] although it is not clear if they were measuring the same protein. This has led to misleading interpretations about 32 kD inheritance. Clearly the 32 kD protein must have some key role in photosynthesis. That role has been elusive. It was postulated that the role was developmental,[94] as there was a flush of

synthesis of the 32 kD protein and its mRNA made during greening.[90-92] This proposal was dropped when it was found that mature tissue continued to make 32 kD and its mRNA. The 32 kD is not directly necessary for photosynthetic processes. The turnover of the 32 kD is far more rapid than other plastid proteins;[95] thus it could be depleted by incubating duckweed plants in chloramphenicol, which rather specifically inhibits plastid protein synthesis. An 85% depletion of the 32 kD caused no more than a 25% depletion of $^{14}CO_2$ fixation[94] or electron transport as measured by ferricyanide reduction.[101] Greater losses of PS II capacity have been found measuring other parameters.[85] If the 32 kD is an integral part of photosynthetic processes, there must be alternative pathways to partially overcome its absence. These may give rise to the less efficient photosynthesis.

3. Photoaffinity Labeling Thylakoid Proteins with Herbicides

The loose nature of the herbicide binding, decreases the possibility of isolating the binding protein with attached herbicide. Similar problems arise in determining hormone binding to loose binding sites. The techniques of photoaffinity labeling were used to isolate the binding site in hormone receptor site studies. This technique was adapted by two groups to show binding of herbicides inhibiting PS II.[81,102] The method is simple; one replaces a group on the herbicide that is not necessary for herbicidal activity with a photoreactive group such as azide. The azidoherbicide is equilibrated with the thylakoid binding-sites and then the azide is activated to form nitrenes or other reactive groups with light. These reactive groups rapidly form covalent linkages to protein. If the herbicide is radiolabeled, proteins can be separated and it is possible to ascertain which protein covalently bound through the azido-linkage. This was first reported with 2-azido-substituted dinoseb, a nitro-phenolic herbicide. The radioactivity was found to be bound to a peptide with a M_r of 41 kD.[102] Results using the same approach were shown at almost the same time using 2-azido-substituted atrazine having ^{14}C in the ring.[81] This material was equilibrated with R and S plastids from *Amaranthus hybridus*, irradiated and the peptides fractionated. The results showed a specific binding to a 32 kD peptide in the S biotype and no binding at herbicidal concentrations in the R biotype (Figure 15A). As the azido-substituted dinoseb and the azido-substituted atrazine bound to different proteins, they concluded that they bind to different sites[104] even though competitive binding of phenolic, urea, and triazine-type herbicides has been reported (Section IV.A.3). When azido-substituted atrazine was used well above the I_{50}, there was covalent binding to peptides other than the 32 kD as well.[105] When azido-substituted ^{14}C-atrazine-labeled thylakoids were trypsinized, the label remained in the undegraded (hydrophobic) core of the 32 kD protein (Figure 15B).[103] The authors[81,103,105] concluded that the 32 kD protein contained the triazine-binding-site. These results seem to contradict those that showed that trypsinized thylakoids with the hydrophilic portion removed were insensitive to atrazine[103] and did not bind atrazine. How can the azido-substituted atrazine covalently bind to the material not removed from the thylakoids by trypsin, if trypsin removed the site? If the binding-site is not found in the material removed by trypsin, removal of these portions of the molecule must cause conformational changes in the hydrophobic binding-site. The trypsinization kinetics and patterns of digestion of the 32 kD protein that bound the azido-substituted atrazine[103] are identical to those of the rapidly turning over thylakoid 32 kD protein from many species[106,108] and are different from other 32 to 34 kD proteins that have been studied. One of these other proteins of similar mass can be seen upon staining, contains lysine, and has been the source of much confusion.[107] Indeed, no difference in proteolysis patterns could be found between the 32 kD protein of R and S biotypes of four species using different enzymes.[108]

There is one drawback and possible artifact that makes evidence from photoaffinity labeling only supportive, but not conclusive evidence. The photoactive group is often placed on the side of the herbicide molecule that is inconsequential for physiological activity. This has

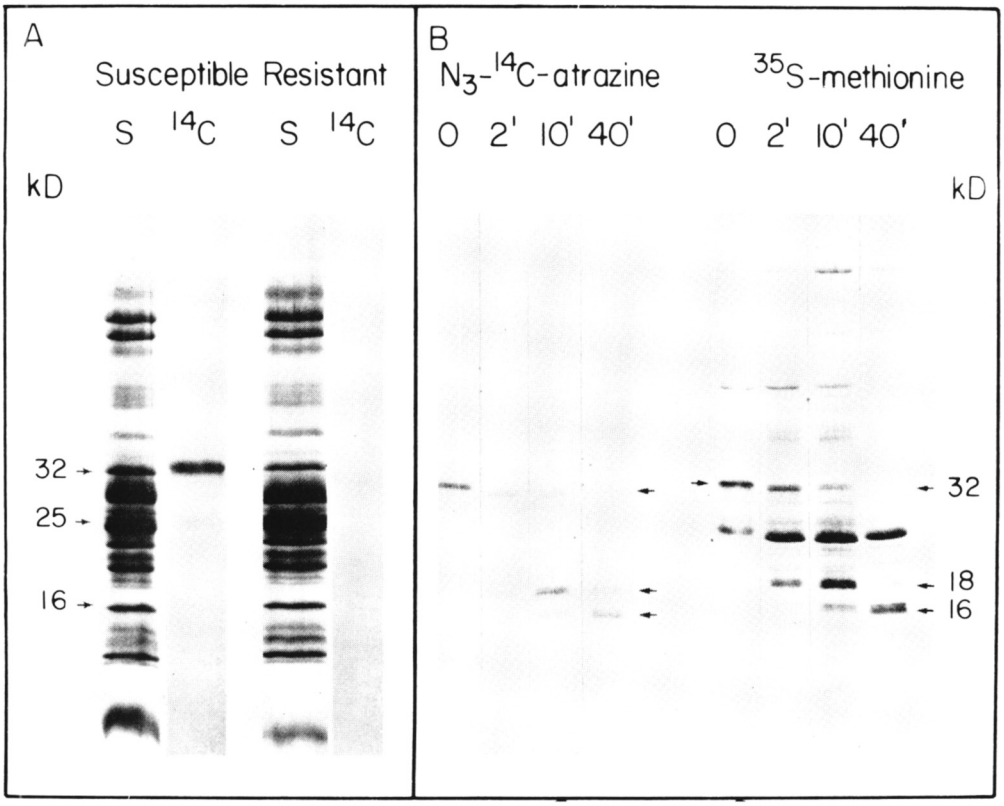

FIGURE 15. Photoaffinity labelling of S and R thylakoid proteins and digested protein pieces with azido-substituted ^{14}C-atrazine. (A) Polyacrylamide gel electrophoresis of thylakoid polypeptides from susceptible and resistant biotypes of *A. hybridus* stained for protein (lanes S) and by fluorography (lanes ^{14}C). Susceptible and resistant membranes were incubated with 0.5 μM azido-substituted (^{14}C)-atrazine under UV light for 10 min prior to detergent solubilization. (Modified from Reference 81.) (B) Binding of azido-substituted ^{14}C-atrazine to the embedded core of the 32 kD protein. Autoradiograms of polyacrylamide gels showing the effects of treating the 32 kD polypeptide radiolabeled by covalent addition of azido (^{14}C)-atrazine or in vivo (^{35}S)-methionine labeled 32 kD with trypsin. After radiolabeling, isolated thylakoid membranes were trypsinized for various durations, solubilized and analyzed by electrophoresis. (Modified from Reference 103.)

been the case with atrazine. The two *N*-alkyl-amino groups at positions 4 and 6 are needed for activity.[109] If either *N*-alkyl amino group is removed, the resulting *N*-dealkylated compound cannot compete with atrazine.[110] The chlorine in position 2 can be replaced by many groups of varying lengths and compositions; the only restriction is that the compound not be too water soluble. There are other views based on molecular orbital considerations as to what constitutes the active site of all the PS II herbicides.[110a] The azide was placed on this 2 position, 180° from the site needed for herbicidal activity. As the azide group is directly opposite the part of the structure required for herbicide activity, a valid alternative hypothesis was postulated that the azido-atrazine may be acting as a bifunctional reagent instead of a photoaffinity label. It is most probable that the ring with the azide is perpendicular to the plane of the electrostatically bound side chains and the adjacent part of the ring. The herbicidally active site of atrazine bound (loosely) to its target protein in thylakoids of sensitive plants and the photoactivation of the azide upon irradiation caused most of the azide moiety to bind primarily to that portion of the 32 kD protein which faces the herbicidal site (Figure 16). Only the covalent part of the bifunctional azido-atrazine would remain bound upon detergent disruption of the thylakoids; the binding to the physiological binding-

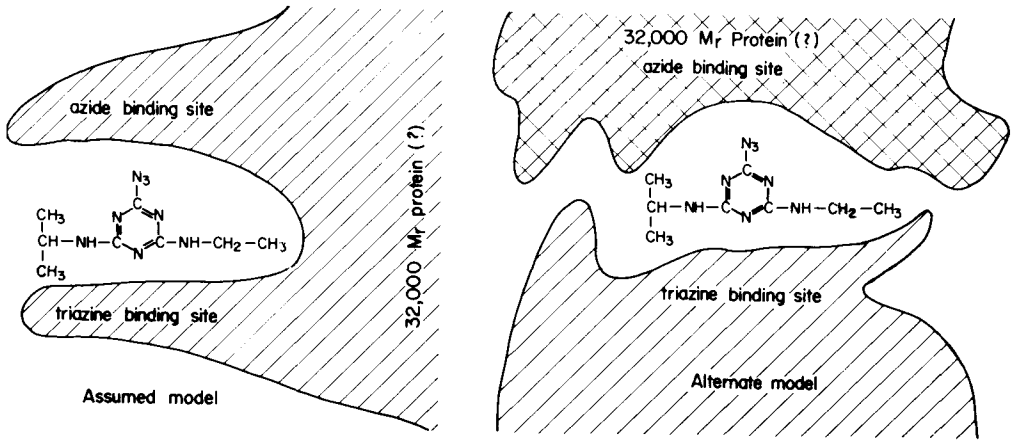

FIGURE 16. The possible binding-sites of azido-atrazine. The section of the atrazine molecule considered to be required for herbicidal activity is drawn facing the physiological herbicide binding-site. (A) The Arntzen model. Both the herbicide and the azido binding-sites are on the same protein. This requires that opposite sides of the loosely bound atrazine face the same protein (B) The alternate view is that the herbicide binding group may bind to a shallow binding-site of one protein and the opposite end of the molecule with the azide pointed away from it, binds to an adjacent or apposing protein. (From Gressel, J., *Plant Sci. Lett.*, 25, 99, 1982. With permission.)

site would be cleaved.[101] As the peptides in PS II must be closely packed to transfer electrons, it seems quite possible that such a bifunctional linkage may occur. This possible problem of the azide not binding to the same position as the herbicide will still be there even if the 32 kD protein is the herbicide binding protein. If one cannot expect to use azido-atrazine to accurately find the micro-site on the binding molecule (Figure 15B); the azide moiety cannot be expected to bind precisely to the same site as the herbicidally active group. It is hard to envisage such molecular flexibility. Thus, if the 32 kD protein contains the triazine binding-site, the site may be in the hydrophilic surface-exposed part (as the mild trypsinization and loss of binding suggested) or in the internal, hydrophobic portion as the photoaffinity suggested. The fact that azido-substituted atrazine bound to the 32 kD and other proteins,[105] and azido-dinoseb bound to a 41 kD protein[102] strongly argues against a single site for herbicide binding for these herbicides. Additional contradictory data can be found in a study where a "core complex" of PS II particles was studied. This core clearly included the 32 kD protein yet the particles lost sensitivity to a triazine herbicide.[111] Conversely, such particles retained their sensitivity to the phenolic herbicide, dinoseb.[112]

4. Amino-Acid Sequence of the 32 kD Protein

Another approach to ascertaining whether the 32 kD protein is responsible for triazine resistance is to show that there are differences in amino-acid sequence between the resistant and sensitive biotypes. The classical method of doing such work was very time consuming. The best classical example of sequencing is hemoglobin in humans and the proof that it carried sickle-cell anemia. Hemoglobin was isolated from many people, and the many sequence variabilities among individual normal humans were shown. Still, there was a consistent variation from the normal sequences in all cases with sickle-cell anemia. In the case of the 32 kD protein such an approach cannot be considered as there are not relatively large quantities of this high-turnover protein. It has been estimated that there is only one molecule of the 32 kD protein per PS II reaction center, i.e., only one molecule of 32 kD per 650 molecules of chlorophyll. The methods of modern molecular biology were used to ascertain its sequence. A chloroplast DNA piece containing the gene was inserted into plasmids and then cloned in *E. coli*. The cloned DNA was made radioactive with each of

the four radioactive nucleotides separately and the sequence determined electrophoretically by standard techniques. From the knowledge of the genetic code, one can quickly deduce which portion of the sequence might code for protein ("open reading frame") and which are noncoding leader and trailer sequences. The 32 kD protein was one of the first plastid-coded proteins sequenced; first in spinach and in a wild-tobacco species (*Nicotiana debneyi*).[113] There was a striking, almost complete similarity between the sequences; no amino-acid differences were found between the two species although there were more than forty differences in the nucleotide sequence that were "silent" i.e., a change from one codon to another codon coding for the same amino-acid. This "conservatism" suggests that the protein must require a very specific amino-acid sequence to perform its function; most variations from the sequence (mutations) would be lethal or detrimental. In contrast to hemoglobin, the data only describe the differences between one DNA molecule from one spinach plant and one from one tobacco plant without any data on intraspecific variability within each species.

Using this approach, DNA molecules originating from one plant each of R and S biotypes of *Amaranthus hybridus* were sequenced.[114] The nucleotide sequences showed not one difference, but three between the R and S DNAs, two of which were silent. The codon that is serine at amino-acid 228 in the S DNA of *A. hybridus*[114] is also serine in the tobacco and spinach[113] and in *Solanum nigrum*.[115,116] There is disagreement about which methionine in the open reading frame is the beginning of the molecule. In the spinach and tobacco nomenclature[96,113] this serine is at position 264. There is one amino-acid difference between the S *Amaranthus* and spinach and tobacco; a few more between other species have been reported at conferences. In the DNA from the R plants glycine is in position 228. A perusal of the amino-acid sequence shows that the serine is in position 228 in a block of rather hydrophobic amino-acids and should be embedded in the lipid matrix of the thylakoid. A computer program was used to obtain a preferred helical structure of the 32 kD and to show which portions are membrane embedded. Serine 228 maps into the membrane in these calculations.[96] Such computer programs were highly successful for bulk membrane proteins such as rhodopsin but are more prone to artifacts with proteins in low concentrations. They do not consider interaction with neighboring peptides. The three nucleotide differences between R and S *A hybridus* clearly indicate that there are intraspecific differences. It is assumed that the R arose from the S biotype by mutation. It is extremely unlikely that a single mutational event to resistance would cause three nucleotide changes. To be equally sure that a mutation in the 32 kD gene actually confers triazine resistance as we are that the hemoglobin gene mutated to confer sickle-cell anemia, we need far more data on intraspecific differences. Maybe the serine to glycine difference is within the allowed intraspecific variation and has nothing to do with triazine resistance. The likelihood is clearly there that if one will sequence any particular gene in two plants of the same species that there will be *a* one amino-acid difference. The 32 kD gene of a diuron-resistant strain of *Chlamydomonas* has also been sequenced. Here the codon for alanine replaces the wild type codon for serine at amino-acid 228.[116a] If azido-atrazine binds to the herbicide binding site on the 32 kD protein, then position 228 cannot be part of the binding site. Azido-atrazine labeled 32 kD protein was trypsinized and the label was attached to a peptide including amino acids 115 to 189.[116b]The reports that the same serine to glycine variation occurs in R and S *S. nigrum*[115,116] lends further credence to the involvement of the 32 kD, but it is not clear-cut either. Different *S. nigrum* biotypes have different dose-response curves (Figure 2) and the I_{50} differentials between R and S biotypes of other species vary considerable (Table 1); presumably the lesion in the gene conferring resistance should be in different amino-acids either at amino acid 228 or at some other locus. How else could be have triazine-resistant *A. hybridus* susceptible to chloroxuron and triazine-resistant *A. retroflexus* totally resistant to the same phenyl-urea herbicide (Table 1)? Indeed, if the standard statistical techniques used by ge-

neticists to determine inheritance were used with these data, one would quickly find that there are not enough of sequenced 32 kD genes from separate individuals to draw conclusions. Some of the best data will clearly come from the algae which can be sexually crossed, such as *Chlamydomonas*. It is much easier to get mutants with algae than with higher plants. Hopefully, the 32 kD from the triazine, phenyl-urea (Table 1), and phenolic herbicide[117] resistant strains will soon be sequenced. Another novel approach that many researchers are contemplating to prove that the 32 kD from R plants confers triazine resistance is to use a plasmid to insert the gene for the 32 kD protein into an organism such as a cyanophyte (blue-green algae) and thereby confer triazine resistance and show that both the amino-acid change and the silent mutations are those of the donor gene-source. Conversely it may be experimentally easier to first mutate the cyanophyte to resistance and insert sensitivity to the triazines. These would probably be the ultimate proof.

V. DIRECTIONS

There is strong circumstantial evidence pointing to the 32 kD protein as the protein conferring resistance to triazine herbicides. There are many such lines of evidence, none of which are wholly conclusive as each has alternative alibis. Most juries would be convinced that the 32 kD is the culprit, but few would find the evidence sufficient for conviction despite the rhetoric of the prosecutors.[118] The evidence is even less persuasive that the 32 kD protein is responsible for binding other PS II-inhibiting herbicides. Even if the 32 kD protein is responsible for triazine-resistance it does not mean that it actually contains the binding-site. The conformational change in the protein responsible for resistance could easily be propagated to a nearby protein which is actually responsible for binding the herbicide.

The discussion of the role of the 32 kD protein in triazine-resistance may well detract too many researchers from the important questions about this peptide: what is its real role in photosynthesis? why is it so highly conserved from species to species? why must it turn over so rapidly (but only when photosynthesis is occurring)? why is its synthesis under such tight controls? Indeed, some recent research has shown that the rate of turnover of the 32 kD is a direct function of PS II activity.[119] This protein must play some key yet still very elusive function in nature.

The plastid gene for triazine-resistance has become of great interest from the point of view of agricultural biotechnology. There are many crops grown in areas where triazines have not been used and these crops could well use atrazine as a cost-effective herbicide with little fear of immediate selection of weed resistance for as many years as crop rotation is used. Breeding techniques have been used to transfer triazine resistance from the weed *Brassica campestris* to *B. napus* (rape-seed for oil).[22] A variety has been released, although its yield *potential* is somewhat lower than the wild-type, but it has proven to be cost effective under farming conditions. Protoplast fusion techniques are being used to transfer this resistance from weeds to related crops in cases where they do not interbreed.[12,31,32] Plans are being promulgated to transfer the 32 kD gene from resistant weeds to crops (unwisely including soybeans which is often grown in rotation with corn).[118] Some basic questions must be seriously asked about the problem before investing large resources in such projects: Is the 32 kD gene really responsible for triazine-resistance? If so, are there various loci in the 32 kD gene that can be mutated to give triazine-resistance? Do all the PS II inhibiting herbicides bind to one, two, or more different proteins? Is there less of a fitness problem in some of these mutants than others? Part of the work showing different plastid properties that might result in inefficiencies were found in R *Amaranthus* (Figures 5, 8B, and 13B), which is claimed to have the same lesion as the resistant biotype of *Solanum nigrum*.[114-116] Much more work on fitness and photosynthesis has been done with the resistant biotype of *Senecio vulgaris* (Figures 6 and 8A). It is unfortunate that we do not have the amino-acid

sequence of the 32 kD of this *Senecio* biotype or that of the *A. retroflexus* with a different sensitivity for chloroxuron (Table 1) for comparison. If all mutants for plastid triazine-resistance have potential yield reductions, using the gene in breeding and genetic engineering may not be agriculturally useful research in the long run, as better selective herbicides will be found for the same crops which do not reduce crop yield. It will be possible to answer many of these basic questions using the alga *Chlamydomonas* which is far superior to higher plants for these types of questions. Because it is a single cell organism, it is possible to apply very high selection pressures, (unavailable in the field with weeds)[4] to get many mutants.[6,8,9,117] Indeed, the fitness of some of the mutants may be lowered as they have a slightly lower growth rate.[8] Because *Chlamydomonas* has genetics with maternal inheritance, the inheritance of resistance and binding can be studied much more easily than has been done so far in a single higher plant.[120] One can also study the inheritance of cross-resistance and amino-acid sequence changes much more easily in *Chlamydomonas*. It is quite probable that if PS II herbicide resistance will be genetically engineered into crop species, the best genes with least loss of fitness will originate from the best *Chlamydomonas* mutants that are extensively tested before transfer, and not from the triazine-resistant weeds.

EPILOGUE

While this chapter was in press, many advances have been made with *Chlamydomonas* genetics and molecular biology which considerably clarify our understanding of the relationships between the 32 kD protein and the binding of PS II inhibiting herbicides. Diuron, atrazine, and bromacil were used separately to isolate more mutants, the cross resistances measured[121] and the sequence of amino acids in the 32 kD protein was deduced from nucleotide sequence of the gene for two of these.[122] This is in addition to mutant D-4 which is diuron- and atrazine-resistant which has already been sequenced and found to have a serine (S) to alanine (R) change at the same position that *Amaranthus* and *Solanum* have transversions to glycine.[116a] These mutants vary from 15 times more resistant to atrazine than the wild type (without cross resistance to bromacil or diuron) to a fivefold increase in resistance to bromacil and diuron (without increase in tolerance to atrazine). There were no silent nucleotide changes between any of the resistant mutants and the wild type, but each had an amino acid substitution at new loci on the 32 kD, the furthest mapping about 40 amino-acids from the substitutions previously reported. Thus, the results clearly show that mutations at different loci on the gene for the 32 kD protein control resistance to these three types of PS II inhibiting herbicides. Whether the conformational change conferred by these amino acid transversions, at considerable distances from each other change the affinity of herbicide binding site(s) on the 32 kD itself or on a nearby thylakoid protein is still an open question. The atrazine-resistant mutant in this group did not have the major changes in electron-transfer capacity noted so far with weeds, and may represent a better gene for biotechnological uses than the triazine-resistant genes hitherto available. Still, as many species are controlled by 30 g/ha atrazine and 2.5 to 4.5 kg/ha are used in corn, the 15-fold increase in tolerance conferred by the newer amino acid transversions may not be enough in agricultural situations.

ACKNOWLEDGMENTS

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REFERENCES

1. **Gressel, J.,** The evolution of herbicide resistant weeds, in *Origins and Development of Adaptation* (Ciba Symp. No. 102), Pitman, London, 1984, 73.
2. **Bandeen, J. D., Stephenson, G. R., and Cowett, E. R.,** Discovery and distribution of herbicide resistant weeds in North-America, in *Herbicide Resistance in Plants*, LeBaron, H. M. and Gressel, J., Eds., John Wiley & Sons, New York, 1982, 9.
3. **Gressel, J., Ammon, H. U., Fogelfors, H., Gasquez, J., Kay, Q. O. N., and Kees, H.,** Discovery and distribution of herbicide resistant weeds outside North America, in *Herbicide Resistance in Plants*, LeBaron, H. M. and Gressel, J., Eds., John Wiley & Sons, New York, 1982, 32.
4. **Gressel, J. and Segel, L. A.,** Interrelating factors controlling the rate of appearance of resistance, in *Herbicide Resistance in Plants*, LeBaron, H. M. and Gressel, J., Eds., John Wiley & Sons, New York, 1982, 325.
5. **Boger, P., Sandmann, G., and Miller, R.,** Herbicide resistance in a mutant of the microalga *Bumilleriopsis filiformis*, *Photosynthesis Res.*, 2, 61, 1981.
6. **Galloway, R. E. and Mets, L.,** Non-mendelian inheritance of 3-(3,4-dichlorophenyl)-1,1-dimethylurea-resistant thylakoid membrane properties in *Chlamydomonas*, *Plant Physiol.*, 70, 1673, 1982.
7. **Laval-Martin, D., Grizeau, D., and Calvayrac, R.,** Characterization of diuron resistant *Euglena:* Greater tolerance for various phenylurea herbicides and increased sensitivity of thylakoids to ethyl-*S*-dipropyl thiocarbamate (EPTC), *Plant Sci. Lett.*, 29, 155, 1983.
8. **Janatkova, H. and Wildner, G. F.,** Isolation and characterization of metribuzin-resistant *Chlamydomonas reinhardii cells*, *Biochim. Biophys. Acta*, 682, 227, 1982.
9. **Tellenbach, M., Gerber, A., and Boschetti, A.,** Herbicide-binding to thylakoid membranes of a DCMU-resistant mutant of *Chlamydomonas reinhardii*, *FEBS Lett.*, 158, 147, 1983.
10. **Thiel, A. and Boger, P.,** Comparative herbicide binding by photosynthetic membranes from resistant mutants, *Pest. Biochem. Physiol.*, (in press).
11. **Meredith, C. P. and Carlson, P. S.,** Herbicide resistance in plant cell cultures, in *Herbicide Resistance in Plants*, LeBaron, H. M. and Gressel, J., Eds., John Wiley & Sons, New York, 1982, 275.
12. **Gressel, J., Ezra, G., and Jain, S. M.,** Genetic and chemical manipulation of crops to confer tolerance to chemicals, in *Chemical Manipulation of Crop Growth and Development*, McLaren, J. S., Ed., Butterworths, London, 1982, 79.
13. **Gressel, J.,** Plant tissue culture systems for screening of plant growth regulators: hormones, herbicides and natural phytotoxins, in *Advances in Cell Cultures*, Vol. 3, Maramorosch, K., Ed., Academic Press, New York, 1984, 93.
14. **LeBaron, H. and Gressel, J., Eds.,** *Herbicide Resistance in Plants*, John Wiley & Sons, New York, 1982, 401 pp.
15. **Gressel, J., Regev, Y., Malkin, S., and Kleifeld, Y.,** Characterization of a *s*-triazine resistant biotype of *Brachypodium distachyon*, *Weed Sci.*, 31, 450, 1983.
16. **Faukner, J. S.,** Breeding herbicide-tolerant crop cultivars by conventional methods, in *Herbicide Resistance in Plants*, LeBaron, H. M. and Gressel, J., Eds., John Wiley & Sons, New York, 1982, 235.
17. **Harvey, B. M. R. and Harper, D. B.,** Tolerance to bipyridylium herbicides, in *Herbicide Resistance in Plants*, LeBaron, H. M. and Gressel, J., Eds., John Wiley & Sons, New York, 1982, 215.
18. **Heap, J. and Knight, R.,** A population of ryegrass tolerant to the herbicide diclofop-methyl, *J. Aust. Inst. Agr. Sci.*, 48, 156, 1982.

18a. **Mudge, L. C., Gossett, B. J., and Murphy, T. R.,** personal communication, 1983.

19. **Schimke, R. T., Kaufman, R. J., Alt, F. W., and Kellems, R. F.,** Gene amplification and drug resistance in cultured murine cells, *Science*, 202, 1051, 1978.
20. **Sawicki, R. and Denholm, I.,** Adaptation of insects to insecticides, in *Origins and Development of Adaptation* (Ciba Symp. No. 102), Pitman, London, 1984.
21. **Jensen, K. I. N.,** The roles of uptake, translocation and metabolism in the differential intraspecific responses to herbicides, in *Herbicide Resistance in Plants*, LeBaron, H. M. and Gressel, J., Eds., John Wiley & Sons, New York, 1982, 133.
22. **Souza-Machado, V.,** Inheritance and breeding potential of triazine tolerance and resistance in plants, in *Herbicide Resistance in Plants*, LeBaron, H. M. and Gressel, J., Eds., John Wiley & Sons, New York, 1982, 257.
23. **Darmency, H. and Gasquez, J.,** Inheritance of triazine resistance in *Poa annua:* consequences for population dynamics, *New Phytol.*, 89, 487, 1981.
24. **Gasquez, J., Darmency, H., and Compoint, J. P.,** Etude de la resistance chloroplastique aux triazines chez *Solanum nigrum L.*, *C. R. Acad. Sci. Paris*, 292D, 847, 1981.
25. **Scott, K. R. and Putwain, P. D.,** Maternal inheritance of simazine resistance in a population of *Senecio vulgaris*, *Weed Res.*, 21, 137, 1981.

26. **Solymosi, P.,** Az *Amaranthus retroflexus* L. triazin rezisztenciajanak oroklodese, *Novenytermeles*, 30, 57, 1981.
27. **Souza-Machado, V. S. and Bandeen, J. D.,** Genetic analysis of chloroplast atrazine resistance in *Brassica campestris* cytoplasmic inheritance, *Weed Sci.*, 30, 281, 1982.
28. **Warwick, S. I. and Black, L.,** Uniparental inheritance of atrazine resistance in *Chenopodium album*, *Can. J. Plant Sci.*, 60, 751, 1980.
29. **Radosevich, S. R. and Holt, J. S.,** Physiological responses and fitness of resistant weed biotypes to triazine herbicides, in *Herbicide Resistance in Plants*, LeBaron, H. M. and Gressel, J., Eds., John Wiley & Sons, New York, 1982, 163.
30. **Arntzen, C. J., Pfister, K., and Steinback, K.,** The mechanism of chloroplast triazine resistance: alterations in the site of herbicide action, in *Herbicide Resistance in Plants*, Le Baron, H. and Gressel, J., Eds., John Wiley & Sons, New York, 1982, 185.
31. **Binding, H., Jain, S. M., Finger, J., Mordhorst, G., Nehls, R., and Gressel, J.,** Somatic hybridization of a triazine resistant biotype of *Solanum nigrum* with *S. tuberosum*. 1. Clonal variations in morphology and atrazine sensitivity, *Theor. Appl. Genet.*, 63, 273, 1982.
32. **Gressel, J., Cohen, N., and Binding, H.,** Somatic hybridization of an atrazine resistant biotype of *Solanum nigrum* with *Solanum tuberosum*. 2. Segregation of plastomes, *Theor. Appl. Genet.*, 67, 131, 1984.
33. **Putwain, P. D.,** personal communication, 1982.
34. **Holliday, R. J. and Putwain, P. D.,** Evolution of herbicide resistance in *Senecio vulgaris:* variation in susceptibility to simazine between and within populations, *J. Appl. Ecol.*, 17, 779, 1980.
35. **Oettmeier, W., Masson, K., Fedtke, C., Konze, J., and Schmidt, R. R.,** Effect of different photosystem II inhibitors on chloroplasts isolated from species either susceptible or resistant toward *s*-triazine herbicides, *Pestic. Biochem. Physiol.*, 18, 357, 1982.
36. **Pfister, K. and Arntzen, C. J.,** The mode of action of photosystem II — specific inhibitors in herbicide-resistant weed biotypes, *Z. Naturforsch.*, 34c, 996, 1979.
37. **Gasquez, J.,** Evolution de la resistance aux triazines chez les especes annuelles: la resistance chloroplastique, COLUMA Versailles 11e Conference, December 1981, 981.
38. **Zanin, G., Vecchio, B., and Gasquez, J.,** Indagini sperimentali su popolazioni di dicotiledoni resistenti all'Atrazine, *Riv. Agron.*, 3-4, 196, 1981.
39. **Renger, G.,** Studies on the structural and functional organization of system II photosynthesis. The use of trypsin as a structurally selective inhibitor at the outer surface of the thylakoid membrane, *Biochim. Biophys. Acta*, 440, 287, 1976.
40. **Souza-Machado, V., Arntzen, C. J., Bandeen, J. D., and Stephenson, G. R.,** Comparative triazine effects upon system II photochemistry in chloroplasts of two common lambsquarters *Chenopodium album* biotypes, *Weed Sci.*, 26, 318, 1978.
41. **Ducruet, J. M. and Gasquez, J.,** Observation of whole leaf fluorescence and demonstration of chloroplastic resistance to atrazine in *Chenopodium album L.* and *Poa annua L.*, *Chemosphere*, 8, 695, 1978.
42. **Trebst, A. and Draber, W.,** Structure activity correlations of recent herbicides in photosynthetic reactions, in *Advances in Pesticide Science, 1978*, Geissbuhler, H., Brooks, G. T., and Kearney, P. C., Eds., Pergamon Press, London, 1979, 223.
43. **Govindjee and van Rensen, J. J. S.,** Bicarbonate effects on the electron flow in isolated broken chloroplasts, *Biochim. Biophys. Acta*, 505, 183, 1978.
44. **Haldane, J. B. S.,** More precise expressions for the cost of natural selection, *J. Genet.*, 57, 351, 1960.
45. **Ahrens, W. H., Arntzen, C. J., and Stoller, E. W.,** Chlorophyll fluorescence assay for the determination of triazine resistance, *Weed Sci.*, 29, 316, 1981.
46. **Ali, A. and Souza-Machado, V.,** Rapid detection of triazine resistant weeds using chlorophyll fluorescence, *Weed Res.*, 21, 191, 1981.
47. **Droppa, M., Horvath, G., and Demeter, S.,** Effect of photosystem II herbicides in triazine susceptible and resistant *Amaranthus retroflexus* L. Biotypes investigated by thermoluminescence, *Z. Pflanzenphysiol.*, 105, 75, 1981.
48. **Cahadia, E., Ducruet, J. M., and Gaillardon, P.,** Whole leaf fluorescence as a quantitative probe of detoxification of the herbicide chlortoluron in wheat, *Chemosphere*, 11, 445, 1982.
49. **Bowes, J., Crofts, A. R., and Arntzen, C. J.,** Redox reactions on the reducing side of photosystem II in chloroplasts with altered herbicide binding properties, *Arch. Biochem. Biophys.*, 200, 303, 1980.
50. **Gasquez, J. and Darmency, H.,** Variation of chloroplast properties between two triazine-resistant biotypes of *Poa annua L.*, *Plant Sci. Lett.*, 30, 99, 1983.
51. **Holt, J. S., Stemler, A. J., and Radosevich, S. R.,** Differential light responses of photosynthesis by triazine-resistant and triazine-susceptible *Senecio vulgaris*, *Plant Physiol.*, 67, 744, 1981.
52. **Holt, J. S., Radosevich, S. R., and Stemler, A. J.,** Differential efficiency of photosynthetic oxygen evolution in flashing light in triazine-resistant and triazine-susceptible biotypes of *Senecio vulgaris L.*, *Biochim. Biophys. Acta*, 722, 245, 1983.

53. **Vermaas, W. F. J. and Arntzen, C. J.,** Synthetic quinones influencing herbicide binding and photosystem II electron transport: the effects of triazine-resistance on quinone binding properties in thylakoid membranes, *Biochim. Biophys. Acta*, 725, 483, 1983.
54. **Burke, J. J., Wilson, R. F., and Swafford, J. F.,** Characterization of chloroplasts isolated from triazine susceptible and triazine resistant biotypes of *Brassica campestris L.*, *Plant Physiol.*, 70, 24, 1982.
55. **Blein, J. P.,** Mise en culture de cellules de jeunes plantes de *chenopodium album L.* sensibles ou resistantes a latrazine, *Physiol. Veg.*, 18, 703, 1980.
56. **Pillai, P. and St.-John, J. B.,** Lipid composition of chloroplast membranes from weed biotypes differentially sensitive to triazine herbicides, *Plant Physiol.*, 68, 585, 1981.

56a. **Lemoine, Y., Dubacq, J. P., and Ducruet, J. M.,** Modification de l'appareil photosynthetique et de sa stabilite thermique chez une population de navette *(Brassica campestris)* resistante a l'atrazine, Proc. 6th. Intl. Rapeseed Conf., 197, 1983.

57. **Grenier, G., Marier, J. P., and Beaumont, G.,** Effects physiologiques de l'atrazine a doses subletales sur *Lemna minor* L. IV. Influence sur la composition lipidique, *Can. J. Bot.*, 57, 1015, 1978.
58. **Browse, J., Roughan, P. G., and Slack, C. R.,** Light control of fatty acid synthesis and diurnal fluctuations of fatty acid composition in leaves, *Biochem. J.*, 196, 347, 1981.
59. **Stout, D. G. and LaCroix, L. J.,** Membrane lipid changes during cold acclimation of spring rape, *Z. Pflanzenphysiol.*, 101, 335, 1981.
60. **Gressel, J.,** unpublished results, 1983.
61. **deWit, C. T.,** On competition, *Versl. Landbouwkd. Onderz. L.*, 66, 1, 1960.
62. **Conard, S. G. and Radosevich, S. R.,** Ecological fitness of *Senecio vulgaris* and *Amaranthus retroflexus* biotypes susceptible or resistant to atrazine, *J. Appl. Ecol.*, 16, 171, 1979.
63. **Pyle, D. W.,** Effects of artificial selection on reproductive fitness in *Drosophila*, *Nature (London)*, 263, 317, 1976.
64. **Bradshaw, A. D.,** Evolution of heavy metal tolerance — an analogy to herbicide resistance, in *Herbicide Resistance in Plants*, LeBaron, H. M. and Gressel, J., Eds., John Wiley & Sons, New York, 1982, 293.
65. **Warwick, S. I. and Black, L.,** The relative competitiveness of atrazine susceptible and resistant populations of *Chenopodium album* and *C. strictum*, *Can. J. Bot.*, 59, 689, 1981.
66. **Ahrens, W. H. and Stoller, E. W.,** Competition, growth rate and CO_2 fixation in triazine-susceptible and resistant smooth pigweed *(Amaranthus hybridus)*, *Weed Sci.*, 31, 438, 1983.

66a. **Ort, D. R., Ahrens, W. H., Martin, B., and Stoller, E. W.,** Comparison of photosynthetic performance in triazine-resistant and susceptible biotypes of *Amaranthus hybridus*, *Plant Physiol.*, 72, 925, 1983.

67. **Bhomik, P. C.,** Differential growth response to temperature between two biotypes of *Chenopodium album*, *Proc. N.E. Weed Sci. Soc.*, 36, 89, 1982.
68. **Ducruet, J. M., Lemoine, Y., and Cahadia, E.,** Activite photosynthetique et labilite thermique de chloroplastes de *Chenopodium album* et *Brassica campestris* sensibles et resistants aux triazines, *Biochimie*, Suppl. 2, 45, 1982.
69. **Gasquez, J. and Compoint, J. P.,** Isoenzymatic variations in populations of *Chenopodium album L.*, *Agro-ecosystems*, 7, 1, 1981.
70. **Gasquez, J. and Darmency, H.,** personal communications, 1982—1983.
71. **Wiest, S. C.,** Kinetic and proteolytic identification of heat-induced conformational changes in the DCMU binding site of isolated *Phaseolus* chloroplast thylakoids, (submitted).
72. **Tischer, W. and Strotmann, H.,** Relationship between inhibitor binding by chloroplasts and inhibition of photosynthetic electron transport, *Biochim. Biophys. Acta*, 460, 113, 1977.
73. **Van Assche, C. J. and Carles, P. M.,** Photosystem II inhibiting chemicals: Molecular interaction between inhibitors and a common target, in *Biochemical Responses Induced by Herbicides*, Moreland, D. E., St. John, J. B., and Hess, F. D., Eds., ACS Symp. Ser. 181, American Chemical Society, Washington, D.C., 1982.
74. **Shipman, L. L.,** Binding sites associated with inhibition of photosystem II, in *Biochemical Responses Induced by Herbicides*, Moreland, D. E., St. John, J. B. and Hess, F. D., Eds., ACS Symp. Ser. 181, American Chemical Society, Washington, D.C., 1982, 23.
75. **Fromm, H. J.,** Use of competitive substrate analogs and alternative substrates for studying kinetic mechanisms, in *Initial Rate Enzyme Kinetics*, Springer-Verlag, Berlin, 1975, chap. 4.
76. **Khanna, R., Pfister, K., Keresztes, A., Van Rensen, J. J. S., and Govindjee,** Evidence for a close spatial location of the binding sites for CO_2 and for photosystem II inhibitors, *Biochim. Biophys. Acta*, 634, 105, 1981.
77. **Van Rensen, J. J. S.,** Molecular mechanisms of herbicide action near photosystem II, *Physiol. Plant.*, 54, 515, 1982.

77a. **Lavergne, J.,** Mode of action of 3-(3,4 dichlorophenyl)-1,1-dimethyl urea. Evidence that the inhibitor competes with plastoquinone for binding to a common site on the acceptor side of photosystem II, *Biochim. Biophys. Acta*, 682, 545, 1982.

77b. **Oettmeier, W. and Soll, H. J.,** Competition between plastoquinone and 3-(3,4-dichlorophenyl)-1,1-dimethylurea at the acceptor side of photosystem II, *Biochim. Biophys. Acta*, 724, 287, 1983.
78. **Vermaas, W. F. J., Arntzen, C. J., Gu, L.-Q., and Yu, C.-A.,** Interactions of herbicides and azidoquinones at a photosystem II binding site in the thylakoid membrane, *Biochim. Biophys. Acta*, 723, 266, 1983.
79. **Moreland, D. E. and Novitzky, W. P.,** Interference by DDT and cyclodiene types of insecticides with chloroplast associated reactions, *Chem. Biol. Interactions*, (in press).
80. **Arntzen, C. J., Ditto, C. L., and Brewer, P. E.,** Chloroplast membrane alterations in triazine-resistant *Amaranthus retroflexus* biotypes, *Proc. Natl. Acad. Sci. U.S.A.*, 76, 278, 1979.
81. **Pfister, K., Steinback, K. E., Gardner, G., and Arntzen, C. J.,** Photoaffinity labeling of an herbicide receptor protein in chloroplast membranes, *Proc. Natl. Acad. Sci. U.S.A.*, 78, 981, 1981.
82. **Trebst, A.,** Inhibition of photosynthetic electron flow by phenol and diphenylether herbicides in control and trypsin treated chloroplasts, *Z. Naturforsch.*, 34c, 986, 1979.
83. **Steinback, K. E., Pfister, K., and Arntzen, C. J.,** Trypsin mediated removal of herbicide binding sites within the photosystem II complex, *Z. Naturforsch.*, 36c, 98, 1981.
84. **Boger, P. and Kunert, K. J.,** Differential effects of herbicides upon trypsin-treated chloroplasts, *Z. Naturforsch.*, 34c, 1015, 1979.
85. **Mattoo, A. K., Pick, U., Hoffman-Falk, H., and Edelman, M.,** The rapidly metabolized 32,000 dalton polypeptide of the chloroplast is the "proteinaceous shield" regulating photosystem II electron transport and mediating diuron herbicide sensitivity, *Proc. Natl. Acad. Sci. U.S.A.*, 78, 1572, 1981.
86. **Van Rensen, J. J. S. and Kramer, H. J. M.,** Short-circuit electron transport insensitive to diuron-type herbicides induced by treatment of isolated chloroplasts with trypsin, *Plant Sci. Lett.*, 17, 21, 1979.
87. **Hagemann, R., Vermaas, W., and Renger, G.,** Participation of a lysine containing polypeptide in herbicide binding at the acceptor side of photosystem II, Proc. 6th Int. Cong. Photosynthesis, 4, 17, 1984.
88. **Renger, G., Hagemann, R., and Dohnt, G.,** The role of lysine residues for excitation energy transfer and herbicide action in system II, *Photobiochem. Photobiophys.*, 5, 273, 1983.
89. **Eaglesham, A. R. J. and Ellis, R. J.,** Protein synthesis in chloroplasts. II. Light-driven synthesis of membrane proteins by isolated pea chloroplasts, *Biochim. Biophys. Acta*, 335, 396, 1974.
90. **Rosner, A., Jakob, K. M., Gressel, J., and Sagher, D.,** The early synthesis and possible function of 0.5×10^6 M_r RNA after transfer of dark grown *Spirodela* plants to light, *Biochem. Biophys. Res. Commun.*, 67, 383, 1975.
91. **Grierson, D.,** Light-stimulated accumulation of 14S RNA during chloroplast development in spinach cotyledons, *Z. Pflanzenphysiol.*, 95, 171, 1979.
92. **Reisfeld, A., Gressel, J., Jakob, K. M., and Edelman, M.,** Characterization of the 32,000 dalton membrane protein. I. Early synthesis during photoinduced plastid development of *Spirodela*, *Photochem. Photobiol.*, 27, 161, 1978.
93. **Grebanier, A. E., Coen, D. M., Rich, A., and Bogorad, L.,** Membrane proteins synthesized but not processed by isolated maize chloroplasts, *J. Cell Biol.*, 78, 734, 1978.
94. **Weinbaum, S., Gressel, J., Reisfeld, A., and Edelman, M.,** Characterization of the 32,000 dalton chloroplast membrane protein. III. Probing its biological function in *Spirodela*, *Plant Physiol.*, 64, 828, 1979.
95. **Reisfeld, A., Mattoo, A. K., and Edelman, M.,** Processing of a chloroplast-translated membrane protein, *In vivo* analysis of the rapidly synthesized 32000-dalton shield protein and its precursor in *Spirodela oligorrhiza*, *Eur. J. Biochem.*, 124, 125, 1982.
96. **Mohana-Rao, J. K., Hargrave, P. A., and Argos, P.,** Will the seven helix bundle be a common structure for integral membrane proteins, *FEBS Lett.*, 156, 165, 1983.
97. **Edelman, M., Mattoo, A. K., and Marder, J. B.,** Three hats of the rapidly metabolized 32 kilodalton protein of the thylakoids, in *Chloroplast Biogenesis*, Ellis, R. J., Ed., Cambridge University Press, Cambridge, 1984, 283.
98. **Edelman, M., Marder, J. B., and Mattoo, A. K.,** A compendium of characteristics for the rapidly-metabolized 32kd protein of the chloroplast membrane, in *Structure and Function of Plant Genomes*, Ciferri, O. and Dure, L., Eds., Plenum Press, New York, 1983, 187.
99. **Marder, J. B., Goloubinoff, P., and Edelman, M.,** Molecular architecture of the rapidly metabolized 32 kD protein of photosystem II; Indications for carboxy-terminal processing of a chloroplast membrane protein, *J. Biol. Chem.*, 259, 3900, 1984.
100. **Leto, K. J. and Miles, D.,** Characterization of three photosystem II mutants in *Zea mays L.* lacking a 32,000 dalton lamellar polypeptide, *Plant Physiol.*, 66, 18, 1980.
101. **Gressel, J.,** Triazine herbicide interaction with a 32,000 M_r thylakoid protein — alternative possibilities, *Plant Sci. Lett.*, 25, 99, 1982.
102. **Oettmeier, W., Masson, K., and Johanningmeier, U.,** Photoaffinity labelling for photosystem II herbicide binding protein, *FEBS Lett.*, 118, 267, 1980.

103. **Steinback, K. E., McIntosh, L., Bogorad, L., and Arntzen, C. J.,** Identification of the triazine receptor protein as a chloroplast gene product, *Proc. Natl. Acad. Sci. U.S.A.*, 78, 7463, 1981.
104. **Oettmeier, W., Masson, K., and Johanningmeier, U.,** Evidence for two different herbicide-binding proteins at the reducing side of photosystem II, *Biochim. Biophys. Acta*, 679, 376, 1982.
105. **Mullet, J. E. and Arntzen, C. J.,** Identification of a 32-34 kilodalton polypeptide as a herbicide receptor protein in photosystem II, *Biochim. Biophys. Acta*, 635, 236, 1981.
106. **Hoffman-Falk, H., Mattoo, A. K., Marder, J. B., and Edelman, M.,** General occurrence and structural similarity of the rapidly synthesized, 32,000-dalton protein of the chloroplast membrane, *J. Biol. Chem.*, 257, 4583, 1982.
107. **Kuwabara, T. and Murata, N.,** An improved purification method and a further characterization of the 33-kilodalton protein of spinach chloroplasts, *Biochim. Biophys. Acta*, 680, 210, 1982.
108. **Mattoo, A. K., Marder, J. B., Gressel, J., and Edelman, M.,** Presence of the rapidly-labelled 32,000-dalton chloroplast membrane protein in triazine resistant biotypes, *FEBS Lett.*, 140, 36, 1982.
109. **Gysin, A. and Knusli, E.,** Chemistry and herbicidal properties of triazine derivatives, *Advan. Pest. Control Res.*, 3, 289, 1960.
110. **Gressel, J., Shimabukuro, R. H., and Duysen, M. E.,** *N*-dealkylation of atrazine and simazine in *Senecio vulgaris* biotypes, a major degradation pathway, *Pestic. Biochem. Physiol.*, 19, 361, 1983.
110a. **Trebst, A., Draber, W., and Donner, W. T.,** Mode of action and MO calculation of two classes of herbicides interacting with the reducing side of PSII, in *Pesticide Chemistry — Human Welfare and Environment*, Miyamoto, J., Ed., Pergamon Press, Oxford, 1983, 91.
111. **Satoh, K., Nakatani, H. Y., Steinback, K. E., Watson, J., and Arntzen, C. J.,** Polypeptide composition of a photosystem II core complex; presence of a herbicide-binding protein, *Biochim. Biophys. Acta*, 724, 142, 1983.
112. **Johanningmeier, U., Newmann, E., and Oettmeier, W.,** Interaction of a phenolic inhibitor with photosystem II particles, *J. Bioenerg. Biomembranes*, 15, 43, 1983.
113. **Zurawski, G., Bohnert, H. J., Whitfeld, P. R., and Bottomley, W.,** Nucleotide sequence of the gene for the M_r 32,000 thylakoid membrane protein from *Spinacia oleracea* and *Nicotiana debneyi* predicts a totally conserved primary translation product of M_r 38,950, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 7699, 1982.
114. **Hirschberg, J. and McIntosh, L.,** Molecular basis of herbicide resistance in *Amaranthus hybridus*, *Science*, 222, 1346, 1983.
115. **Hirschberg, J. and McIntosh, L.,** unpublished findings cited in Reference 114.
116. **Goloubinoff, P., et al.,** unpublished personal communication, 1983.
116a. **Erickson, J. M., Rahire, M., Bennoun, P., Delepelaire, P., Diner, B., and Rochaix, J. D.,** Herbicide resistance in *Chlamydomonas reinhardtii* results from a mutation in the chloroplast gene for the 32kD protein of photosystem II, *Proc. Natl. Acad. Sci. U.S.A.*, 81, 3617, 1984.
116b. **Wolber, P. K. and Steinback, K. E.,** Identification of the herbicide binding region of the Q_B-protein by photoaffinity labeling with azido-atrazine, *Z. Naturforsch.*, 39C, 425, 1984.
117. **Pucheu, N., Oettmeier, W., Heisterkamp, U., Masson, K., and Wildner, G. F.,** Metribuzin resistance and the herbicide binding protein in *Chlamydomonas reinhardtii*, Proc. 6th Int. Cong. Photosynthesis, 4, 29, 1984.
118. **Marx, J. L.,** Plants' resistance to herbicide pinpointed, *Science*, 220, 41, 1983.
119. **Mattoo, A. K., Hoffman-Falk, H., Marder, J. B., and Edelman, M.,** Regulation of protein metabolism: Coupling of electron transport to in vivo degradation of the rapidly metabolized 32000 dalton protein of the chloroplast membranes, *Proc. Natl. Acad. Sci. U.S.A.*, 81, 1380, 1984.
120. **Darr, S., Souza-Machado, V., and Arntzen, C. J.,** Uniparential inheritance of a chloroplast photosystem II polypeptide controlling herbicide binding, *Biochim. Biophys. Acta*, 634, 219, 1981.
121. **Galloway, R. E. and Mets, L. J.,** Atrazine, bromacil, and diuron resistance in *Chlamydomonas*, *Plant Physiol.*, 74, 469, 1984.
122. **Erickson, J. M. and Rochaix, J.-D.,** unpublished personal communication, 1984.

Chapter 8

HERBICIDE ABSORPTION AND TRANSLOCATION AND THEIR RELATIONSHIP TO PLANT TOLERANCES AND SUSCEPTIBILITY

F. Dan Hess

TABLE OF CONTENTS

I. INTRODUCTION

For herbicides to affect weeds they must come in contact with the symplastic (living) system of the plant. At the very least the chemical must contact the plasmalemma surrounding the symplasts. To achieve this contact, the herbicide must be absorbed into the plant and translocated to the site of action. The absorption can be in root, stem, or leaf tissue and translocation can be short distance (movement through a few cell layers) or long distance (movement in the xylem and phloem). The degree of absorption and translocation can govern the efficiency of weed control and can be the limiting factor in the usefulness of a herbicide for a particular situation. In most instances, only a small portion of the applied herbicide ever reaches the site of action. By understanding the mechanism of herbicide absorption and translocation we can perhaps manipulate these processes to increase the overall efficiency of herbicide use. The first part of this chapter will describe the mechanisms of absorption and translocation of preemergence and postemergence applied herbicides. Maximizing herbicide absorption and translocation in all plant species may not always be a desirable goal. Many uses require crop plants to be tolerant of the applied herbicide. One mechanism for crop tolerance and weed susceptibility to a particular herbicide is differential absorption or translocation between the crop plant and weed. The second part of this chapter will discuss the involvement of absorption and translocation in plant tolerance to herbicides.

II. ABSORPTION MECHANISMS

A. Absorption After Preemergence Applications

Absorption of soil-applied herbicides can occur in root and shoot tissue in direct contact with herbicide dissolved in the soil solution or present in the vapor phase. Whereas root absorption is commonly recognized as important after preemergence application, shoot absorption can also be significant during germination, soil emergence, and early growth.

1. Root Absorption

Even in seedling plants, root tissue constitutes a surprisingly large surface area of absorption and the single-celled root hairs (outgrowths of epidermal cells) increase this surface area substantially. As plants continue to grow, their root systems become massive. A 4-month-old rye (*Secale cereale* L.) plant was estimated to have a total root system measuring 626 km in length, with 233 m^2 of surface area.[1] Adding root hairs brought the total surface area to over 638 m^2![1] New root hairs were estimated to develop at a rate of more than 100 million per day.[2] Root hairs differentiate behind the meristematic and cell elongation regions, in the zone of differentiation. In most species an individual root hair functions for only a few days. As the root tip grows through the soil, new root hairs are constantly being differentiated and old ones are dying. Because of this continual growth, the root tip provides a uniform tissue with a constant physiological age.

Besides anchoring the plant to the soil, the primary function of the root system is to absorb water and nutrients from the soil. During growth, plants require the influx of large amounts of water. The absorption of this water occurs primarily at the root hair zone. Those substances dissolved in the water (e.g., herbicides) come in contact with the root surface and can be absorbed along with the water. The root tip area, including the root hair zone, of some species has been reported to be covered by a thin layer of lipid-like substance.[3] This substance is not a significant barrier to penetration because water, minerals, and herbicides readily penetrate through the epidermal layer.

Herbicide molecules in contact with the root, from the meristematic region through the functioning root hair zone, appear to move into the root by simple diffusion. For example, atrazine concentration in excised velvetleaf (*Abutilon theophrasti*, Medic) roots became equal

to the external atrazine concentration by 30 min and did not accumulate to concentrations above the external solution.[4] In addition, Q_{10} values for atrazine absorption in this tissue were between 1.3 and 1.4, suggesting a passive diffusion. Over longer periods of time (e.g., 24 h) herbicides can accumulate to above the concentration of the external medium; however, the accumulation may be the result of nondissociated, lipid-soluble herbicide molecules partitioning into the lipid constituents of the roots.[5]

2. Shoot Absorption

During germination and growth, prior to soil emergence, shoot tissue is exposed to the soil solution and any herbicide dissolved in the soil solution. In addition, volatile herbicides present in the vapor phase of the soil can come in contact with shoot tissue. Shoot tissue below the soil surface does have a surface cuticle, although the degree of development is less than above-ground tissue. Herbicide dissolved in the water and vapor phase, in contact with below ground shoot tissue, can be absorbed into the shoot. The amount of injury associated with shoot absorption is dependent on the species and herbicide. In many instances, shoots of grass species are particularly sensitive to shoot exposure. EPTC, diallate, and propham induced injury to oats (*Avena sativa* L.) after coleoptile exposure.[6] These herbicides are growth inhibitors and the site of action in oats is the growing coleoptile and the shoot meristem at the base of the coleoptile. Therefore, in this case the site of absorption is close to the site of action. In giant foxtail (*Setaria faberi* Herrm.), atrazine, propachlor, linuron, and trifluralin caused more than a 50% reduction in dry weight of shoot tissue 2 weeks after the herbicides were incorporated into the soil only in the shoot zone.[7]

B. Postemergence Applications

With acceptance of reduced tillage farming practices, the interest and use of postemergence herbicides is increasing. The amount of herbicide adsorption is first dependent on the amount contacting the shoot tissue. The amount reaching the plant surface and then adhering is in turn dependent on leaf orientation with respect to incoming spray, density of plant canopy, leaf pubescence (Figure 1A and 1B), surface waxiness (Figure 1C and D), and surface tension of the spray solution. Once herbicide has reached the plant surface five things can happen:[8]

1. The herbicide may not remain on the leaf surface. It can run off while still in the liquid form, it can be washed off by rain, or it can volatilize from the surface.
2. The herbicide can dry down on the leaf surface as an amorphous deposit (Figure 2A) or crystallize after solvent evaporation (Figure 2B).
3. If lipophilic, the herbicide can penetrate into the cuticle and remain associated with the lipoidal components.
4. The herbicide can absorb across the cuticle and then move in the apoplastic system of the cell walls and xylem.
5. As in 4, the herbicide can absorb across the cuticle and enter the cell wall system, then absorb into the symplast of parenchyma or phloem cells.

1. Absorption Through Plant Cuticles

The most significant barrier to herbicide absorption into shoot tissue is the cuticle, which covers the surface of essentially all epidermal cells of all above-ground shoot systems. The cuticle is a complex and variable layer. Cuticle development varies between plant species and different plant parts within a species. The degree of cuticle development even varies at different locations on the same leaf. In addition, the amount of cuticle present is dependent on age of the plant part and environmental conditions during cuticle deposition.

The cuticle has three distinct components, each having a unique chemical and physical

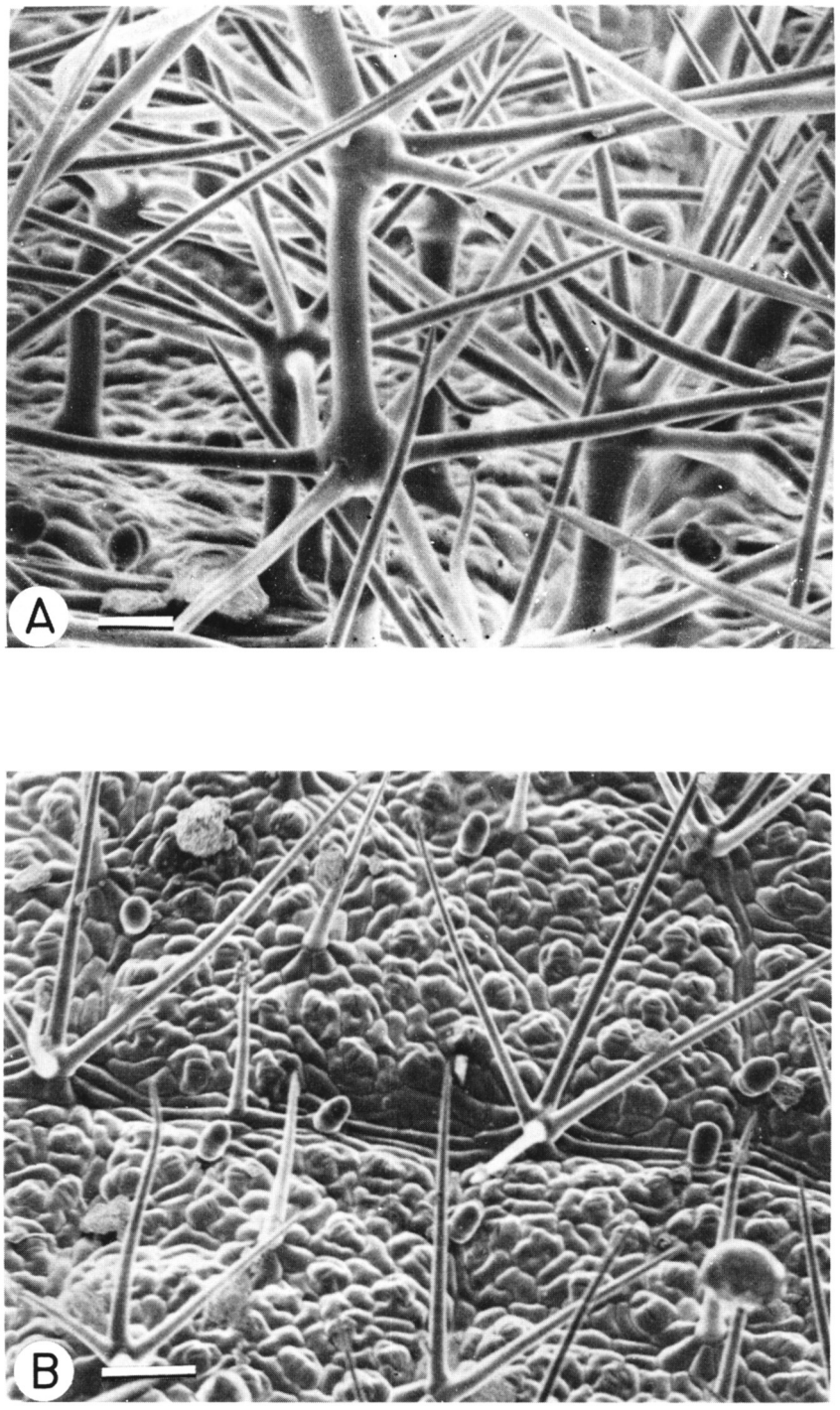

FIGURE 1. Scanning electron micrographs of leaf surfaces. (A) Common mullein (*Verbascum thapsus* L.), scale marker = 50 μm. (B) Lower epidermis of velvetleaf (*Abutilon theophrasti* Medic) scale marker = 100 μm. (C) Cabbage (*Brassica oleracea* var *capitata* L.), scale marker = 25 μm. (D) Sugar beet (*Beta vulgaris* L.), scale marker = 25 μm.

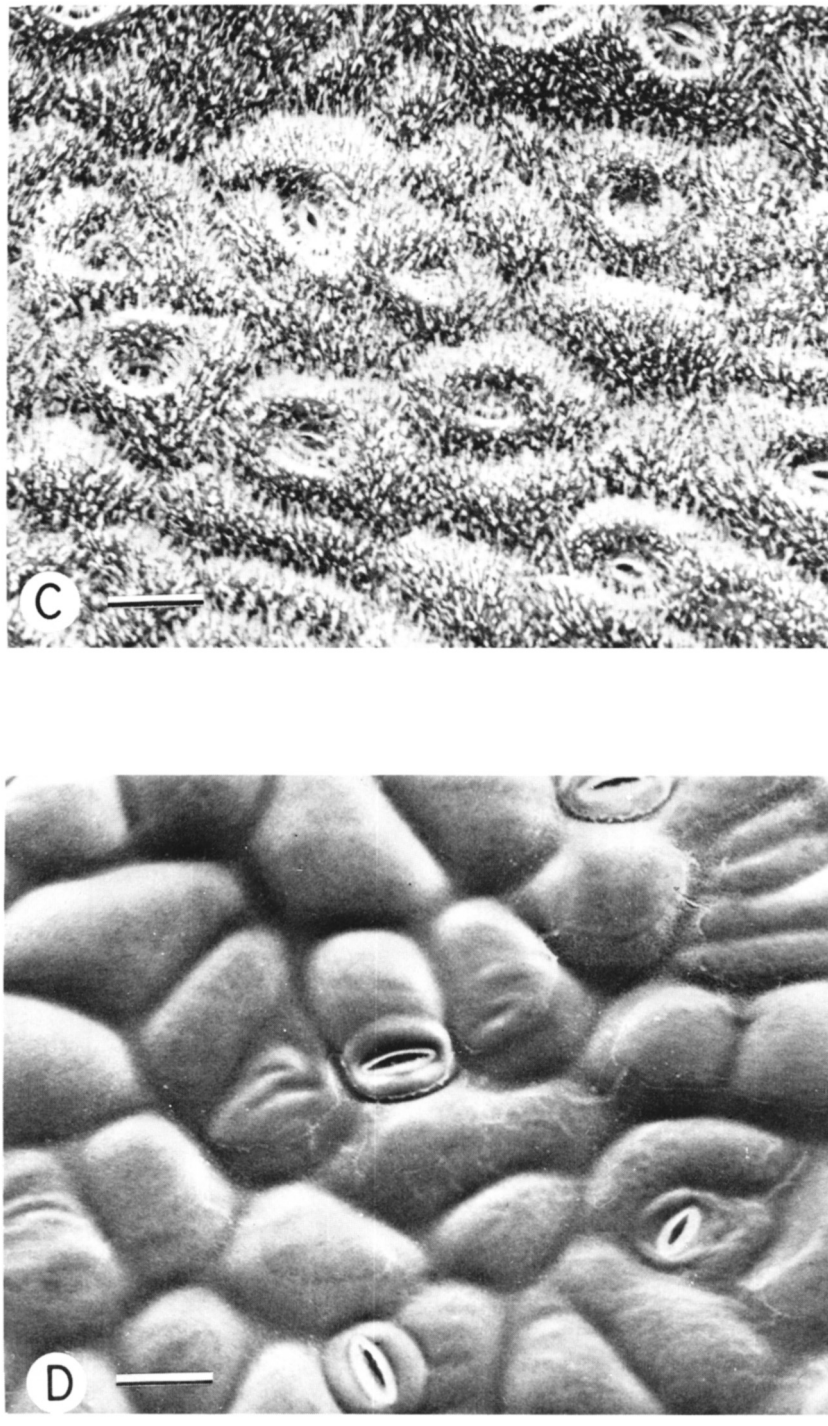

FIGURE 1. C and D.

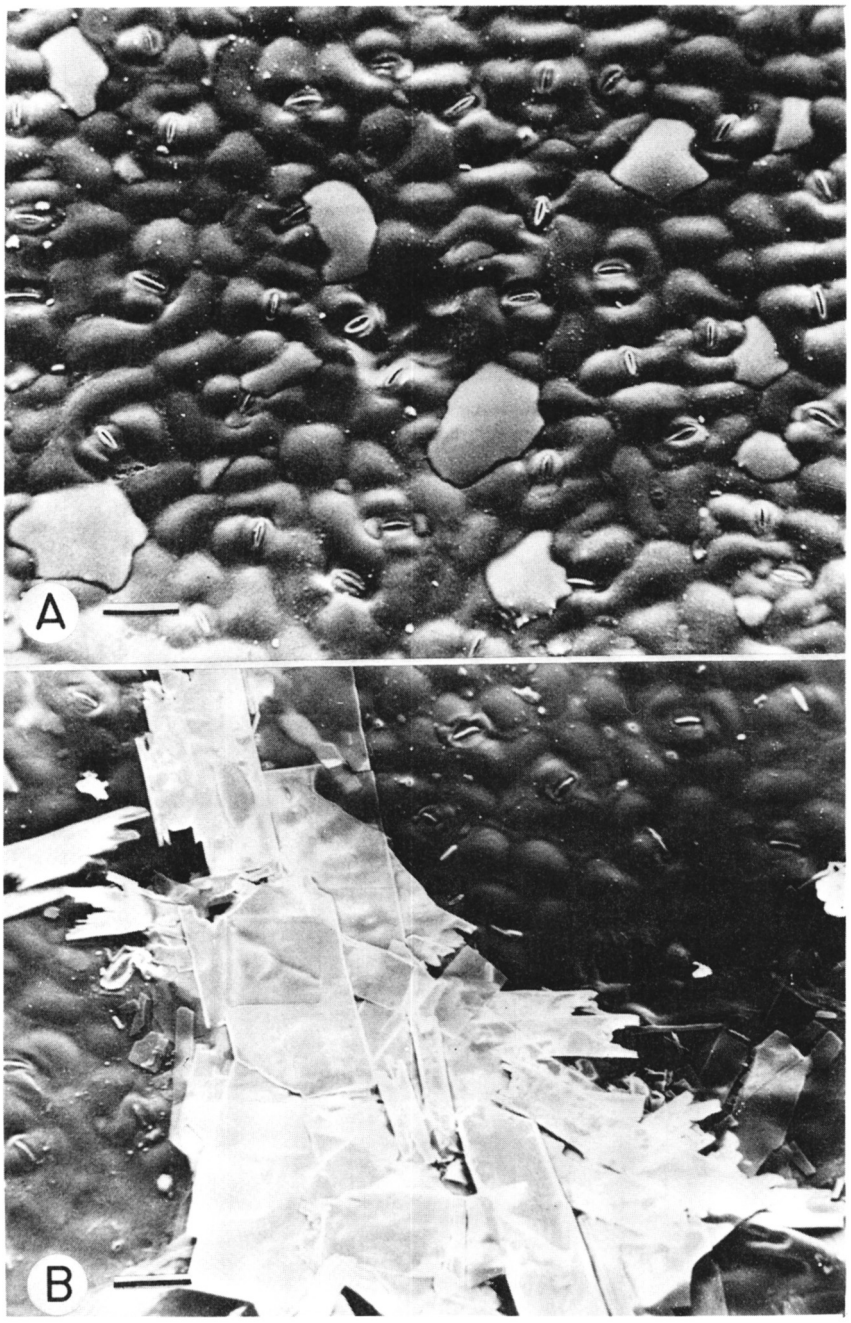

FIGURE 2. Scanning electron micrographs of sugar beet leaf surfaces sprayed with 5.6 kg/ha propanil. A and B are different commercial emulsifiable concentrate formulations. Scale markers = 50 μm. (From Hess, F. D., Bayer, D. E., and Falk, R. H., *Weed Sci.*, 29, 224, 1981. With permission.)

structure. One component, the wax, is divided between that occurring on the surface (epicuticular wax) and that occurring within the cuticle proper (embedded wax). These waxes are primarily composed of long straight or branched chain alkanes, fatty ketones, fatty aldehydes, fatty alcohols (primary and secondary), saturated and unsaturated fatty acids,

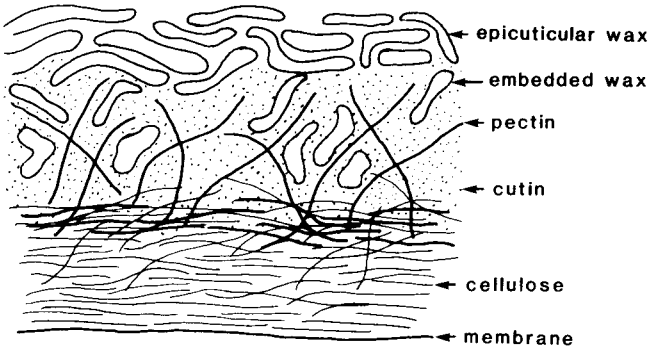

FIGURE 3. Diagrammatic representation of the location of components within plant cuticles.

and fatty esters with chain length ranging from C_{21} to C_{35}.[9] The composition of the epicuticular wax markedly influences the wettability characteristics of the cuticle.[10] In addition to a characteristic composition, epicuticular wax often has a unique ultrastructure that varies between species and within species in different environments.[9] The characteristic ultrastructure of these waxes has been divided by Amelunxen et al.[11] into 6 categories (granules, rods, plates, crusts, aggregates, and amorphous). A second component, cutin, is less complex. Cutin is composed of hydroxylated fatty acids and fatty acids bound together by ester linkages.[9] The C_{16} to C_{18} chain lengths predominate. Both mono- and dicarboxylic acids are reported to be present and the one to three hydroxyl groups may be terminal or intermediate on the chain. A portion of the polar groups remain free, therefore, cutin is more hydrophilic than wax. Because of this increased hydrophilic nature, cutin has the ability to become hydrated in the presence of water. The final structural component in the cuticle is pectin, the most hydrophilic component of the cuticle. Pectin is primarily polysaccharide polymers rich in uronic acid which are present in the form of strands. These pectin strands are either present in a layer near the cutin-cellulose microfibril interface or interspersed in the cutin layer. The exact structural arrangement of the cuticular components is unknown, however, many suggest it may look somewhat like the structure of a sponge. The matrix of the sponge corresponds to the cutin, whereas the holes correspond to the embedded wax. The surface of the sponge is coated with different shapes of epicuticular wax and pectin strands extending into, but not through the sponge (Figure 3). Therefore, the cuticle composition and structure is such that both lipophilic and hydrophilic pathways are present for herbicide penetration.[9] In general, cuticle deposition is a finite process. Deposition occurs during leaf expansion and its termination coincides approximately with the cessation of leaf blade expansion.[12]

With respect to herbicide movement through plant cuticles, one major complication is the influence of environmental conditions on cuticle development. Because of this interaction the amount, composition, and hydration state of cuticle present on a given species will not be consistent during different herbicide applications. In a 1982 review written by Hull et al.,[9] they state "the present state of our knowledge is such that the applicator relies primarily on the recurrence of a set of environmental conditions for the desired herbicidal effect. When climatic, plant, edaphic, or any one of many other factors change, the applicator can only make an educated guess as to the expected result under these new field conditions." Of those published reports dealing with the influence of environmental conditions on cuticle development, most agree environmental stress induces a change in subsequently synthesized cuticle composition, quantity, and structure, and these changes influence herbicide penetration. For example, Skoss[12] reported that tree tobacco (*Nicotiana glauca* Graham) grown

under water stress had a thicker cuticle and a lower wettability than plants not under stress. Environmental parameters commonly recognized as influencing cuticle composition and structure during deposition are soil moisture, relative humidity, temperature, and light intensity.

Dry soil and associated water stress can induce development of thick cuticles. Unfortunately, whether enhanced cuticle development is the result of relative humidity or available soil moisture is not always clearly defined.[10] Leaves of peas (*Pisum sativum* L.), sugar beet (*Beta vulgaris* var. *saccharifera* L.), and strawberry (*Fragaria* x *ananassa* Duch.) grown at 40% pot capacity soil moisture had up to three times as much wax as those grown at 100% pot capacity.[13] This increased wax deposition decreased penetration rates of cuticular-applied substances. Amer and Williams,[14] however, found that when geranium (*Pelargonium zonale* Ait.) was grown under moisture stress conditions, subsequent leaf cuticle thickness, as measured by electron microscopy, did not differ from plants receiving normal amounts of water. Hammerton[15] discusses other instances where water stress had a variable effect on leaf surface wettability and concluded the relationship between water stress and post-emergence herbicide efficacy, prior to and at spraying, appears to be far from clear.

Several investigators have reported consistent trends with the relationship between relative humidity and cuticle development. Decreased relative humidity during cuticle deposition generally results in increased cuticle thickness per unit area of leaf. For example, Tribe et al.[16] reported lipid content in cuticles of oat and barley (*Hordeum vulgare* L.) was inversely proportional to relative humidity. In brussels sprouts (*Brassica oleracea* var. *gemmifera* Zenker), low relative humidity caused an increase in wax deposition and modified the size, configuration, and distribution of surface wax structures.[17]

Temperature is thought to have a greater influence on the quantity and quality of wax than on that of the cutin.[9] In most instances maximum cuticle is produced when temperatures are intermediate rather than excessively high or low.[10] In tree tobacco, leaf cuticle development was greater at 17°C day temperature than at higher or lower temperatures, whereas the ratio of wax to cutin increased with increasing temperature.[12] In mesquite (*Prosopis juliflora* Swartz) seedlings, cuticular wax content was maximum at a day/night temperature of 30/26°C.[18]

In most instances, cuticle development is positively correlated with light intensity. In oat and barley, Tribe et al.[16] found the total amount of cuticle was proportional to light intensity. Epicuticular wax production on pea leaves increased as light intensity was increased from 900 to 5000 fc.[19] In field rape (*Brassica napus* L.), shading reduced epicuticular wax at all temperatures studied.[20] Skoss[12] suggested the increased cuticle production at high light intensities could be the result of localized areas of water stress caused by rapid transpiration in the sun.

2. Stomatal Penetration

Stomata consist of guard cells surrounding a pore (stoma). Stomata open and close to provide a pathway for exchange of gas between the internal leaf and the environment. The density of stomata varies with species. In most instances there are more stomata on the abaxial (lower) surface of leaves. For example, Canada thistle (*Cirsium arvense* (L.) Scop.) has 4,600 and 12,300, barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.) has 11,100 and 11,200, redroot pigweed (*Amaranthus retroflexus* L.) has 13,000 and 17,500, and common lambsquarter (*Chenopodium album* L.) has 8,000 and 10,400 stomata per cm^2 on the adaxial and abaxial surface, respectively.[21]

With regard to herbicide penetration, stomata can potentially be involved in two ways. First, the cuticle over the guard cell appears thinner and more permeable to substances than cuticle over other epidermal cells. For example, Neumann and Jacob reported that when aminoisobutyric acid was applied to leaves, the material became preferentially located in guard cells.[22] Second, spray solution can conceivably move through the pore of an open

stomata and into the substomatal chamber. The most recognized work is that of Dybing and Currier.[23] They followed the penetration of a fluorescent dye after mixing it in different surfactant solutions. With an appropriate surfactant concentration (0.05 to 0.5%), entry through open stomata occurred after leaves were dipped in treatment solutions for 5 min. The dye did not enter the substomatal chamber when the stomata were closed or a surfactant was not included in the application solution. Different species required different amounts of surfactant to achieve stomatal penetration. As discussed by Bukovac (Reference 5 and personal communication), stomatal penetration of spray droplets from a water-based spray solution is a complex process and is probably of minor importance in field applications of herbicides. Important considerations are the surface tension of the liquid, the contact angle produced by the liquid on the plant surface (see Chapter 6), and the morphology and chemistry of the stoma pore wall.[5] The degree of stomatal opening is of little importance.[24] Stomatal pore penetration can occur if the surface tension of the spray solution is such that complete wetting (where the surface tension of the liquid is less than the leaf critical surface tension) occurs.[24] When complete wetting does not occur, stomatal pore penetration can only occur if the contact angle between the spray drop and cuticle surface is smaller than the wall angle of the pore wall.[24] Even if stomatal pore penetration is achieved, most species are believed to have a cuticle on the cell surfaces of the substomatal chamber. As concluded by Ashton and Crafts[8] and Bukovac,[5] stomatal pore penetration of water-based spray cannot be depended upon as an efficient or reliable means of herbicide absorption.

3. Plasmalemma Penetration

With the exception of herbicides that may act at the plasmalemma surface, herbicides must move across the plasmalemma prior to reaching the site of action. Herbicide movement across cell membranes is generally concluded to be passive. There are four important concepts with respect to passive diffusion across membranes:[1]

1. Cells must be functioning normally. Abnormal cell metabolism generally results in membranes becoming more permeable to all solutes.
2. Water molecules and dissolved gases (O_2, N_2, CO_2) penetrate cell membranes freely.
3. Water-soluble (hydrophilic) molecules penetrate membranes at a rate inversely related to their molecular size.
4. Lipid-soluble (hydrophobic) molecules penetrate membranes at a rate positively related to their lipid solubility and fairly independently of molecular size.

Of the herbicides adequately studied, movement across membranes has been primarily shown to be passive. Atrazine absorption into corn (*Zea mays* L.) protoplasts was shown to be complete in 10 sec.[25] The theoretical time for simple diffusion to reach equilibrium in these protoplasts was calculated to be 7 sec. Internal concentrations in these protoplasts were 36% higher than the external solution, however, this accumulation was thought to result from partitioning of atrazine into cellular lipids.[25] In potato (*Solanum tuberosum* L.) tuber slices, atrazine and diuron rapidly (30 min) penetrated the entire tissue volume and then were able to diffuse freely out of the tissue.[26] This strongly suggests a passive diffusion across the plasmalemma. The one documented exception to passive diffusion is the phenoxy herbicides. In barley roots, absorption of exchangeable and nonexchangeable 2,4-D appeared to depend on a supply of metabolic energy.[27] However, when parenchyma tissue from potato tubers was treated with inhibitors of ATP anabolism and catabolism, 2,4-D accumulation was not significantly affected.[28] Using a cell suspension of crown gall cells from ivy (*Parthenocissus tricuspidata* Planch), Rubery concluded, however, there was a saturable carrier-mediated component to 2,4-D anion absorption in addition to the passive diffusion of the undissociated acid.[29] At pH 6.5, 2,4-D accumulated in corn root protoplasts to a level higher

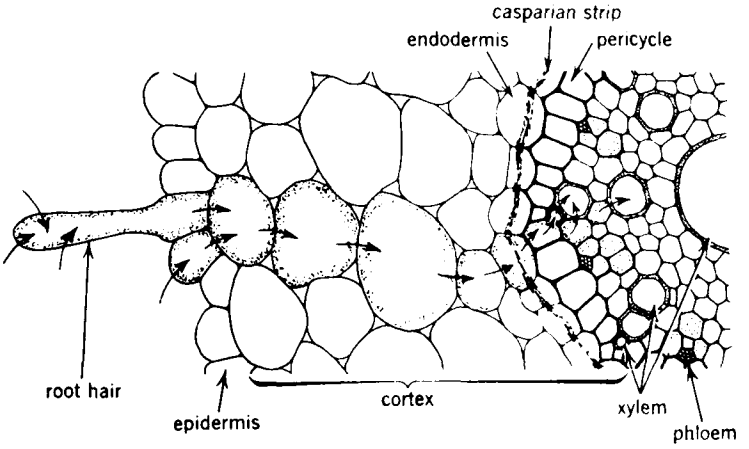

FIGURE 4. Diagram of the endodermis of plant roots with associated Casparian strip. (Reproduced with permission from Esau, K., *Plant Anatomy*, John Wiley & Sons, New York, 1965, 517.)

than predicted.[25] This supports the hypothesis of a carrier being involved in the movement of the 2,4-D anion across plant membranes.

III. TRANSLOCATION MECHANISMS

A. Short Distance Translocation

Once herbicide is absorbed into root or shoot tissue, further movement must occur before reaching the site of action. Many compounds, for example growth inhibitors applied preemergence or photosynthesis inhibitors applied postemergence, only need to move a few cell layers to reach the site of action. This movement can be in the cell walls (apoplastic) or within cells (symplastic). Herbicide movement in cell walls can be by diffusion or with any mass flow of water. Once absorbed into the cell, symplast herbicide movement can occur during cytoplasmic streaming. Movement from cell to cell may be achieved by movement through the cell to cell cytoplasmic connections (plasmodesmata). The density of plasmodesmata are often estimated at greater than 1 million per mm^2 of cell wall surface.[1] Whereas movement through plasmodesmata has not been specifically proven for herbicides, various other solutes have been shown to move through plasmodesmata.[1]

Movement of herbicide from the root surface to the xylem is an important short distance translocation system that deserves special attention. The pathway can be symplastic from epidermis to xylem. However, the xylem is an apoplastic system so the herbicide must leave the symplasm at this location. Movement of herbicide can be apoplastic from the epidermis to the endodermis, but the endodermis contains a barrier that limits apoplastic movement beyond this point. All radial and transverse walls of the endodermis contain a banded area where the walls are impregnated with suberin (Figure 4). Suberin is a complex mixture of lipophilic substances; primarily long chain (>16 carbons) fatty acids and alcohols and phenolic compounds (principally ferulic acid). Because of this band, termed the Casparian strip, water and dissolved substances must enter the symplast at this location in the root. As discussed in the plasmalemma penetration section (Section II.B.3), herbicides are apparently able to readily penetrate the plasmalemma, so the Casparian strip is not a significant barrier to most herbicide translocation. Once inside the endodermal layer, the herbicide can move to the xylem surface in either the apoplast or symplast.

B. Long Distance Translocation

When trying to control weeds beyond the seedling stage of growth, long distance translocation in the xylem and phloem become important. Without adequate translocation, the whole weed is not killed and regrowth will reintroduce the weed problem. Achieving translocation is particularly important when postemergent compounds are used to control perennial weeds.

1. Xylem Translocation

The functional xylem system is a continuous network of cells extending from the zone of differentiation (root hair zone) of all roots to all mature and developing leaves. The principle purpose of the xylem is translocation of water and dissolved substances from roots to leaves. For a detailed discussion of xylem morphology, see Reference 2. The xylem elements are nonliving when functional, and thus are considered part of the apoplastic system. Water moves in the xylem by two mechanisms. In small plants, under high soil moisture and high relative humidity, water can move as a result of root pressure. In large plants, when the relative humidity is less than saturated, water is pulled up the xylem as a result of water evaporating from the leaf surface (transpiration). This second mechanism is by far the most common type of xylem translocation of water and dissolved substances. The water evaporating from the leaf can provide an adequate driving force (pull) for this mechanism to function. At 100% relative humidity the water potential of air equals zero. At 98% relative humidity the water potential of air decreases to -27.5 bars, which would be adequate to support a column of water 281 m high.[1] As the relative humidity decreases, the water potential continues to decrease. So even when the relative humidity is high, there is a substantial water potential gradient from the soil, through the plant, to the leaf surface. If the plant is transpiring, a large negative water potential is created in the leaves and a water potential gradient then exists through the continuous water column in the xylem network to the roots. The roots have a more negative water potential than the soil solution, so the rate of diffusion of water, and substances dissolved in the water, into the roots will increase. The overall mass movement of water, and hence dissolved substances (e.g., herbicides), will be towards the more negative water potential (the evaporating surface of the leaf).

Because of the mass flow of water and dissolved substances in the xylem system during transpiration and the apparent diffusion of herbicides through the plasmalemma at the endodermis, most herbicides are xylem mobile. Not all herbicides, however, are xylem mobile. There are several explanations for a lack of xylem mobility:

1. Herbicides may adsorb to apoplastic or symplastic cellular components.
2. Herbicides may become compartmentalized in cellular components (e.g., vacuoles or plastids.
3. Herbicides may become conjugated to cellular substrates that are not xylem mobile.

Because of the need for nearly all herbicides to enter the symplast to reach their sites of action, it is unlikely many herbicides are not xylem mobile as a result of an inability to cross the plasmalemma at the endodermis.

2. Phloem Translocation

After photosynthesis, the products must be moved from their point of production (source) to their point of utilization (sink). One very important location for utilization of photosynthesis products (photosynthate) is roots. Once stored products from the seed endosperm are utilized, all carbon substrates needed for root maintenance and growth must be supplied by the shoot tissue. Other sinks are stems, young leaves, flowers, developing fruits, rhizomes, and tubers. Sink areas either utilize or store the products imported by the phloem. The degree of

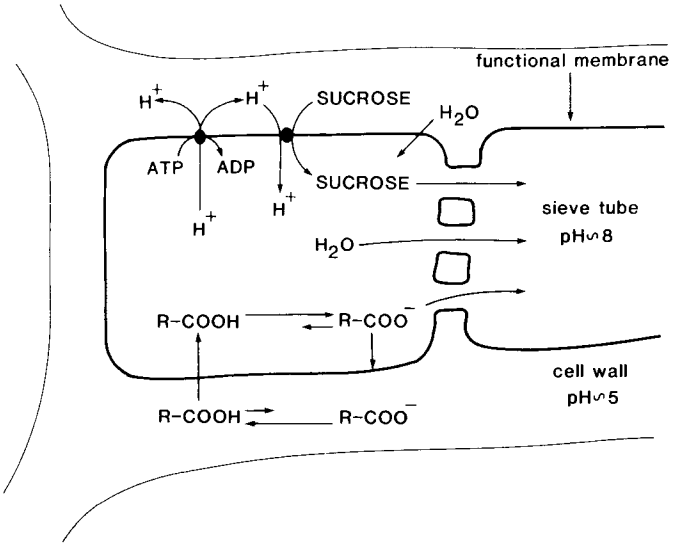

FIGURE 5. Ion trapping model for phloem loading of herbicides. The acid cell wall favors the protonated herbicide (R−COOH), which can readily diffuse across the plasmalemma. The basic pH of the sieve element favors the nonprotonated herbicide (R−COO⁻), which cannot readily diffuse across the plasmalemma. Sucrose is loaded, by a co-transport system, against a concentration gradient. Water enters the phloem by osmosis, and as the pressure increases, water and substances dissolved in the water flow towards sink areas where the pressure is lower.

translocation from a source is positively correlated to the rate of utilization and storage of photosynthate at the various sink areas. The magnitude of individual sinks changes during different times of the year and should be considered prior to application of a phloem-mobile herbicide. For example, when plants flower and set fruit, the structures involved become significant sinks. These sinks may not be the preferred location for herbicide accumulation. In addition, leaves located at different shoot areas tend to translocate phloem substances to different sink areas. For example, lower leaves of herbaceous plants tend to translocate substances to the roots, whereas upper leaves favor translocation of substances to developing leaves and the shoot meristem.[1] Considering phloem translocation of herbicides is particularly important when trying to control perennial weeds with extensive vegetative reproductive tissues dispersed throughout a soil profile.

The principle cell type in the phloem of angiosperms are sieve elements that when joined end to end are termed sieve tubes. Sieve elements are living cells (symplast) that are devoid of nuclei. The end walls (sieve plates) of sieve elements contain connective pores that join sieve elements together. Loading and unloading of photosynthate (primarily sucrose) into and out of phloem at sources and sinks are important in the understanding of the movement of substances in the phloem. Photosynthate appears to be secreted by leaf mesophyll cells into the cell wall (apoplast). The photosynthate then enters companion cells or directly into sieve elements. Photosynthate is moved from the apoplast, across the plasmalemma, to the symplast of the companion cell or sieve element, against a concentration gradient, by the use of metabolic energy (ATP) (Figure 5). The sugar concentration is from 1.5 to 2 times higher in the sieve elements than in the mesophyll cells. Photosynthate in sink sieve elements is low, as a result of utilization and storage, so unloading of the phloem may be by simple diffusion. The most widely accepted theory for movement of substances in the phloem is the Münch pressure flow hypothesis, which is a passive transport model. The different sugar

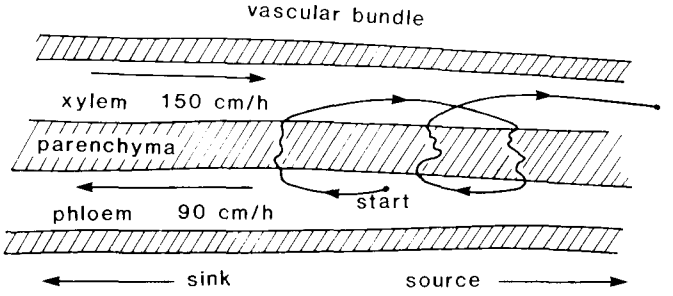

FIGURE 6. Diagram of why lipophilic, nonionizable herbicides are not able to effectively translocate out of a source leaf. Assume the vascular bundle is located in a leaf petiole and that the herbicide remains in the xylem, phloem, and associated parenchyma for 2 min each.

concentration in sieve elements and surrounding parenchyma cells at sources and sinks causes osmosis to occur. Water flows in at the source, building up pressure. With the storage or utilization of sugar in the sink the osmotic concentration of the phloem is reduced and water flows out. Because water is moving in at the source and out at the sink, and because the two systems are connected there is mass flow of water in the sieve tubes. Substances dissolved in the water (e.g., sugars, herbicides) flow along with the water.

A question arises as to why some herbicides are able to move in the phloem and others are not. Based on collected data (see Section II.B.3) herbicides are readily able to move across membranes, so most herbicides are apparently able to enter the phloem. The reason why some herbicides do not translocate any appreciable distance in the phloem is they are too able to diffuse across membranes! Atrazine, for example, can diffuse into the phloem but as the water (and dissolved atrazine) in the phloem move, the atrazine diffuses back out. In leaves and petioles the xylem and phloem systems are in proximity (vascular bundles). Xylem and phloem flow is in opposite directions and water flow in the xylem (transpiration stream) is more rapid than in the phloem. The net direction of movement in the vascular bundle for herbicides (e.g., the triazines) that freely move between the symplast (phloem) and the apoplast (xylem), will be in the direction of the transpirational stream (Figure 6).[26] In fact, when the direction of the transpirational stream was reversed in an excised segment of a corn leaf, basipetal movement of simazine did occur.[30]

Why then, can some herbicides remain in the phloem long enough to translocate? An explanation becomes apparent when the structure of phloem mobile herbicide molecules is studied. All herbicides, with one exception to be discussed later, have ionizable groups (mostly carboxyl groups), which can exist as a protonated (noncharged) or a nonprotonated anionic) form. These forms are in equilibrium, with the favored species being dependent on the pH of the surrounding medium. The pH of the apoplast around the phloem is approximately 5 and the pH of the symplast of the sieve elements is approximately 8.[31] This gradient is established during the active loading of sucrose into sieve elements by the involvement of an ATPase. In the apoplast, therefore, ionizable groups with appropriate pK_a values will be primarily protonated and because the overall characteristic of the molecule will be nonpolar, will readily penetrate the plasmalemma of the sieve element. Because of the high pH of the sieve element, the equilibrium will be shifted toward the nonprotonated (anionic) form. This form is not as readily able to penetrate the plasmalemma, so the herbicide becomes trapped in the sieve element. A diagram of this principle is shown in Figure 5. By attaching a free carboxyl group to atrazine or defenuron, Jacob and Neumann[31] were able to demonstrate phloem mobility. For this principle to work, herbicides must have significantly

different degrees of dissociation between pH 5 and pH 8.[31] Penetration into the phloem can be inhibited with uncouplers of oxidative phosphorylation; however, the inhibition may be the result of degradation of the pH gradient rather than a direct inhibition of herbicide penetration. The exception to all phloem-mobile compounds containing an cationic group with an appropriate pK_a is amitrole. There have been suggestions, however, that amitrole may translocate in the phloem as a conjugate.[32]

Because herbicides move in the phloem dissolved in water, which is moving by mass flow from source to sink, the amount of herbicide that will move to a sink area is relative to the degree of water and dissolved sugar flow to that area.What happens to the herbicide at the sink area? If sugar diffuses out of the phloem, as suggested by many physiologists, the pH gradient, caused by active loading, will not be present. Without the pH gradient there is no difference in the degree of herbicide ionization between the inside and outside of the sieve element. The unionized form will rapidly move across the sieve element membrane, whereas the ionized form will more slowly diffuse across the membrane.

IV. ABSORPTION AND TRANSLOCATION AS A BASIS FOR HERBICIDE TOLERANCE

There are numerous examples of tolerance of one species (usually a crop) and susceptibility of another species (usually a weed) being the result of differential absorption or translocation. These differences occur in both preemergence and postemergence applications. Unfortunately, tolerance and susceptibility can usually not be pinned to a single explanation. For example, differential translocation may be the result of differential degradation at the site of absorption yielding some products that will translocate and other products that will not translocate. In addition, differential absorption will usually result in differential translocation. Even though there is sometimes no clear or simple explanation of reasons for tolerance or susceptibility, there are useful principles and examples that can lead to an increased understanding of the associated mechanisms.

A. Tolerance Associated with Absorption

Because of different germination and seedling emergence characteristics, root and shoot morphology, and cuticle development between plants, it is not surprising that differences in herbicide absorption can explain tolerance and susceptibility. The mechanisms involved are different for preemergence and postemergence applications and thus will be discussed separately.

1. Tolerance by Differential Absorption After Preemergence Application

If herbicide absorption is primarily by the root, selectivity can occur as a result of different depths of root-absorbing tissue between the crop and weed.[33] Weeds usually germinate near the soil surface and many annual crops can be planted below this weed emergence zone, so during early growth the roots of weeds, but not those of the crop, will contact a herbicide band near the soil surface. Root depth is a particularly important selectivity mechanism in many perennial tree crops. Morphological differences between grass and broadleaf species may be responsible for absorption differences and hence tolerance differences between species. When napropamide was applied preemergence to sensitive corn and less sensitive tomato (*Lycopersicon esculentum* Mill.), absorption studies showed total napropamide levels in corn roots (fibrous root system) were 60% higher than in tomato (tap root system).[34] Tomato translocated the herbicide apoplastically within 0.5 h, whereas little translocation was reported in corn after this time interval. During the 8-h experiment, no metabolism differences between species were detected.[34] There are, however, examples where tolerance differences are the result of different locations of the herbicide site of action between the

crop and weed. This is most common for growth-inhibiting herbicides affecting meristems. In one study, the growth-inhibitor herbicides EPTC, chlorpropham, and propham in contact with shoot tissue did not affect pea shoot growth but did reduce corn shoot growth.[35] The reason may be the location of the sensitive apical growing regions. In pea, the apical meristem is located on top of the plant, away from treated soil, whereas, in corn seedlings it is located at or just below the soil surface.

Different grass species vary in morphology of their emerging shoots, which can account for differences in susceptibility to herbicides. In 1960, Baker reported a comparison of rice (*Oryza sativa* L.) and barnyardgrass susceptibility to chlorpropham.[36] Because of mesocotyl (the internode between the caryopsis and coleoptile) elongation, barnyardgrass produces the first node (which contains the shoot meristem and is the site of chlorpropham action) near the herbicide band on the soil surface. The mesocotyl of rice does not expand, so the shoot meristem was well below the herbicide band. A similar explanation was given for the control of wild oat (*Avena fatua* L.) in barley and wheat by diallate[37] and for green foxtail (*Setaria viridis* L.) in wheat (*Triticum aestivum* (L.) Beauv.) by trifluralin.[38] If trifluralin was applied directly to the coleoptilar node of wheat, damage occurred.[38] In some instances more than just the coleoptile node area is sensitive to herbicide absorption in grass species. Wheat, barley, and oats were injured only when EPTC was applied near the coleoptile node area; however, sorghum (*Sorghum bicolor* (L.) Moench) was injured at all shoot application locations.[39] Sorghum was the most sensitive species tested and absorbed two times more EPTC into shoot tissue than wheat.[39]

Not all differences in absorption are caused by morphological variation. Susceptible snap beans (*Phaseolus vulgaris* L.) absorbed more terbutryn and fluometuron during germination than tolerant cotton (*Gossypium hirsutum* L.).[40] After 96 h, snap beans absorbed four times more terbutryn than cotton. Cotton accumulated most of the herbicides in the seed coat, which was shed following emergence. In addition to differential absorption, there was very little translocation in cotton, whereas translocation in snap beans was to the cotyledon and then to seedling leaves. Absorption and subsequent translocation of tetrafluron was limited in tolerant peanuts (*Arachis hypogaea* L.) and high in susceptible jimsonweed (*Datura stramonium* L.) and prickly sida (*Sida spinosa* L.).[41] After 6 h, jimsonweed absorbed 50% of applied tetrafluron and peanut absorbed only 10%.[41] Differences in absorption, as an explanation for tolerance, has even been reported in the same species. A hybrid of corn (PAG-644) susceptible to butylate absorbed 66% more than a tolerant hybrid (Pioneer 3030) over a 10-h period.[42] Both hybrids translocated the same percentage of absorbed herbicide. There was, however, a significant difference in metabolism between the two hybrids. The tolerant hybrid inactivated substantially more butylate than the susceptible hybrid.[42]

2. Tolerance by Differential Absorption After Postemergence Application

For postemergence applications of herbicides the amount absorbed into shoot tissue is dependent on the amount adhering to the surface of the plant. The amount adhering is governed by spray solution surface tension, leaf area, leaf orientation with respect to the angle of incidence of the spray drops, spray volume, and foliage characteristics (for example, leaf waxiness).[33,43] In a study reported by Sharma et al.,[44] wild oat was found to be susceptible and flax (*Linum usitatissimum* L.) tolerant of asulam. Wild oat retained four times more spray solution (commercial formulation) than flax. Absorption was more rapid in flax, but contact injury prevented translocation. In oats, asulam distribution was throughout the plant.

There are instances when differential penetration is important in tolerance and susceptibility, yet the difference cannot be explained by different amounts of spray retention. Five days after application of 5 $\mu\ell$ of herbicide solution, absorption of cyanazine by tolerant corn was only 20% of that absorbed by susceptible green foxtail and 5% of that absorbed by susceptible fall panicum (*Panicum dichotomiflorum* Michx.).[45] After 48 h, there was

more acifluorfen absorption into susceptible common ragweed (*Ambrosia artemisiifolia* L.) and common cocklebur (*Xanthium pensylvanicum* Wallr.) than in tolerant soybean (*Glycine max* L.).[46] In addition, soybeans were able to rapidly metabolize acifluorfen.[46] For ioxynil solutions containing 0.1% Tween 20, susceptible mustard (*Brassica* sp. L.) absorption was seven times greater than tolerant pea after 2 and 4 h.[47] Absorption was not related to stomatal entry, so differences in cuticular penetration were concluded to have "contributed substantially" to selectivity.[47] DMSA and MSMA provided 80% more control of yellow nutsedge (*Cyperus esculentus* L.) than purple nutsedge (*Cyperus rotundus* L.).[48] Yellow nutsedge absorbed more of these herbicides than did purple nutsedge. Degradation of the herbicides was minor in both species The authors concluded differential penetration contributed "substantially" to the control difference.[48] In addition to species within the same genus absorbing different amounts of herbicide, slightly different herbicides can absorb differently in the same species. Kirkwood et al.[49] evaluated absorption of MCPA and MCPB in broad bean (*Vicia faba* L.). Broad bean was found to be more tolerant to MCPB than MCPA. From 12 to 26% of the applied MCPB was dissolved in the epicuticular wax, thus resulting in less absorption of MCPB in comparison to MCPA. This proposed cause of differential tolerance was supported by different partitioning ability between water and hexane for the sodium salt of MCPA (250:1) and MCPB (10:1). In addition, when epicuticular wax was removed by chloroform prior to treatment, the absorption and translocation of MCPB was increased.[49]

Differential absorption between species, after postemergence application, is often associated with differences in cuticles. The tolerance level of different cabbage (*Brassica oleracea* var *capitata* L.) cultivars to nitrofen has been associated with wax content.[50] The resistant cultivar, Hybelle, had more wax (2.95 mg/dm^2) on the primary and secondary leaf than the susceptible cultivar, Rio Verde (2.33 mg/dm^2). The absorption of nitrofen into the low wax cultivar was twice that of the high wax cultivar. After removing the wax from Hybelle (by rubbing the leaf surface with glass wool) its susceptibility was increased. In this instance tolerance was clearly associated with epicuticular wax thickness. Norris[51] concluded, however, that cuticle composition may be as important as thickness. He measured cuticle thickness of nine species (range; 1.4 μm to 10.8 μm) and then found no correlation between cuticle thickness and absorption of 2,4-D. In addition, if wax was removed by chloroform there was no correlation between the amount of wax removed and the increase in 2,4-D absorption.

3. Environmental Conditions Changing Tolerance

The penetration characteristics of most foliar-applied herbicides have been investigated, however, in most instances the influence of environmental conditions are not known. Commonly, radioactive herbicide is applied to greenhouse grown plants and subsequent penetration monitored. The cuticle is an important barrier because in nearly all instances complete absorption of applied herbicide is not reported. For example, in hemp dogbane (*Apocynum cannabinum* L.), application of the isopropylamine salt of glyphosate and 1.0% cationic surfactant to the newest fully-expanded leaf of greenhouse-grown plants resulted in 51% penetration 12 days after application.[52] Averaged over absorption periods ranging over a 7-day period, 45% of the sodium salt of bentazon remained on the surface of wild mustard (*Brassica kaber* (DC.) L.C. Wheeler), 63% on the surface of redroot pigweed, and 58% on the surface of soybean after applying the herbicide to greenhouse-grown plants (20°C).[53] Forty eight hours after applying acifluorfen to the midveins of greenhouse-grown common ragweed, cocklebur, and soybean, 89%, 89%, and 96% remained on the leaf surface, respectively.[46] Averaged over field applications in March, July, and November, Drummond's goldenweed (*Isocoma drummodii* (T. & G.) Greene) only absorbed 43% of the applied 2,4-D (diethylamine salt) and 34% of the applied picloram (potassium salt) 5 days after treatment.[54]

Where environmental influences on herbicide penetration have been investigated, the

parameter studied was usually relative humidity (RH) during and after application. Pallas[55] reported greater absorption of 2,4-D into leaves at 70 to 74% RH than at 34 to 38% RH. Glyphosate injured cotton and purple nutsedge more when plants were placed in 100% RH than in 40% RH after spraying.[56] At all temperatures tested, leaf absorption of acifluorfen into crotalaria (*Crotalaria spectabilis* Roth) was three to four times greater at 100% RH than at 40% RH.[57] When the sodium salt of asulam was applied to growth chamber (20°C) grown wild oat plants, 10% penetrated at 40% RH and 39% penetrated at 90% RH 24 h after application.[44] In johnsongrass (*Sorghum halepense* (L.) Pers.) absorption and translocation of glyphosate were often greater at 100% RH than 45% RH.[58] In addition, absorption increased when the air temperature was increased from 24 to 35°C and when the soil moisture was increased from 12% (near the wilting point) to 20% (field capacity). In another report on the effect of soil moisture, Ahmadi et al.[59] reported an increase of glyphosate absorption into barnyardgrass from 20% in the low soil moisture regime (-37 bars) to 62% in the high soil moisture regime ($-1/8$ bar).

Few correlations between environmental effects on cuticles and herbicide penetration have been made. In general, if environmental conditions at spraying include ample soil moisture and high relative humidity, the pectin and cutin are hydrated and hydrophilic herbicides can penetrate as can lipophilic herbicides. Another factor thought to be important in absorption following postemergence spray application is the rate of drop drying. Savory et al.[60] reported the potency of ioxynil and bromoxynil was decreased with increased wind speeds, which they equated to increased rates of drop drying. In another study Prasad et al.[61] reported the rate of drop drying apparently contributed to the observed increased absorption of dalapon at high relative humidity. Because spray droplets dried less quickly at high relative humidity, the period of effective dalapon absorption was prolonged. They observed that at low relative humidity, periodic rewetting of the droplet area enhanced absorption. With increasing cuticle desiccation caused by low relative humidity and dry soil, following spray application, the hydrophilic route is reduced and hydrophilic herbicides are not able to readily penetrate the cuticle.[9] The lipophilic herbicides, however, are still able to penetrate the cuticle because of their dominate lipophilic nature. For example, Morrison and Akey[62] reported the absorption of the methyl ester of dichlofop into wild oat leaves 12, 24, or 48 h after application did not differ in plants growing in soil-moisture stressed conditions when compared to nonstressed conditions.

Environmental conditions prior to application can also influence herbicide absorption. As discussed in Section II.B.1, the environment can have a substantial effect on cuticle development during leaf expansion. This change in cuticle can change herbicide tolerance. For example, Savory et al.[60] reported the increased potency of ioxynil and bromoxynil salts and esters when plants were grown in low light and high relative humidity could be partially ascribed to these environmental effects on wax production. Change in herbicide efficacy in plants with different cuticles may be due to changes in the penetration rate per unit area or may be due to changes in distribution of herbicide over the leaf surface. Hess et al.[63] showed the spray distribution patterns were different on different species even though the spray parameters were the same. The conclusion is that inadequate information is known about the detailed influence of specific environmental stress conditions on herbicide distribution, penetration, and efficacy in weeds important to crop production.

4. Preemergence Herbicides Influencing Tolerance to Postemergence Applications

The most well recognized case is that in which preemergence herbicide application reduces the production of epicuticular wax on shoot tissue. This can lead to altered postemergence herbicide selectivity patterns as a result of altered herbicide absorption. In 1966, Gentner[64] reported EPTC inhibited wax formation on cabbage. The amount of inhibition was related to rate of application, with maximum wax inhibition reaching more than 90%. This reduced

wax deposition resulted in an increase in absorption of alkanolamine salts of DNBP as measured by increased phytotoxicity. When wax was significantly reduced, spray retention increased and contact angle decreased. Later, Flore and Buckovac[65] reported EPTC inhibited wax production on cabbage and altered its chemical composition. EPTC did not, however, influence cutin composition or quantity. In another study, these same authors found 2.24 kg/ha EPTC increased the cuticular permeability of NAA (1-naphthaleneacetic acid) and increased cuticular transpiration.[66] Absorption of NAA was increased 200% in snap beans, 121% in sugar beet, and 621% in cabbage. In addition to a reduction in wax production (40% for cabbage), there was a shift in wax chemistry from nonpolar to polar constituents, which may have also influenced penetration of NAA.[66]

Other preemergence herbicides have been reported to reduce epicuticular wax. When peas were sprayed postemergence with propanil after preemergence applications of diallate, there was an increase in phytotoxicity.[67] These researchers found diallate reduced pea epicuticular lipid content 50%. The composition and ratio of wax lipid components were unchanged, except the primary alcohols were reduced. Duncan et al.[68] reported ethofumesate applied preemergence to sugar beet "severely" decreased epicuticular wax deposition. The alkanes and secondary ketones were reduced and the long-chain fatty esters were increased. In this same study TCA was reported to decrease alkanes and ketones. Ethofumesate plus TCA applied preemergence increased the postemergence absorption of ^{14}C-ethofumesate and ^{14}C-desmedipham.[68]

An interesting report on the influence of metolachlor and the protectant CGA-43089 [α-(cyanomethoximino)-benzacetonitrile] on wax deposition was published by Ebert.[69] Metolachlor was shown to inhibit wax formation on sorghum stem tissue, thus allowing more metolachlor absorption during the course of early growth. This enhanced absorption resulted in growth inhibition. By adding the protectant CGA-43089 to the treatment, the loss of epiculticular wax was reduced and thus absorption of metolachlor into sorghum was reduced to a level below that causing growth inhibition. In one study, sorghum plants were pretreated for 7 days and then absorption of ^{14}C-metolachlor into coleoptiles was followed over a 6.5-h period. The nonpretreated coleoptiles absorbed 6.5 μg metolachlor per gram tissue (fresh weight). Metolachlor pretreatment resulted in 54.3 μg metolachlor absorption per gram tissue and adding protectant to the pretreatment reduced metolachlor absorption to 26.8 μg. Pretreating the plants with protectant only, resulted in metolachlor absorption the same as the nonpretreated plants.

B. Translocation

Several methods are used to determine herbicide translocation. Most commonly, translocation is determined by autoradiography of plants treated with radioactive herbicide, usually ^{14}C. By comparing the patterns on the autoradiograph with the plant used, a pattern of translocation can be obtained. Autoradiography is primarily a qualitative measure of translocation, but gross differences in herbicide concentrations can be assessed by differences in densities of dark areas on the X-ray film. Autoradiography is a powerful tool, however, there is one major problem. Autoradiography can only determine the presence of radioactivity, not its molecular form. If degradation or conjugation of applied herbicide occurs, the different products may exhibit different translocation characteristics.[70] When differences in translocation are observed, the potential of herbicide degradation being responsible must be evaluated. If the form of the translocated herbicide is not identified, nothing can be concluded about the translocation characteristics of the parent herbicide.

1. Tolerance by Differential Translocation After Preemergence Application

This discussion will be limited to changes in long distance translocation, principally in the xylem, causing a shift in herbicide tolerance. Root accumulation coupled with a lack of

translocation out of the root has been related to tolerance of carrot (*Daucus carota* L.) and parsnip (*Pastinaca sativa* L.) to the photosynthetic inhibitor linuron.[71] Carrot translocated 13% and parsnip 4% of that absorbed. The sensitive species lettuce (*Lactuca sativa* L.) and turnip (*Brassica rapa* L.) translocated 64% and 80%, respectively, of that absorbed. Linuron degradation in the root was low for all species, and thus cannot explain the difference in translocation. The amount of herbicide translocated was related to transpiration rate, but could not account for the significant difference in translocation. The authors concluded tolerance in carrot and parsnip was the result some type of "root fixation" of the linuron causing a reduction in translocation. There was substantial degradation of translocated linuron in shoot tissue of carrot and parsnip, which was also thought to contribute to the tolerance. Hogue and Warren[72] also reported linuron remained in the root of parsnip and concluded this was partially responsible for tolerance in comparison to susceptible tomato, where significant translocation occurred. Parsnip shoots contained 4,000 DPM whereas the roots contained 19,000 DPM. As a comparison, tomato contained 78,000 DPM in the shoot tissue and 2,700 DPM in the root tissue. These authors also reported no degradation in roots, but linuron breakdown did occur in shoots of parsnip. Even within the same species, tolerance has been associated with photosynthesis-inhibiting herbicides remaining in roots. In a diuron tolerant and susceptible sugar cane (*Saccharum officinarum* L.) cultivar, root absorption was the same, but the tolerant cultivar retained twice the amount of herbicide and its metabolites in the roots.[73] In another study, soybean cultivar variation in metribuzen sensitivity was related to differences in translocation.[74] The amount of root absorption was not different between cultivars. Less metribuzin was found in the leaflets of tolerant when compared to sensitive cultivars 24, 48, and 72 h after treatment. The authors suggested a glucose conjugate was formed in the roots of resistant cultivars, which limited availability of free metribuzin for translocation.

Using rates of application that yielded selectivity, chloramben translocated more in redroot pigweed (susceptible) than in soybeans (tolerant).[75] In this study the reduced translocation in soybean may have been associated with formation of a *N*-glycoside conjugate. Stoller[76] reported that following root exposure to ^{14}C-chloramben in nutrient solution, tolerant wheat translocated 1.4% of the absorbed chloramben and susceptible barnyardgrass translocated 74.3%. The author concluded this substantial difference in translocation could account for the 50-fold difference in observed tolerance. As in the other chloramben case, retention in the roots by the tolerant species was thought to be the result of conjugation to form the nontransportable glucosyl derivative of chloramben. When the bases of excised shoots of these species were treated with chloramben the translocation and metabolism differences were not observed. The translocation of ethofumesate in tolerant and susceptible species has also been reported to be different. Susceptible redroot pigweed and common lambsquarter translocated more ^{14}C-ethofumesate than tolerant sugar beet.[77] As in many other instances, radioactivity accumulated in sugar beet roots, perhaps as a water-soluble conjugate.[77]

An interesting translocation pattern was reported by Shone and Wood.[78] They suggested tolerance of blackcurrent (*Ribes nigrum* L.) to simazine could partly be associated with reduced translocation as a result of formation of metabolites in the roots. However, 60% of the atrazine found in leaves was the parent compound, yet the plants were tolerant. When autoradiographs were observed, the majority of the radioactivity in the leaves of blackcurrent was associated with the veins. Simazine not moving into the mesophyll and palisade cells of leaves may explain how the species was able to tolerate unaltered atrazine in the shoot tissue. Smith and Wilkinson[74] published autoradiographs from metribuzin-treated soybean plants where tolerant cultivars retained most of the radioactivity in the veins, whereas susceptible cultivars had radioactivity primarily in the interveinal leaf tissue. In tolerant soybeans, Leather and Foy[79] found the radioactivity that did translocate after preemergence treatment with ^{14}C-bifenox was confined to the primary and secondary leaf vein areas. In

susceptible velvetleaf, distribution was throughout the shoot tissue. The total amount of radioactivity in the shoot tissue was similar for soybean and velvetleaf; however, root degradation of bifenox in soybean was substantially higher than in velvetleaf. Accumulation in leaf veins could account for selectivity if the herbicide was restricted to the apoplast (xylem and nearby cell walls) or if the site of action was not present in phloem. The reports of differential vein accumulation have not identified if tolerant species are able to retain parent herbicide whereas susceptible species are unable to retain the parent compound. Although this does suggest an interesting mechanism of selectivity, in studies thus far published, the differential accumulation could be the result of differential ability to retain metabolites and parent herbicide near the vein area.

2. Tolerance by Differential Translocation After Postemergence Application

As in preemergence cases, it is important to determine if observed differences in translocation are the expression of nonrelated events. For example, changes in translocation may be a result of changes in absorption or different degrees of herbicide metabolism. In these instances, differential translocation between tolerant and susceptible species is a secondary response to a primary change.

In most instances the reason for differential translocation is not identified. If reaching the site of action requires long distance translocation, and if parent herbicide translocation is reduced or inhibited in the tolerant species, then differential translocation is important in explaining tolerance. Ashton[80] found in tolerant sugar cane 94% of recovered 2,4-D remained in the leaf, whereas in susceptible bean, only 23% remained in the leaf even though both species readily absorbed the herbicide. This difference in translocation resulted in a difference in concentration at the growing point, an important site of action. The percent recovery as parent 2,4-D was the same in both species. In tolerant wheat, Quimby and Nalewaja[81] found dicamba translocation was primarily apoplastic after leaf treatment. In susceptible buckwheat (*Polygonum convolvulus* L.), translocation from treated leaves was symplastic and accumulation occurred in sensitive meristem areas. There was no consistent correlation with absorption, thus this was not the reason for differential translocation. In wheat, however, there was more inactivation of dicamba than in buckwheat, so tolerance and susceptibility of these species may be the result of both translocation and metabolism differences.

Herbicides containing carboxyl groups do not absorb efficiently when they are in the ionized form. To overcome the absorption barrier, the herbicides are often applied to the foliage as esters. The esters are usually not biologically active and do not efficiently translocate in the phloem. Fortunately, most herbicide esters of carboxylic acid groups rapidly deesterify (with the assistance of esterase enzymes) to yield the parent acid, which is biologically active and can translocate in the symplast. Species with different abilities to deesterify applied herbicide have been identified and this has been implicated in tolerance mechanisms. In 1975, Jeffcoat and Harries[82] reported tolerance of barley to flamprop-isopropyl was the result of a lack of deesterification. Sensitive wild oats, however, were able to efficiently deesterify the applied herbicide. The ester did not translocate in the symplast, whereas the acid did. After 2 weeks wild oat contained three times more of the acid derivative than barley. In addition to the deesterification difference, acid that was found in barley was detoxified by conjugation. Benzoylprop ethyl also differs in deesterification between tolerant wheat (deesterification low) and susceptible wild oats (deesterification high). A carboxylesterase was isolated from wild oat that could hydrolyze the herbicide ester.[83] This esterase could not be isolated from wheat. Shaner et al.[84] reported the tolerance of wheat to AC 222,293 [methyl 6(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)*m/p*-toluate] was the inability of wheat to deesterify and then translocate the methyl ester. Wild oat was able to deesterify the ester to the free acid and subsequently three times more of the herbicide translocated out of susceptible wild oat leaves when compared to tolerant wheat leaves. When the free acid form of the herbicide was sprayed on wheat, the tolerance was lost.

The antagonism of one herbicide on the efficacy of another herbicide has been implicated to be the result of changes in translocation. Olson and Nalewaja[85] clearly showed downward (symplastic) translocation of ^{14}C-diclofop in wild oat was reduced by concurrent MCPA application. MCPA also inhibited the symplastic translocation of ^{14}C-sucrose and ^{14}C-glucose, suggesting a general reduction in symplastic translocation. The reduction in phloem translocation induced by MCPA was similar to that caused by placing plants in the dark. These authors showed the reduction in translocation was not the result of reduced absorption.

A final interesting topic with regard to translocation is the potential of manipulating translocation with spray additives. It would be of significant benefit to perennial weed control if symplastic translocation in weeds could be enhanced after postemergence herbicide application. The ethylene-releasing agent [(2-chlorethyl)phosphonic acid plus *N*-methylpyrrolidone], (GAF 141), has been shown to increase the basipetal translocation of glyphosate, dicamba, and acifluorfen in bean seedlings.[86] To achieve this effect, however, it was necessary to apply the GAF 141, 24 h prior to the herbicide being injected into the cotyledonary node of bean seedlings.

REFERENCES

1. **Salisbury, F. B. and Ross, C. W.,** *Plant Physiology*, 2nd ed., Wadsworth, Belmont, Calif., 1978.
2. **Weier, T. E., Stocking, C. R., and Barbour, M. G.,** *Botany An Introduction to Plant Biology*, 5th ed., John Wiley & Sons, New York, 1974.
3. **Scott, F. M., Bystrom, B. G., and Bowler, E.,** Root hairs, cuticle and pits, *Science*, 140, 63, 1963.
4. **Price, T. P. and Balke, N. E.,** Characterization of rapid atrazine absorption by excised velvetleaf (*Abutilon theophrasti*) roots, *Weed Sci.*, 30, 633, 1982.
5. **Buckovac, M. J.,** Herbicide entry into plants, in *Herbicides: Physiology, Biochemistry, Ecology*, Vol. 1, 2nd ed., Audus, L. J., Ed., Academic Press, New York, 1976, chap. 11.
6. **Appleby, A. P., Furtick, W. R., and Fang, S. C.,** Soil placement studies with EPTC and other carbamate herbicides on *Avena sativa*, *Weed Res.*, 5, 115, 1965.
7. **Knake, E. L. and Wax, L. M.,** The importance of the shoot of giant foxtail for uptake of preemergence herbicides, *Weed Sci.*, 16, 393, 1968.
8. **Ashton, F. M. and Crafts, A. S.,** *Mode of Action of Herbicides*, 2nd ed., John Wiley & Sons, New York, 1981, chap. 4.
9. **Hull, H. M., Davis, D. G., and Stolzenberg, G. E.,** Action of adjuvants on plant surfaces, in *Adjuvants for Herbicides*, Weed Science Society of America, Champaign, Ill., 1982, chap. 3.
10. **Hull, H. M., Morton, H. L., and Wharrie, J. R.,** Environmental influences on cuticle development and resultant foliar penetration, *Bot. Rev.*, 41, 421, 1975.
11. **Amelunxen, F., Morgenroth, K., and Picksak, T.,** Untersuchungen an der epidermis mit dem stereoscan-elektronenmikroskop, *Z. Pflanzenphysiol.*, 57, 79, 1967.
12. **Skoss, J. D.,** Structure and composition of plant cuticles in relation to environmental factors and permeability, *Bot. Gaz.*, 117, 55, 1955.
13. **Baker, E. A.,** Effect of cuticular components on foliar penetration, *Pest. Sci.*, 11, 367, 1980.
14. **Amer, F. A. and Williams, W. T.,** Drought-resistance in *Pelargonium zonale*, *Ann. Bot.*, 22, 369, 1958.
15. **Hammerton, J. L.,** Environmental factors and susceptibility to herbicides, *Weeds*, 15, 330, 1967.
16. **Tribe, I. S., Gaunt, J. K., and Perry, D. W.,** Cuticular lipids in the gramineae, *Biochem. J.*, 109, 8, 1968.
17. **Baker, E. A.,** The influence of environment or leaf wax development in *Brassica oleracea* var. *gemmifera*, *New Phytol.*, 73, 955, 1974.
18. **Hull, H. M.,** The effect of day and night temperature on growth, foliar wax content, and cuticle development of velvet mesquite, *Weeds*, 6, 133, 1958.
19. **Juniper, B. E.,** Growth, development, and effect of the environment on the ultra-structure of plant surfaces, *J. Linn. Soc. London Bot.*, 56, 413, 1958.
20. **Whitecross, M. I. and Armstrong, D. J.,** Environmental effects on epicuticular wax of *Brassica napus* L., *Aust. J. Bot.*, 20, 87, 1972.

21. **Ormrod, D. J. and Renney, A. J.,** A survey of weed leaf stomata and trichomes, *Can. J. Plant Sci.*, 48, 197, 1968.
22. **Neumann, S. and Jacob, F.,** Aufnahme von α-aminoisobuttersäure durch die blätter von *Vicia fabia* L., *Naturwissenschaften*, 55, 89, 1968.
23. **Bybing, C. D. and Currier, H. B.,** Foliar penetration by chemicals, *Plant Physiol.*, 36, 169, 1961.
24. **Schönherr, J. and Bukovac, M. J.,** Penetration of stomata by liquids, *Plant Physiol.*, 49, 813, 1972.
25. **Darmstadt, G. L., Balke, N. E., and Schrader, L. E.,** Use of corn root protoplasts in herbicide absorption studies, *Pest. Biochem. Physiol.*, 19, 172, 1983.
26. **Peterson, C. A. and Edgington, L. V.,** Entry of pesticides into the plant symplast as measured by their loss from an ambient solution, *Pestic. Sci.*, 7, 483, 1976.
27. **Donaldson, T. W., Bayer, D. E., and Leonard, O. A.,** Absorption of 2,4-dichlorophenoxyacetic acid and 3-(*p*-chlorophenyl)-1,1-dimethylurea (Monuron) by barley roots, *Plant Physiol.*, 52, 638, 1973.
28. **Smith, A. E.,** Accumulation of (2,4-dichlorophenoxy)acetic acid and (2,4,5-trichlorophenoxy)acetic acid by parenchyma tissue as influenced by metabolic inhibitors and lecithin, *Physiol. Plant.*, 27, 338, 1972.
29. **Rubery, P. H.,** The specificity of carrier-mediated auxin transport by suspension-cultured crown gall cells, *Planta*, 135, 275, 1977.
30. **Davis, D. E., Funderburk, H. H., and Sansing, N. G.,** The absorption and translocation of C^{14}-labeled simazin by corn, cotton, and cucumber, *Weeds*, 7, 300, 1959.
31. **Jacob, F. and Neumann, S.,** Qualitative determination of mobility of xenobiotics and criteria of their phloem and xylem mobility, in *Proc. 5th Int. Cong. Pesticide Chemistry*, Vol. 1, Doyle, P. and Fujita, T., Eds., Pergamon Press, New York, 1983, 357.
32. **Herrett, R. A. and Bagley, W. P.,** The metabolism and translocation of 3-amino-1,2-4-triazole by Canada thistle, *J. Agric. Food Chem.*, 12, 17, 1964.
33. **Holly, K.,** Selectivity in relation to formulation and application methods, in *Herbicides: Physiology, Biochemistry, Ecology*, Vol. 2, 2nd ed., Andus, L. J., Ed., Academic Press, New York, 1976, chap. 8.
34. **Barrett, M. and Ashton, F. M.,** Napropamide uptake, transport, and metabolism in corn (*Zea mays*) and tomato (*Lycoperiscon esuletum*), *Weed Sci.*, 29, 697, 1981.
35. **Prendeville, G. N.,** Shoot zone uptake of soil-applied herbicides, *Weed Res.*, 8, 106, 1968.
36. **Baker, J. B.,** An explanation for the selective control of barnyardgrass in rice with CIPC, *Weeds*, 8, 39, 1960.
37. **Parker, C.,** Factors affecting the selectivity of 2,3-dichloroallyldiisopropylthiolcarbamate (di-allate) against *Avena* spp. in wheat and barley, *Weed Res.*, 3, 259, 1963.
38. **Rahman, A. and Ashford, R.,** Selective action of trifluralin for control of green foxtail in wheat, *Weed Sci.*, 18, 754, 1970.
39. **Prendeville, G. N., Oliver, L. R., and Schreiber, M. M.,** Species differences in site of shoot uptake and tolerance to EPTC, *Weed Sci.*, 16, 538, 1968.
40. **Rubin, B. and Eshel, Y.,** Absorption and distribution of terbutryn and fluometuron by germinating seeds of cotton (*Gossypium hirsutum*) and snapbean (*Phaseolus vulgaris*), *Weed Sci.*, 26, 378, 1978.
41. **Pinto, H. and Corbin, F. T.,** Absorption and translocation of tetrafluron in cotton (*Gossypium hirsutum*), jimsonweed (*Datura stramonium*), peanut (*Arachis hypogaea*), and prickly sida (*Sida spinosa*), *Weed Sci.*, 28, 557, 1980.
42. **Wright, T. H. and Rieck, C. E.,** Differential butylate injury to corn hybrids, *Weed Sci.*, 21, 194, 1973.
43. **Blackman, G. E., Bruce, R. S., and Holly, K.,** Studies in the principles of phytotoxicity. V. Interrelationships between specific differences in spray retention and selective toxicity, *J. Exp. Bot.*, 9, 175, 1958.
44. **Sharma, M. P., Vandenborn, W. H., and McBeath, D. K.,** Spray retention, foliar penetration, translocation and selectivity of asulam in wild oats and flax, *Weed Res.*, 18, 169, 1978.
45. **Kern, A. D., Meggitt, W. F., and Penner, D.,** Uptake, movement, and metabolism of cyanazine in fall panicum, green foxtail, and corn, *Weed Sci.*, 23, 277, 1975.
46. **Ritter, R. L. and Coble, H. D.,** Penetration, translocation, and metabolism of acifluorfen in soybeans (*Glycine max*), common ragweed (*Ambrosia artemisiifolia*) and common cocklebur (*Xanthium pensylvanicum*), *Weed Sci.*, 29, 474, 1981.
47. **Davies, P. J., Drennan, D. S. H., Fryer, J. D., and Holly, K.,** The basis of the differential phytotoxicity of 4-hydroxy-3-5-di-iodobenzonitrile, *Weed Res.*, 8, 233, 1968.
48. **Keeley, P. E. and Thullen, R. J.,** Control of nutsedge with organic arsenical herbicides, *Weed Sci.*, 19, 601, 1971.
49. **Kirkwood, R. C., Dalziel, J., Matlib, A., and Somerville, L.,** The role of translocation in selectivity of herbicides with reference to MCPA and MCPB, *Pestic. Sci.*, 3, 307, 1972.
50. **Pereira, J. F., Splittstoesser, W. E., and Hopen, H. J.,** Mechanism of intraspecific selectivity of cabbage to nitrofen, *Weed Sci.*, 19, 647, 1971.
51. **Norris, R. F.,** Penetration of 2,4-D in relation to cuticle thickness, *Am. J. Bot.*, 61, 74, 1974.

52. **Schultz, M. E. and Burnside, O. C.,** Absorption, translocation, and metabolism of 2,4-D and glyphosate in hemp dogbane *(Apocynum cannabinum),Weed Sci.*, 28, 13, 1980.
53. **Nalewaja, J. D. and Adamczewski, K. A.,** Uptake and translocation of bentazon with additives, *Weed Sci.*, 25, 309, 1977.
54. **Mayeux, H. S., Jr. and Scifres, C. J.,** Foliar uptake and transport of 2,4-D and picloram by Drummond's goldenweed *(Isocoma drummondii), Weed Sci.*, 28, 678, 1980.
55. **Pallas, J. E., Jr.,** Effects of temperature and humidity on foliar absorption and translocation of 2,4-dichlorophenoxyacetic acid and benzoic acid, *Plant Physiol.*, 35, 575, 1960.
56. **Wills, G. D.,** Effect of temperature, relative humidity, soil moisture and surfactant on toxicity of glyphosate to cotton and purple nutsedge, 1974 Meeting of the Weed Science Society of America, Las Vegas, Nevada, Abstr. No. 275, Weed Science Society of America, Champaign, Ill.
57. **Wills, G. D. and McWhorter, C. G.,** Effect of environment on the translocation and toxicity of acifluorfen and showy crotalaria *(Crotalaria spectabilis), Weed Sci.*, 29, 397, 1981.
58. **McWhorter, C. G., Jordan, T. N., and Wills, G. D.,** Translocation of ^{14}C-glyphosate in soybeans (*Glycine max*) and johnsongrass *(Sorghum halepense), Weed Sci.*, 28, 113, 1980.
59. **Ahmadi, M. S., Haderlie, L. C., and Wicks, G. A.,** Effects of growth stage and water stress on barnyardgrass (*Echinochloa crus-galli*) control and on glyphosate absorption and translocation, *Weed Sci.*, 28, 277, 1980.
60. **Savory, B. M., Hibbitt, C. J., and Catchpole, A. H.,** Effect of climatic factors on the potency of ioxynil and bromoxynil, *Pestic. Sci.*, 6, 145, 1975.
61. **Prasad, R., Foy, C. L., and Crafts, A. S.,** Effects of relative humidity on absorption and translocation of foliarly applied dalapon, *Weeds*, 15, 149, 1967.
62. **Akey, W. C. and Morrison, I. N.,** Effect of moisture stress on wild oat (*Avena fatua*) response to diclofop, *Weed Sci.*, 31, 247, 1983.
63. **Hess, F. D., Bayer, D. E., and Falk, R. H.,** Herbicide distribution patterns. III. As a function of formulation, *Weed Sci.*, 29, 224, 1981.
64. **Gentner, W. A.,** The influence of EPTC on external foliage wax deposition, *Weeds*, 14, 27, 1966.
65. **Flore, J. A. and Bukovac, M. J.,** Pesticide effects on the plant cuticle. III. EPTC effects on the qualitative composition of *Brassica oleracea* L. leaf cuticle, *J. Am. Soc. Hort. Sci.*, 103, 297, 1978.
66. **Flore, J. A. and Bukovac, M. J.,** Pesticide effects on the plant cuticle. IV. The effect of EPTC on the permeability of cabbage, bean, and sugar beet cuticle, *J. Am. Soc. Hort. Sci.*, 106, 189, 1981.
67. **Still, G. S., Davis, D. G., and Zander, G. L.,** Plant epicuticular lipids: Alteration by herbicidal carbamates, *Plant Physiol.*, 46, 307, 1970.
68. **Duncan, D. N., Meggitt, W. F., and Penner, D.,** Basis for increased activity from herbicide combinations with ethofumesate applied on sugar beet *(Beta vulgaris), Weed Sci.*, 30, 195, 1982.
69. **Ebert, E.,** The role of waxes in the uptake of metolachlor into sorghum in relation to the protectant CGA 43089, *Weed Res.*, 22, 305, 1982.
70. **Jensen, K. I. N.,** The role of uptake, translocation, and metabolism in the differential intraspecific responses to herbicides, in *Herbicide Resistance in Plants*, LeBaron, H. M. and Gressel, J., Eds., John Wiley & Sons, New York, 1982, chap. 8.
71. **Walker, A. and Featherstone, R. M.,** Absorption and translocation of atrazine and linuron by plants with implications concerning linuron selectivity, *J. Exp. Bot.*, 24, 450, 1973.
72. **Hogue, E. J. and Warren, G. F.,** Selectivity of linuron on tomato and parsnip, *Weed Sci.*, 16, 51, 1968.
73. **Osgood, R. V., Romanowski, R. R., and Hilton, H. W.,** Differential tolerance of Hawaiian sugar cane cultivars to diuron, *Weed Sci.*, 20, 537, 1972.
74. **Smith, A. E. and Wilkinson, R. E.,** Differential absorption, translocation and metabolism of metribuzin [4-amino-6-tert-butyl-3-(methylthio)-as-triazine-5(4H)one] by soybean cultivars, *Physiol. Plant.*, 32, 253, 1974.
75. **Colby, S. R.,** The mechanism of selectivity of amiben, *Weeds*, 14, 197, 1966.
76. **Stoller, E. W.,** Mechanism for the differential translocation of amiben in plants, *Plant Physiol.*, 46, 732, 1970.
77. **Duncan, D. N., Meggitt, W. F., and Penner, D.,** The basis for selectivity of root-applied ethofumesate in sugarbeet *(Beta vulgaris)* and three weed species, *Weed Sci.*, 30, 191, 1982.
78. **Shone, M. G. T. and Wood, A. V.,** Factors responsible for the tolerance of blackcurrents to simazine, *Weed Res.*, 12, 337, 1972.
79. **Leather, G. R. and Foy, C. L.,** Differential absorption and distribution as a basis for the selectivity of bifenox, *Weed Sci.*, 26, 76, 1978.
80. **Ashton, F. M.,** Absorption and translocation of radioactive 2,4-D in sugar cane and bean plants, *Weeds*, 6, 257, 1958.
81. **Quimby, P. C., Jr. and Nalewaja, J. D.,** Selectivity of dicamba in wheat and wild buckwheat, *Weed Sci.*, 19, 598, 1971.

82. **Jeffcoat, B. and Harries, W. N.,** Selectivity and mode of action of flamprop-isopropyl, isopropyl(±)-2-[*N*-(3-chloro-4-fluorophenyl)benzamido] propionate, in the control of *Avena fatua* in barley, *Pestic. Sci.*, 6, 283, 1975.
83. **Hill, B. D., Stobbe, E. H., and Jones, B. L.,** Hydrolysis of the herbicide benzoylprop ethyl by a wild oat esterase, *Weed Res.*, 18, 149, 1978.
84. **Shaner, D. L., Simcox, P. D., Robson, P. A., Mangels, G., Reichert, B., Ciarlante, D. R., and Los, M.,** AC-222,293-translocation and metabolic selectivity, *Proc. British Plant Protection Conference*, London, 1983, 333.
85. **Olson, W. and Nalewaja, J. D.,** Effect of MCPA on ^{14}C-diclofop uptake and translocation, *Weed Sci.*, 30, 59, 1982.
86. **Chykaliuk, P. B., Peeper, T. F., and Basler, E.,** Stimulation of basipetal herbicide translocation with GAF-141, *Weed Sci.*, 30, 6, 1982.

Chapter 9

DETOXICATION OF HERBICIDES

Richard H. Shimabukuro

TABLE OF CONTENTS

I. INTRODUCTION

The basis for herbicide selectivity is the ability of crop plants to tolerate or survive the application of a herbicide at a specific rate while the weeds are injured or killed. Herbicide selectivity may be attained by formulating and applying the herbicide in a manner whereby the target species receives a greater portion of the herbicide than the nontarget species or by using compounds that are intrinsically more phytotoxic to the weeds than to the crops. The latter method has a physiological basis due to differences in herbicide absorption and translocation, rates and nature of herbicide activation and detoxication, and intrinsic differences in sensitivity at the site(s) of action. This chapter will consider only activation and detoxication, but the other important aspects are discussed in Chapters 7 and 8.

Much is known about the metabolism and degradation of pesticides in plants but detailed knowledge of biotransformation reaction mechanisms and the effects on biological activity is often lacking.[1] Pesticide (including herbicide) metabolism in plants has been reviewed extensively.[1-7] Two recently published reviews are excellent references for a detailed discussion on herbicide chemistry, metabolism, and biological activity.[8,9] It is not the intent of this review to present an exhaustive coverage of herbicide metabolism but to illustrate reactions and examples of herbicide metabolism resulting in detoxication and their influence on selectivity.

II. HERBICIDE METABOLISM IN PLANTS

Plants metabolize herbicides or other xenobiotics (foreign compounds) through a series of intermediates ultimately to insoluble residues. Complete oxidation of organic herbicides to CO_2 is an exception rather than the rule. The terms, metabolism and biotransformation, are often used interchangeably and they refer to the alteration of the herbicide's chemical structure within plant cells that may or may not be catalyzed enzymatically.

The metabolism of herbicides and other xenobiotics may occur as a three-phase process in plants in contrast to a two-phase process in animals (Table 1).[7,10,11] Phase I reactions generally detoxify the herbicide or predispose the molecule to phase II conjugations.[7,8,12] Conjugation of herbicides in phase II usually results in the loss of any phytotoxic activity remaining after phase I reactions. Therefore, phase I metabolism is most important to biological activity of herbicides and it may be the major factor influencing herbicide selectivity.[1] Although not very common, activation or increased phytotoxicity of herbicides may result from phase I metabolism.[7-9] Some examples of herbicide activation such as EPTC, paraquat, and esters of organic acid herbicides will be discussed.

Phase III metabolism is peculiar to plants. In this phase herbicide conjugates from phase II are converted to secondary conjugates or insoluble bound residues.[1,13] Phase III appears to exist in plants because excretion of conjugated herbicide metabolites from phase II is not significant in plants as it is in animals. It is generally assumed that intermediates involved in phase III metabolism are no longer phytotoxic. The identity and fate of most insoluble residues in plants are still unknown.

III. HERBICIDE DETOXICATION AND TOLERANCE

For a herbicide to be effective it must reach the sensitive site(s) in a plant in its toxic form at a concentration sufficient to cause severe disruption of normal growth and development. If severe injury is sustained for a long period, it may reach a threshold level where irreversible damage results and death becomes inevitable. Metabolism resulting in detoxication is an effective mechanism for reducing the toxic concentration of the herbicide and increasing the plant's tolerance toward the chemical.

Table 1
GENERALIZED SUMMARY OF CHANGES IN PESTICIDE PROPERTIES AND THEIR FATE IN PLANTS

Characteristics	Initial properties	Phase I	Phase II	Phase III
Reactions	Parent compound →	Oxidation Reduction Hydrolysis →	Conjugation →	Secondary conjugation or incorporation into biopolymers (insoluble residues)
Solubility	Lipophilic	Amphophilic	Hydrophilic	Hydrophilic and insoluble
Translocation	Selective mobility	Modified or reduced mobility	Limited or immobile	Immobile
Phytotoxicity	Toxic	Modified or less toxic	Greatly reduced or nontoxic	Nontoxic
Bioavailability[a]	+ + +	+ + +	+ +	+ or unavailable

[a] + + +, Readily absorbed in GI tract of animals; + +, less absorption; +, limited absorption.

The criteria for demonstrating herbicide detoxication and its role in selectivity are

1. Isolate, characterize and synthesize the metabolite, apply the metabolite to the plant, and determine its biological activity.
2. Correlate the kinetics of metabolite formation in a plant (or its parts) with the extent of injury and recovery of the affected biological activity.
3. Isolate and characterize the enzyme that catalyzes the formation of the metabolite and compare its activity between resistant and susceptible species.

Generally, all of the criteria are not fulfilled for a specific metabolite, but a partial fulfillment of all or some of the criteria has identified many metabolites as detoxication products. This discussion will be limited to such examples. Many reports indicate metabolism and detoxication of parent herbicide molecules to "water-soluble" metabolites. However, emphasis will be given to reports where structures of metabolites have been determined.

IV. DETOXICATION REACTIONS

The basic biochemical reactions in higher plants that generally result in herbicide detoxication are oxidation, reduction, hydrolysis, and conjugation. The first three reactions are generally associated with phase I metabolism and conjugation occurs frequently in phase II metabolism.[7,8,10] One of the first definitive examples of detoxication was that of simazine.[14] The major metabolite in root-fed tolerant maize (*Zea mays* L.) was identified as the analogue, hydroxysimazine (2-hydroxysimazine). The nonenzymatic catalyst, benzoxazinone (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one), was isolated and characterized from maize.[15,16] Crude plant juice from maize containing benzoxazinone hydrolyzed simazine to hydroxysimazine and the metabolite was not phytotoxic when administered to simazine-susceptible oat and wheat.[17] Subsequent research revealed that enzymatic conjugation with glutathione is the major detoxication reaction of *s*-triazines rather than nonenzymatic hydrolysis.[18] Nevertheless, the early example above with simazine is a classical illustration of the relationship between metabolism, detoxication, and selectivity.

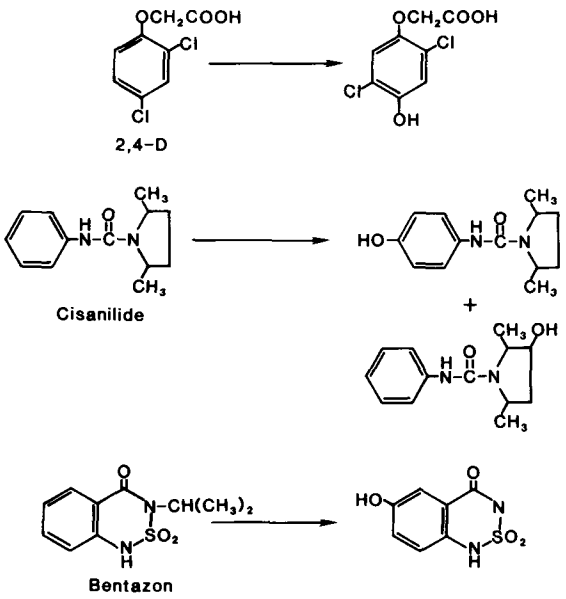

FIGURE 1. Aryl hydroxylation of the herbicides 2,4-D, cisanilide and bentazon.

A. Oxidation

Oxidation reactions of herbicides are most common in plants and frequently are primary reactions that result in either detoxication or activation of the herbicide. Major oxidative reactions such as *N*-dealkylation, aromatic hydroxylation, alkyl oxidation, epoxidation, sulfur oxidation, and *O*-dealkylation are catalyzed by monooxygenases collectively referred to as mixed-function oxidases (mfo).[4] However, direct evidence for mfo catalysis (participation of cytochrome P 450) in herbicide metabolism exists for only a few of the reactions and many of the reactions presumed to be mfo-catalyzed may be catalyzed by peroxidases or other oxygenases.[4,6]

Examples of herbicide metabolism by oxidation are shown in Figure 1. Aryl hydroxylation of 2,4-D in grass and broad-leaf species to 2,5-dichloro-4-hydroxyphenoxyactic acid (4-OH-2,5-D) is a major pathway for 2,4-D metabolism.[19-22] The epoxide intermediate was not isolated but chlorine migration or the NIH shift is probable evidence for an epoxide intermediate.[23,24] The 4-OH-2,5-D metabolite had no auxin activity like the parent, 2,4-D, and was considered to be a detoxication product. However, aryl hydroxylation was not related to the tolerance of grasses to 2,4-D since both grasses and susceptible broad-leaf species formed 4-OH-2,5-D.[19,22] It is possible that the *Avena* coleoptile test for auxin activity is not a specific indicator of the phytotoxic herbicidal action of 2,4-D or its metabolites.

Aryl hydroxylation may be the most common reaction in herbicide metabolism.[8] Other examples with no direct evidence for the involvement of mfo are the hydroxylation of chlorpropham (Figure 11),[25] cisanilide (Figure 1),[26] bentazon (Figure 1),[27] and diclofop-methyl (Figure 16).[28,29] Generally, the hydroxylated metabolites do not accumulate as the free phenols but are conjugated rapidly as glycosides.[30]

N-Dealkylation is also a very common oxidative reaction in herbicide metabolism.[8] Generally, *N*-dealkylation of herbicides is a detoxication reaction in contrast to some insecticides.[7] *N*-Demethylation of monuron is one example where the mfo-catalyzed reaction mechanism for a herbicide in plants has been demonstrated (Figure 2).[31] Other examples of *N*-dealkylation are atrazine and diphenamid (Figure 3).[32,33] Fluchloralin and other *N*-alkyl substituted din-

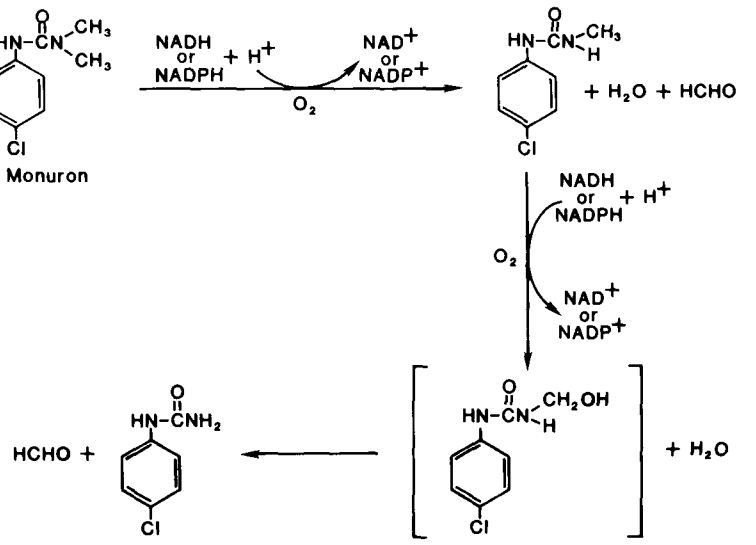

FIGURE 2. The mechanism for mfo-catalyzed *N*-demethylation of monuron.

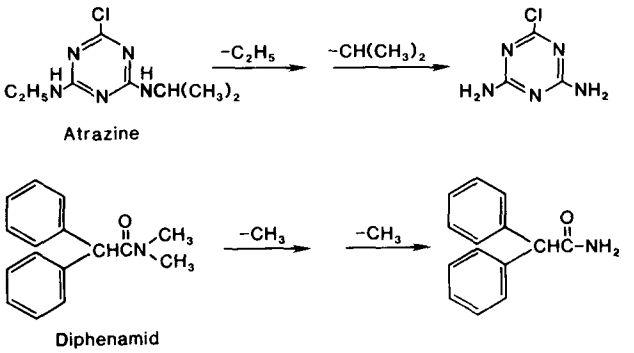

FIGURE 3. *N*-Dealkylation of atrazine and diphenamid in plants.

itroaniline herbicides are likely candidates for *N*-dealkylation in plants but these compounds preferably undergo cyclization rather than *N*-dealkylation.[34] The *N*-hydroxymethyl intermediate in the *N*-dealkylation of diphenamid was identified as the aglycone upon hydrolysis of water-soluble metabolites,[33] as was the unstable intermediate of monuron.[31] Both diphenamid and monuron undergo sequential *N*-demethylation and aryl hydroxylation reactions.[31,33] However, the stable glucose conjugates are either that of the *N*-hydroxymethyl or the aryl-hydroxylated metabolite but not of both groups simultaneously on the same molecule. This agrees with the mfo-catalyzed reaction mechanism for monuron, which indicates that unless the unstable *N*-hydroxymethyl intermediate is conjugated it readily decomposes to the *N*-demethylated product and formaldehyde (Figure 2).[35] The *O*-glucosides of the hydroxymethyl intermediates may be nonphytotoxic. However, hydrolysis of these conjugates by endogeneous hydrolases could conceivably regenerate the hydroxymethyl intermediate, which may decompose to the stable and less phytotoxic *N*-demethylated metabolites such as that of monuron.

O-Dealkylation, alkyl oxidation, epoxidation, and nitrogen oxidation are not common reactions of herbicide metabolism in plants. *O*-Demethylation of methoxy-*s*-triazines to 2-

hydroxy-*s*-triazines and of dicamba to salicyclic acid derivatives is only a minor reaction.[36,37] However, *O*-demethylation of chlomethoxynil to its hydroxy derivative was the major metabolite in rice (*Oryza sativa* L.).[38] The demethylated metabolites of the above herbicides are probably nonphytotoxic and most do not persist in their free hydroxyl forms in plants, but as water-soluble conjugates. The biological activities of the free hydroxy metabolites may be nearly impossible to determine in whole plants but their activities may be determined in specific bioassays or in vitro assays where conjugation does not occur.

Nitrogen and sulfur oxidation are not common herbicide metabolism reactions in plants.[4,8] The intermediate in the cyclization of fluchloralin to substituted benzimidazole was a benzimidazole *N*-oxide.[34] Substituted dinitroaniline herbicides such as fluchloralin are metabolized through multiple pathways and consequently only small amounts of numerous metabolites accumulate in plants. Therefore, a metabolite such as the *N*-oxide of fluchloralin is probably not involved in the phytotoxic action of the herbicide. Indeed, the major difference between a resistant and susceptible plant after herbicide treatment was the concentration of fluchloralin remaining in tissues at various time periods.[34]

Insecticides are activated readily in plants by oxidation to their sulfoxides and sulfones but only limited examples are known of herbicides undergoing similar reactions.[2] The oxidation of prometryn to its sulfoxide and sulfone has been reported, but this reaction has not been confirmed.[39] Prometryn probably does not exist in an oxidized form in plants since the sulfoxide is rapidly conjugated nonenzymatically with glutathione.[13,39] The oxidation of EPTC in maize and oat (*Avena sativa* L.) is the best known example of sulfur oxidation and activation of a herbicide in plants (Figure 12).[40,41] EPTC activation and detoxication is discussed in a later section.

B. Reduction

Reduction is less common than oxidation in the metabolism of herbicides and other pesticides in plants. Aryl nitroreduction is only a minor reaction in the metabolism of fluorodifen (Figure 4)[42] and fluchloralin.[34] However, aryl nitroreduction can be a significant reaction as shown by the reduction of the fungicide, pentachloronitrobenzene (PCNB) to pentachloroaniline in several plants (Figure 4).[43] Up to 42% of the absorbed PCNB was reduced to pentachloroaniline in peanut roots after 20 days. Aryl nitroreductase from peanut (*Arachis hypogaea* L.) and soybean (*Glycine max* (L.) Merr.) reduced PCNB to its aniline derivative and dinoben to chloramben under a nitrogen atmosphere.[44,45] Reduction of chlomethoxynil to its amino derivative followed by conjugation was a minor pathway in rice and barnyard millet (*Panicum crus-galli* L. var. *frumentaceum* Trin.) (Figure 4).[38] The reduction of chlomethoxynil and other diphenylether herbicides occurred at a greater rate in unsterilized soil under flooded (low oxygen tension) conditions than under aerobic upland conditions.[46] Therefore, reduction reactions may be due primarily to soil microbial activity, especially under low oxygen conditions, rather than aryl nitroreductase activity in plants.

Reduction does not appear to be an important detoxication mechanism in plants. Aryl nitroreduction, the most common herbicide reduction reaction in plants, appears to be competitive with glutathione conjugation, the major pathway for PCNB and fluorodifen metabolism.[43,47,48] Pentachloroaniline, the major metabolite of PCNB, was metabolized slowly by GSH conjugation.[43] Aminofluorodifen, a minor metabolite of fluorodifen, was not a substrate for GSH conjugation, but it was a strong competitive inhibitor of fluorodifen cleavage by glutathione *S*-transferase.[48]

C. Hydrolysis

Hydrolysis of ester, amide, and nitrile herbicides is a common phase I reaction in plants that is important as a selective mechanism for some herbicides (Figure 5). The hydrolysis of propanil by an aryl acylamidase in rice has been studied extensively.[4,7,8] The hydrolytic enzyme is specific for the anilide linkage and was more active in resistant rice than in

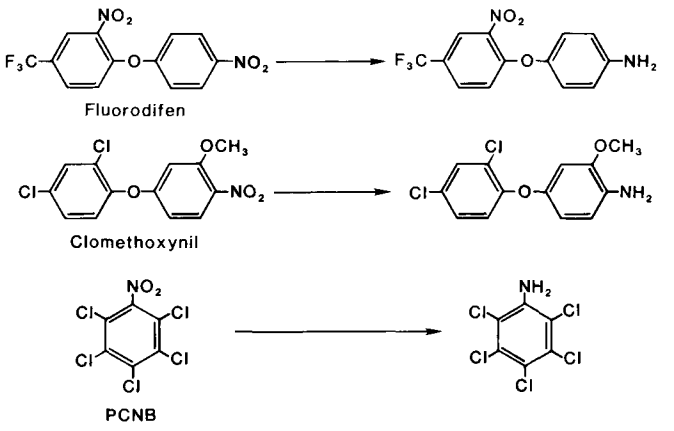

FIGURE 4. Reductive metabolism of fluorodifen, clomethoxynil, and PCNB in plants.

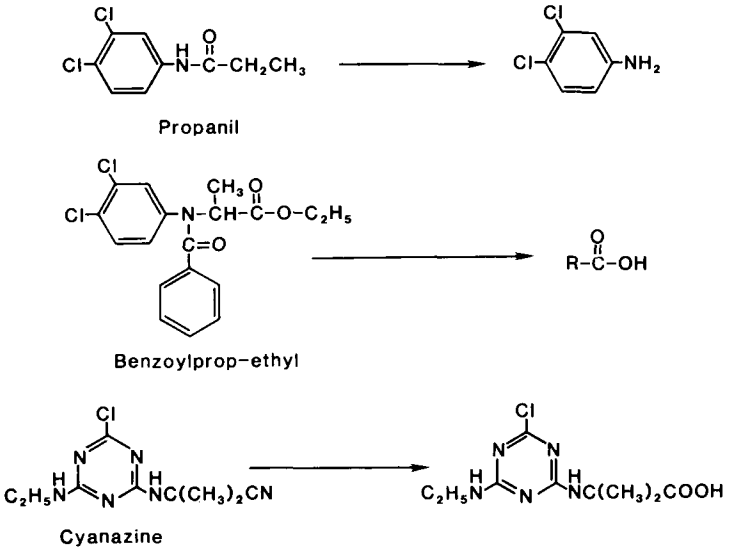

FIGURE 5. Hydrolytic metabolism of propanil, benzoylprop-ethyl, and cyanazine in plants.

susceptible barnyard grass (*Echinocloa crus-galli* (L.) Beauv.). Many carboxylic acid ester herbicides are hydrolyzed readily to their free acids in plants. The formulated esters of 2,4-D are hydrolyzed to the free acid once they penetrate the leaf surface.[49] The grass herbicides benzoylprop-ethyl, chlorfenprop-methyl, and diclofop-methyl are hydrolyzed to their presumed active acids.[29,50,51] The hydrolysis and metabolism of these grass herbicides and the selectivity between closely related grasses are discussed in more detail in another section.

Compounds with cyano groups such as cyanazine and bromoxynil are hydrolyzed to their amides and acids (Figure 5).[52,53] The herbicidal activity of cyanazine following hydrolysis to the carboxylic acid metabolite is unknown. However, the hydrolysis of the chlorine substituent to give the hydroxy-*s*-triazine analogue of the carboxylic acid metabolite detoxified the molecule.[52] The hydrolysis of the herbicide, dichlobenil, in contrast to bromoxynil has not been identified positively in plants. Instead, dichlobenil is aryl hydroxylated and

conjugated.[30] It is likely that the hindered, 2,6-dichloro substitution in dichlobenil prevents hydrolysis of the cyano group in contrast to the 3,5-dibromo substitution in bromoxynil.

D. Conjugation

Conjugation as applied to pesticide metabolism may be viewed as the in vivo reaction of a pesticide metabolite, usually resulting from a phase I reaction, with an endogenous substrate(s) to form a new compound of higher molecular weight.[54] Generally, conjugation is a mechanism whereby plants convert lipophilic parent herbicides into more polar, water-soluble metabolites. In animals conjugation is often preliminary to the excretion of the pesticide from the body. In plants excretion is not a major process. Therefore, conjugation is often an intermediate in the formation of terminal bound pesticide residues.

Conjugation is a major mechanism in herbicide detoxication and selectivity in plants. Conjugation increases the polarity of the herbicide or its metabolite; thus making the conjugated metabolite extremely difficult to isolate, purify and characterize. Therefore, only few of the herbicide conjugates found in plants have been characterized successfully. Most workers report the presence of unknown, polar or water-soluble metabolites that may be hydrolyzed enzymatically or chemically to derivatives formed in phase I metabolism. Most conjugates have not been synthesized and tested directly for biological activity since their chemical structures have not been determined.

Conjugation reactions of herbicides that yield polar, water-soluble metabolites are generally considered as detoxication reactions. Detoxication by conjugation may occur either directly or indirectly.[54] If conjugation occurs with the active herbicide or its metabolite to yield an inactive conjugate, this is considered to be direct detoxication. Indirect detoxication by conjugation occurs when the precursor (usually a herbicide metabolite) to conjugation is inactive. An example of direct detoxication is the nucleophilic displacement reaction between atrazine and glutathione (GSH) to give the inactive glutathione conjugate (Figure 8).[4] Many GSH conjugations of herbicides involve the active parent molecule and GSH in an S_N2 reaction.[4]

Indirect detoxication by conjugation occurs predominantly after phase I metabolism. Herbicides undergo phase I reactions that yield phenols, alcohols, acids, amines, and anilines. These metabolites are often presumed to be detoxified parent molecules and do not usually accumulate in plants in their free form. These metabolites are conjugated rapidly to the *O*- and *N*-glucosides and other complex glycosides.[30] Some examples are the rapid glucosylation of aryl hydroxylated cisanilide,[26] bentazon,[27] and dichlofop-methyl.[28,29] If the phenolic or alcoholic metabolites are biologically active, the *O*-glycosides may influence herbicide selectivity by acting as a regulating mechanism for controlling the levels of toxic metabolites. Examples of such herbicide conjugates are not common in plants but one example may be the *O*-glucoside of 2-hydroxychlorpropham, a metabolite of chlorpropham with considerable biological activity.[25] This example is discussed in more detail later. It is also known that the hydroxylated forms of the insecticide carbaryl are highly toxic to mammals and they are detoxified as the *O*-glucosides in several plants.[54,55]

Ester conjugation of acidic herbicides to polar, water-soluble metabolites is very likely a direct detoxication mechanism. Chloramben and diclofop-methyl following hydrolysis do not accumulate in plants as their free acids but are rapidly conjugated to ester conjugates.[29,56] Because of the reversibility of these reactions, these conjugates may play a critical role in herbicide selectivity, analogous to the role of the IAA-ester conjugate in the bioregulation of free IAA available for controlling plant growth and development.[57]

1. Glucose Conjugation

Glucose conjugation occurs with hydroxyl, carboxyl, or amino functional groups in herbicides or their metabolites. Glucose conjugation of many herbicides are discussed in Hatzios

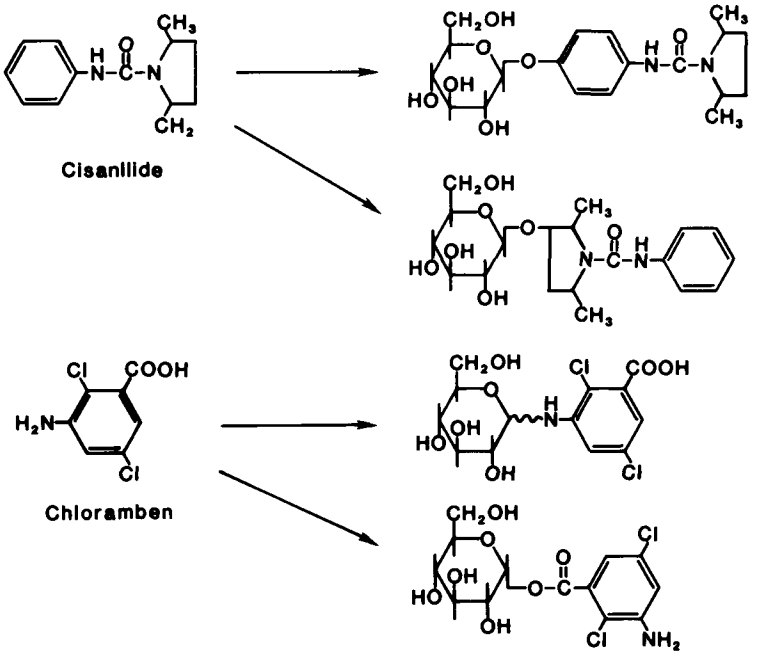

FIGURE 6. *O*-Glucoside metabolites of cisanilide and the *N*-glucoside and *O*-glucosyl ester metabolites of chloramben.

and Penner,[8] and Frear.[30] Typical examples are *O*-glucosides of cisanilide (Figure 6)[26] and chlorpropham (Figure 11),[58] *N*-glucosides of chloramben (Figure 6)[30] and propanil,[59] and glucose esters of chloramben (Figure 6),[56] diclofop-methyl (Figure 16),[29] and 2,4-D.[22] Most of the *O*- and *N*-glucosides that have been characterized are predominantly conjugated as the β-anomer of glucose. However, the glucose ester of chloramben was conjugated as the α-anomer (Figure 6).[56] The physiological significance of this is unknown.

Complex glycoside formation is probably an indirect detoxication reaction. Therefore, the reaction may have no effect on the herbicidal activity of the parent compound or its metabolites. Examples of these conjugations are the metabolism of diphenamid to the *O*-gentiobioside,[60] fluorodifen to the malonylglucoside,[39,61] and flamprop-methyl to the malonylglucose ester (Figure 7).[62] In each of these examples the precursor metabolite was probably the detoxified glucose conjugate before the transfer of a glycosyl residue to form the gentiobioside in diphenamid, and the transfer of a malonyl residue to form the phenolic acylglucoside conjugate in fluorodifen and the acylglucose ester conjugate in flamprop-methyl.[30] Of the three examples, only the acylglucose conjugate of flamprop-methyl may have an influence on herbicide selectivity since its hydrolysis would regenerate the herbicidally active acid, flamprop. Microsomal oxidation of diphenamid to the *N*-hydroxymethyl intermediate prior to glucose conjugation is very likely a detoxication reaction. Fluorodifen was detoxified by cleavage of the diphenylether bond by glutathione *S*-transferase to yield the *p*-nitrophenol that was conjugated with glucose followed by acylation.

2. Amino Acid Conjugation

Herbicides known to form amino acid conjugates through an α-amide bond in plants are predominantly acidic herbicides.[63] Amino acid conjugation of 2,4-D is the best known example of a herbicide undergoing such a reaction.[22,63] The major conjugates were those of glutamic (2,4-D-Glu) and aspartic (2,4-D-Asp) acids with minor conjugates being those of alanine, valine, leucine, phenylalanine, and tryptophan.[63] The amino acid conjugates of 2,4-

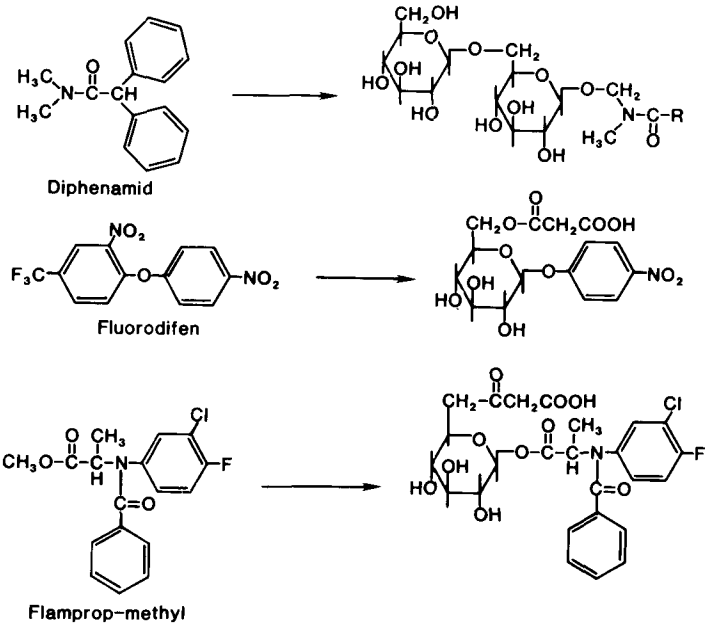

FIGURE 7. Complex glycoside metabolites of diphenamid, fluorodifen, and flamprop-methyl.

D have been isolated and characterized primarily from callus cultures, in contrast to its aryl-hydroxylated metabolites that have been isolated from bean (*Phaseolus vulgaris* L.) plants.[19]

Conjugated metabolites resulting from phase II metabolism are usually hydrophilic, non-phytotoxic polar products. However, the amino acid conjugates are extracted into ether together with free 2,4-D at pH 3.0.[63] The detoxified glucose conjugates and hydroxylated metabolites remain in the water-soluble fraction. The amino acid conjugates of 2,4-D are biologically active and they stimulated soybean callus growth and induced elongation of *Avena* coleoptiles.[64,65] The auxin activity was not due to hydrolysis of the amino acid conjugates to 2,4-D. 2,4-D-Glu stimulated soybean callus growth by more than 50% over that of 2,4-D at an equimolar concentration of 0.1 μM and yet only 4% of the absorbed 2,4-D-Glu was hydrolyzed to 2,4-D.[64] Apparently, the auxin properties of the amino acid conjugates are not stereospecific since both the D- and L- forms of the Glu and Asp conjugates were more active than 2,4-D in stimulating soybean callus growth.[65] The amino acid conjugates of 2,4-D gave evidence of auxin activity in sensitive auxin bioassay systems. However, it is unknown whether these conjugates have herbicidal activity against broadleaf species similar to that of 2,4-D.

The amino acid conjugates of 2,4-D appear to be biologically active as the conjugated molecules, whereas the glucosyl esters of 2,4-D and IAA are believed to be inactive until hydrolysis yields the parent acids.[66,67] The auxin activity of the amino acid conjugates, IAA-alanine and IAA-aspartate, is dependent on the rate of hydrolysis of these conjugates to IAA. The hydrolytic enzyme is also stereospecific for the L- and not the D-amino acid conjugate of IAA.[68] The reasons for the conflicting reports on the biological activities between the parent acids, IAA and 2,4-D, and their ester and amide conjugates are not known. Many factors, including absorption, and intracellular compartmentation, may contribute to the conflicting results.[22,68]

3. Glutathione Conjugation

Glutathione (GSH) conjugation is recognized as a major herbicide detoxication pathway

in plants. Several examples of herbicide metabolism in plants by GSH conjugation have been reported since the first example in 1970.[69] The significance of this pathway in plants is emphasized in several reviews.[1,4,7,8,18,43] GSH conjugation is extremely important in plants because (1) the reaction has a wide range of potential substrates, (2) it is a detoxication mechanism and a major factor in herbicide selectivity, and (3) it influences the nature of terminal herbicide residues in plants.

Glutathione conjugation is the initial reaction leading to the synthesis and excretion of mercapturic acids in mammals.[70] Conjugation with reduced glutathione is catalyzed by glutathione *S*-transferases with different substrate specificities. Sequential removal of glutamic acid and glycine residues yields the *S*-cysteine conjugate that is *N*-acetylated to give the mercapturic acid, the terminal excretion product in mammals. In plants the sequential removal of amino acids is reversed and the mercapturic acids are not the terminal products. The *S*-cysteine conjugate is metabolized to compounds such as the lanthionine conjugate of atrazine[71] and the *N*-malonylcysteine conjugate of fluorodifen[7] and other herbicides such as propachlor, metolachlor, butachlor, EPTC, acifluorfen, chlorpropham, and prometryn[39] that may be intermediates in the formation of insoluble phase III metabolites.

Herbicide molecules that have an electrophilic center and an appropriate leaving group or one that can be chemically activated by oxidation are probable candidates for glutathione conjugation and detoxication.[4] Herbicide classes with compounds known to undergo GSH conjugations are the 2-chloro-*s*-triazines, thiocarbamate sulfoxides, α-chloroacetamides, and diphenylethers.[4] Specific examples of herbicides from some of these classes will be discussed in detail later. The examples of GSH conjugation involve predominantly reactions between the parent herbicide molecules and GSH in a nucleophilic displacement reaction. Therefore, GSH conjugation appears to be a direct rather than an indirect detoxication reaction as with most glycoside conjugations of herbicide metabolites.

Glutathione conjugation of atrazine and its catabolism in plants are well established (Figure 8).[71] The GSH conjugates of hindered α-chloroacetamides such as propachlor and CDAA (Figure 8),[77] and alachlor and metolachlor have also been characterized.[73] The GSH conjugation of α-chloroacetamides is very likely a detoxication mechanism in resistant plants. Greater than 90% of the propachlor in maize was converted to water-soluble metabolites, including the GSH conjugate, within 18 to 72 h.[72] The α-chloroacetamides are highly reactive with GSH and will form GSH conjugates nonenzymatically.[72,73] This was true of the herbicides propachlor, CDAA, alachlor, and metolachlor.

Glutathione conjugation of a herbicide does not necessarily occur in both plants and animals. Atrazine, simazine, and cyprazine all formed GSH conjugates in several plant species but not in vivo in mammals.[7,74] In vitro mouse and rat liver preparations of glutathione *S*-transferase successfully conjugated atrazine.[75] Cyanazine, on the contrary, is metabolized readily by GSH conjugation to its mercapturic acid in the rat but its conjugation with glutathione is very limited in plants.[7,76] Fluorodifen, in contrast to the 2-chloro-*s*-triazines above, is rapidly metabolized by glutathione conjugation in both plants and mammals (Figure 8).[47,77]

Direct conjugation with cysteine was reported as a detoxication mechanism for chlorfenprop-methyl and the aryl-hydroxylated metabolite of chlorpropham (Figure 9).[78,79] Chlorfenprop-methyl inhibits growth in susceptible grasses by antagonizing IAA-induced cellular processes. In resistant wheat (*Triticum aestivum* L.) chlorfenprop-methyl was hydrolyzed to its acid and conjugated with cysteine by substitution of the chlorine in the alkyl side chain. At relatively high concentrations (1 m*M*) chlorfenprop-methyl, its acid, and the cysteine conjugate inhibited wheat coleoptile growth by 88, 78, and 14%, respectively.[78] Part of these differences in the inhibitory activities is probably due to differences in tissue penetration between the three chemical forms. Nevertheless, cysteine conjugation appears to detoxify the acid. The metabolite of chlorpropham, isopropyl-3′-chloro-4′-hydroxycar-

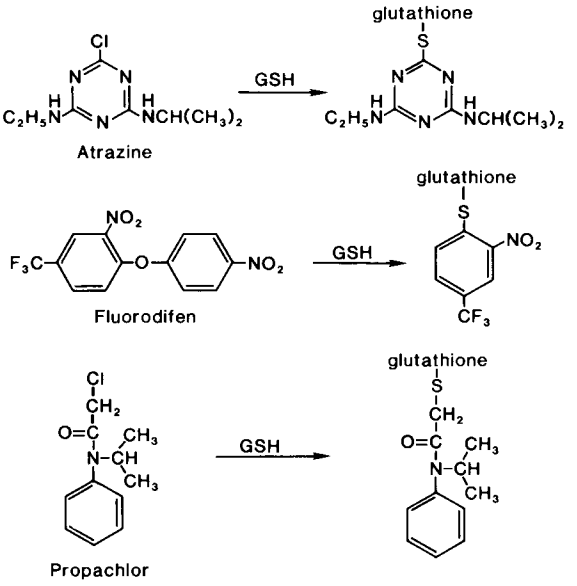

FIGURE 8. Glutathione conjugates of atrazine, fluorodifen, and propachlor.

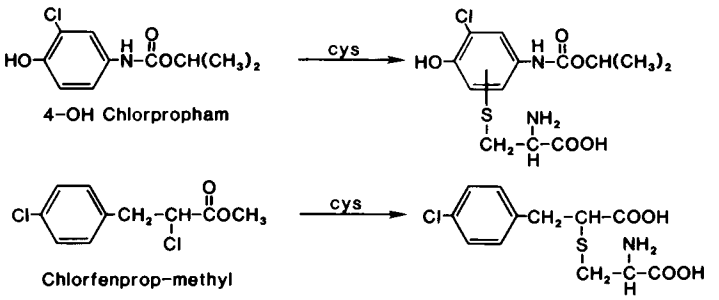

FIGURE 9. Cysteine conjugates of 4-hydroxychlorpropham and chlorfenprop-methyl.

banilate (4-hydroxychlorpropham), isolated from shoots of susceptible oat was conjugated in the aryl moiety to yield an *S*-cysteinylhydroxychlorpropham metabolite that retained both the aryl hydroxy and chlorine substituents (Figure 9.) The metabolite, isopropyl-5′-chloro-2′-hydroxycarbanilate (2-hydroxy-5-chlorpropham), isolated from resistant species was not conjugated with either cysteine or glutathione. The 2-hydroxy-5-chlorpropham was the active inhibitor of mitochondrial respiration and oxidative phosphorylation and not the 4-hydroxychlorpropham.[80] Therefore, cysteine conjugation of 4-hydroxychlorpropham was not a detoxication reaction. This is in contrast to cysteine conjugation of chlorfenprop-methyl.

V. SPECIFIC DETOXICATION AND ACTIVATION OF HERBICIDES

A. Atrazine

The metabolism of atrazine best serves to illustrate the criteria discussed earlier for demonstrating herbicide detoxication and its role in selectivity. Resistance to atrazine due to intrinsic differences at the site of action is an important factor in atrazine selectivity

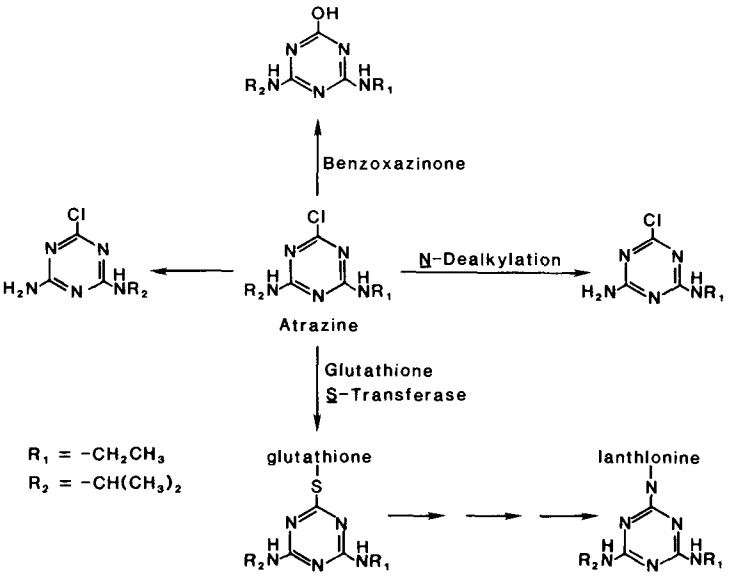

FIGURE 10. The metabolic pathways for atrazine metabolism and detoxication in plants.

(Chapter 7). However, in major crop plants such as maize and sorghum (*Sorghum bicolor* (L.) Moench) and in many weeds metabolism resulting in detoxication is the major factor in atrazine resistance and selectivity.

The two types of resistance to atrazine may be differentiated by a time course study of photosynthetic inhibition. Atrazine should show little or no inhibition of photosynthesis in plants with intrinsic resistance even at high atrazine concentrations. If metabolism and detoxication are the basis for resistance, an initial inhibition of photosynthesis is followed by an expected recovery in highly resistant species.[18]

Atrazine is detoxified by three different metabolic pathways (Figure 10). Each of these pathways has been confirmed by one or more of the criteria to be a detoxication mechanism. The metabolites, hydroxyatrazine and hydroxysimazine were identified as the hydrolysis products in maize. As indicated earlier, a catalyst, benzoxazinone, was isolated and characterized from maize and the hydroxy-*s*-triazines were nonphytotoxic when readministered to susceptible plants.[15-17,81] The *N*-dealkylated metabolites have also been isolated and identified from plants although the enzyme catalyzing this reaction has not been isolated.[32] *N*-Dealkylation is only a partial detoxication mechanism since the I_{50} concentration for inhibition of the Hill reaction was 23 times greater for the *N*-desethyl metabolite than for atrazine.[82] Susceptible oat showed intermediate injury when treated with the 2-chloro-4-amino-6-isopropylamino-*s*-triazine whereas the same species showed no signs of injury when treated with the 2-hydroxy analogue of the same compound.[81]

The chloro-*s*-triazines require a chlorine substituent in the 2-position of the *s*-triazine ring and one or more intact *N*-alkyl side chains for biological activity. Sorghum completely dealkylated atrazine to 2-chloro-4,6-diamino-*s*-triazine (diamino-*s*-triazine), which had very little herbicidal activity.[83] Diamino-*s*-triazine reduced fresh weight by only 12% as compared to 91% for atrazine when both compounds were applied to susceptible oat at 10 μM concentrations.[83] Hill reaction in isolated chloroplasts was virtually unaffected by 100 μM diamino-*s*-triazine as compared to a half-maximal inhibitor concentration (I_{50}) of 2 μM for atrazine and 46 μM for the mono-*N*-dealkylated metabolite.[14,82,83] Therefore, either dis-

placement of the chlorine with a polar substituent or oxidative removal of *N*-alkyl side chains is a detoxication reaction with 2-chloro-*s*-triazine herbicides.

The nucleophilic displacement of chlorine by glutathione is a major detoxication reaction as expected (Figure 10). The glutathione conjugate of atrazine and subsequent metabolites have been isolated and characterized.[71] The enzyme, glutathione *S*-transferase, responsible for catalyzing the atrazine conjugation reaction has been isolated and characterized from resistant maize and is present in high concentrations in other atrazine-resistant species such as johnsongrass (*Sorghum halepense* (L.) Pers.), sorghum, sudangrass (*Sorghum* sudanense (Piper) Stapf), and sugarcane (*Saccharum officinarum* L.).[4,84] Susceptible species such as barley (*Hordeum vulgare* L.), wheat, oat, and pigweed (*Amaranthus retroflexus* L.) lacked this specific enzyme activity. Therefore, the correlation between the enzyme activity in plants and their resistance to atrazine is clearly implicated with the metabolism of atrazine to the glutathione conjugate (GS-atrazine). Furthermore, the transient inhibition of photosynthesis (oxygen evolution) from leaf discs of resistant sorghum and maize and subsequent recovery within 7 h concomitant with the metabolism of tissue atrazine to GS-atrazine implicated GSH conjugation as an effective detoxication mechanism.[18] In root-treated intact plants the net CO_2 exchange (NCE) rates were inhibited initially by atrazine in resistant maize, moderately susceptible large crabgrass (*Digitaria sanguinalis* Scop.) and bristly foxtail (*Setaria verticillata* L.), and susceptible oats. However, within 12 h the NCE recovery rates reflected the species sensitivity to atrazine and their ability to metabolize atrazine primarily to GS-atrazine.[85] The NCE recovered completely in maize within 5 to 6 h, a gradual recovery occurred over a period of 12 h in crabgrass and foxtail, but no recovery occurred in oats.

Three successive periodical treatments of resistant maize seedlings with atrazine affected the recovery time of atrazine-inhibited photosynthesis. The recovery times were shortened after each successive treatment ranging from 21.4 to 4 h. The faster recovery of photosynthesis was accompanied by a more rapid metabolism of atrazine to GS-atrazine after each successive exposure to atrazine.[86] Therefore, atrazine pretreatment of resistant species may further increase its tolerance to atrazine by enhancing the plant's ability to detoxify the herbicide by GSH conjugation. It was not certain whether this enhancement was due to an effect on the enzyme, glutathione *S*-transferase, or to an increase in the level of glutathione.[86] It appears that enhancement was probably through an effect on the enzyme rather than the substrate.

B. Chlorpropham

Chlorpropham is a carbanilate herbicide containing an ester and an amide bond in the same molecule. Therefore, expected degradation reactions would include both ester and amide hydrolysis. However, in contrast to metabolism in the soil and animals where hydrolysis of the carbamate bond is the predominant reaction,[87] chlorpropham is not hydrolyzed in plants. The major degradation reactions are aryl hydroxylation and conjugation (Figure 11). Water-soluble metabolites accumulated rapidly in plants and this led researchers to postulate hydrolysis followed by conjugation as the primary degradation pathway as in animals.[87] However, this was not so as indicated by extensive research on chlorpropham metabolism.[25,58,79] The chlorpropham example illustrates the importance of the first criterion in demonstrating herbicide detoxication in plants, i.e., the need to isolate and chemically characterize the metabolites.

The parent carbamate molecule or subsequent metabolites of chlorpropham must be the biologically active form of the herbicide. This is in contrast to the esters of the phenoxyalkanoic acid and phenoxy-phenoxyalkanoic acid herbicides where it is assumed that the inactive esters are hydrolyzed to yield the herbicidally active acids. Tissue specificity was also observed in the metabolism of chlorpropham in soybean. Both 2-hydroxychlorpropham

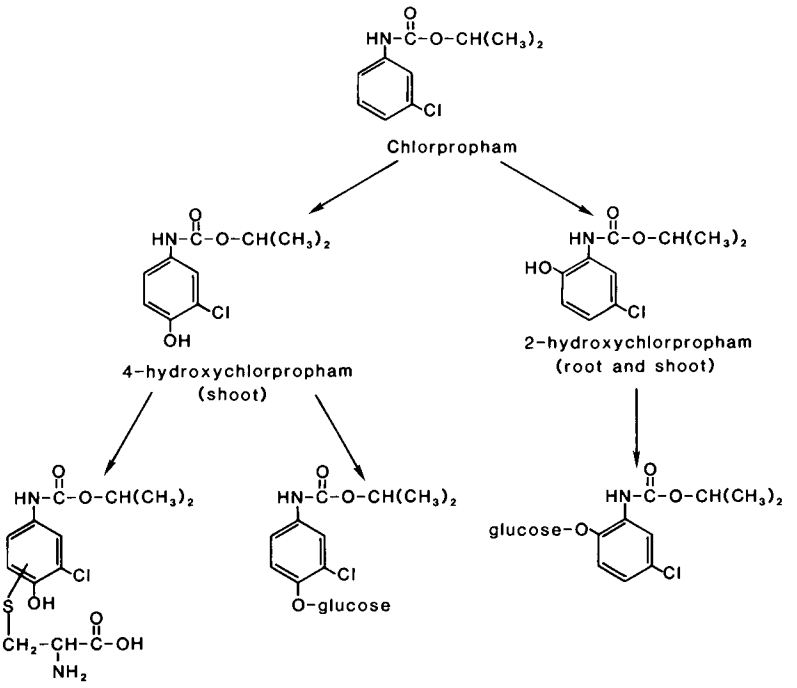

FIGURE 11. The activation and detoxication of chlorpropham in plants.

and 4-hydroxychlorpropham were formed in a 1:1 ratio in shoots but only 2-hydroxychlorpropham was found in roots.[25]

Aryl hydroxylation of herbicides is generally assumed to be a detoxication reaction. However, the herbicidal activities of hydroxylated metabolites are difficult to determine by application of the metabolites to intact plants since aryl-hydroxylated compounds are conjugated rapidly to their inactive glucosides. With the use of in vitro assays and tissue culture techniques,[80,88] both 2-hydroxy- and 4-hydroxychlorpropham were found to be equally or more active than chlorpropham. The activities of the hydroxylated metabolites were determined at physiological concentrations of less than 100 μM, thus minimizing secondary effects due to the use of high concentrations.

The 2-hydroxychlorpropham was an effective uncoupler of phosphorylation similar to 2,4-dinitrophenol (DNP). The metabolite at 100 μM inhibited mitochondrial NADH-linked respiration in both resistant and susceptible plants by 30% and oxidative phosphorylation by 100%, and stimulated ATPase activity two-fold. The 2-hydroxychlorpropham was inactive when glucosylated to 2-*O*-glucosylchlorpropham.[80] This is one example where the activities of a hydroxylated metabolite and its glucosyl conjugate were determined successfully. Generally, the biological activities of herbicide conjugates cannot be determined unequivocally by application to intact plants or plant parts. This is due to the uncertainty of whether the conjugates are absorbed and translocated to the site of action as the intact molecule.

Chlorpropham at 100 μM inhibited NADH-linked respiration but had no effect on oxidative phosphorylation. 4-Hydroxychlorpropham had no effect on mitochondrial activity.[80] Growth of soybean cell cultures was inhibited by chlorporpham at 50 μM but not by 2-hydroxy- and 4-hydroxychlorpropham, whereas the growth of carrot cell cultures was inhibited by all three compounds.[88] Cell mitosis was inhibited by chlorpropham in soybean root tips, resulting in the buildup of prophase and metaphase nuclei; 2-hydroxychlorpropham inhibited all phases of cell mitosis, whereas 4-hydroxychlorpropham had little or no effect.[88] All three compounds

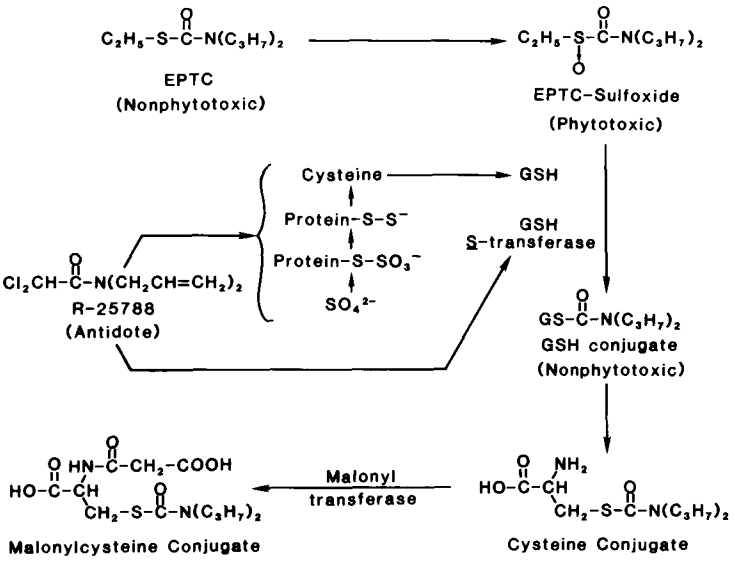

FIGURE 12. The metabolic pathway for oxidative activation of EPTC and antidote-enhanced detoxication in plants.

penetrated the tissues readily since they were metabolized to water-soluble conjugates when added to soybean cell cultures.

The assays used in measuring biological activities of chlorpropham and its metabolites may not be related to the primary mode of action of chlorpropham. However, it is clear that hydrolysis of the ester or amide bond of the parent molecule is not necessary for herbicidal activity. It is also clear that aryl hydroxylation per se is not necessarily a detoxication reaction in chlorpropham, but the conjugation of the hydroxylated metabolites to hydrophilic compounds results in the loss of biological activity. Therefore, information on the quantitative and qualitative aspects of herbicide metabolism is extremely important when determining the basis for selectivity and mode of action of a herbicide.

C. EPTC

EPTC is a thiocarbamate herbicide used as a preplant incorporated compound primarily for the control of annual and perennial grasses. Little is known about the mechanism of action of EPTC. Until recently its metabolism and degradation was believed to occur primarily via hydrolysis, *N*-dealkylation, and incorporation into natural products.[89] The detailed metabolism and detoxication of EPTC in plants was elucidated only recently (Figure 12).[39-41,90]

EPTC is very likely oxidized by an mfo system to EPTC-sulfoxide, which is an excellent carbamoylating agent for tissue thiols.[90] Glutathione was carbamoylated to *S*-carbamyl-GSH (GSH conjugate) catalyzed by a glutathione *S*-transferase. Multiple reactions catalyzed by peptidases yielded the cysteine conjugate that is malonylated to the *N*-malonylcysteine conjugate as the probable terminal metabolite.[39]

EPTC and its oxidized metabolite, EPTC-sulfoxide, are of interest physiologically because of their herbicidal activities and the action of the herbicide antidote, R-25788 (*N,N*-diallyl-2,2-dichloroacetamide).[91] EPTC is an excellent example of a progenitor herbicide that is activated metabolically to a phytotoxic metabolite that is detoxified subsequently by conjugation. EPTC-sulfoxide was more phytotoxic to susceptible weeds than EPTC, whereas maize was unaffected by the sulfoxide, but was injured considerably when treated with

EPTC.[40] In maize roots, the active EPTC-sulfoxide was detoxified rapidly by conjugation with glutathione.[41,92]

EPTC-sulfoxide is apparently the herbicidally active form. Its activity may result from its carbamoylating action of tissue thiols although the target sites or receptors have not been defined.[92] The activated sulfoxide is a better carbamoylating agent than EPTC.[90,93] Carbamoylation of GSH was reported to occur nonenzymatically but this conclusion requires further evidence.[94]

The antidote, R-25788, is specific for protecting maize from injury by EPTC and other thiocarbamate herbicides.[91] Several mechanisms for antidote activity by R-25788 have been proposed. The prevalent hypothesis is that R-25788 increases the level of the substrate, GSH,[41,93,95,96] and stimulates the activity of the enzyme, GSH *S*-transferase, resulting in an accelerated rate of EPTC-sulfoxide detoxication.[41,90,93] Maize roots treated with R-25788 for 24 h had a two-fold increase in GSH *S*-transferase activity with a threshold level for antidote stimulation between 0.014 to 0.14 μM. Maximum stimulation of enzyme activity occurred at 1.4 μM R-25788 and it occurred within 12 hr and was complete by 48 h.[93] Higher concentrations of R-25788 were required to increase the GSH content in maize roots and the time required was similar to that for stimulating GSH *S*-transferase activity.[93]

R-25788 may be a competitive inhibitor of EPTC for the same site of action.[97,98] Antidote activity was higher in compounds with structures similar to the thiocarbamate herbicides. However, the evidence is based on bioassay techniques only. Therefore, the action of R-25788 as a competitive inhibitor of EPTC is still speculative. This mechanism excludes interaction of R-25788 with the detoxication mechanism of EPTC-sulfoxide. Therefore, it is not consistent with the known stimulation of EPTC-sulfoxide detoxication by R-25788. The potency of several compounds as antidotes was generally correlated with their ability to elevate GSH *S*-transferase activity and GSH content.[93]

R-25788 may increase the rate of EPTC sulfoxidation followed by rapid detoxication nonenzymatically to the GSH-conjugate.[73] This mechanism implicates R-25788 as a stimulant of the mfo system, but no evidence for such action was presented.

R-25788 may antagonize EPTC by overcoming EPTC inhibition. An early site of EPTC action in maize cell cultures was the inhibition of ^{14}C-acetate incorporation into lipids.[96] R-25788 alone increased immediately the incorporation of ^{14}C-acetate or decreased the inhibition by EPTC when the two compounds were added as a combination to maize cell cultures.[96] There was a 12-h lag period before R-25788 increased the cell GSH level, but EPTC was detoxified within the first 8 h. Therefore, it was postulated that R-25788 had an early sparing effect on maize cells until the normal GSH conjugation mechanism detoxified the EPTC. Thus, the role of increased GSH concentration induced by R-25788 in the antidote action against EPTC was questioned.[96] However, much of the maize cell responses to EPTC and R-25788 were determined at concentrations of 500 to 1000 μM EPTC and 150-500 μM R-25788. The biochemical and physiological responses of maize cell cultures at lower concentrations are unknown.

The elevation of GSH levels and GSH S-transferase activity by R-25788 appears to be the probable antidotal mechanism. The recent report that R-25788 enhanced $^{35}SO_4^{2-}$ metabolism and incorporation into cysteine and other protein is consistent with the above hypothesis (Figure 12).[99]

D. Paraquat

Paraquat and diquat are the two most widely used bipyridylium herbicides (Figure 13). These compounds are nonselective, general contact herbicides that are applied to the foliage for the control of annual grasses or applied for selective weed control where contact with the crop is avoided.[100]

Paraquat is not metabolized to any extent in plants.[100] Therefore, tolerance or resistance

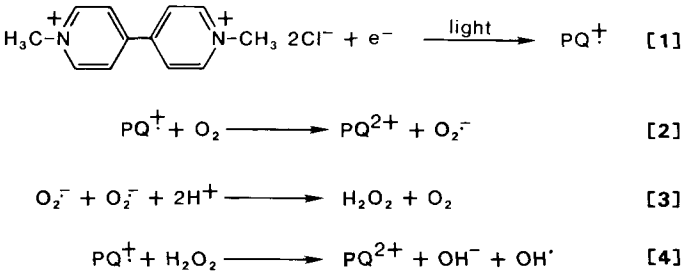

FIGURE 13. The formation of paraquat free radical [1] and the reactions of the free radical resulting in the formation of the toxic hydroxyl free radical [4].

to paraquat cannot be due to biotransformation and detoxication of the herbicide or its activated metabolites as with atrazine, chlorpropham, and EPTC. Uptake and distribution of paraquat in plants did not differ between susceptible and resistant species.[101] Tolerance to paraquat appears to be due to the ability of a plant to detoxify or neutralize secondary toxic forms of oxygen that are induced by paraquat in its mechanism of action.[101,102] Therefore, detoxication and resistance as applied to paraquat differ from the classical examples discussed.

The mode of action of paraquat and diquat in green plants appears to be mediated through the generation of phytotoxic hydrogen peroxide and superoxide radical (O_2^-).[103,104] The phytotoxic action of paraquat and diquat requires light, chlorophyll, and oxygen. Reactive singlet oxygen, superoxide radical, hydrogen peroxide, and hydroxyl radical are generated through normal photochemical and biochemical reactions in photosynthesis.[103] However, green plants possess natural protective mechanisms that prevent or minimize damage by the reactive forms of oxygen. The reactive or toxic forms of oxygen become significant herbicidally when their formation becomes excessive and the natural protective mechanisms are unable to quench, neutralize, or detoxify them to prevent irreversible damage. Paraquat and diquat enhance the generation of the reactive forms of oxygen and cause the natural defenses to be swamped, leading to the eventual death of plants.

Paraquat acts as a catalyst.[105] Paraquat (PQ^{2+}) is reduced photochemically by electrons from photosystem I to yield the paraquat radical (PQ^+) [1] (Figure 13). Paraquat radical undergoes rapid autoxidation by transfer of single electrons to molecular O_2 to yield the superoxide radical [2].[105] This is similar to the autoxidation of reduced ferredoxin to yield O_2^- under natural conditions.[103] The superoxide radical is somewhat unreactive and undergoes a dismutation reaction to $O_2 + H_2O_2$ [3]. This reaction is accelerated by superoxide dismutase.[106] Hydrogen peroxide may react with O_2^- or reduced ferredoxin to yield the hydroxyl radical.[103] The electron donor may also be a paraquat radical to give OH^- and the hydroxyl radical, OH·[4].[105]

Hydroxl radicals are the most reactive oxygen species known and will attack and damage nearly all molecules in a living cell.[103] The membranes of cell organelles, the tonoplast and plasmalemma are particularly sensitive to damage by paraquat. Damage to membranes is consistent with the mechanism of action of reactive free radicals resulting in hydrogen abstraction of unsaturated lipids (A,B) (Figure 14), lipid peroxidation (C), abstraction of hydrogen from an unsaturated lipid (A) to yield the hydroperoxide (D) and breakdown.[107] Hydrogen abstraction initiates a chain reaction that is autocatalytic and highly destructive to membranes.

Superoxide dismutase (SOD) catalyzes reaction [3] (Figure 13) to prevent O_2^- from accumulating as a potential source for highly reactive OH·. The natural pathways for eliminating H_2O_2 [Reactions 5,6,7] (Figure 15) in plants should detoxify the excess H_2O_2 generated by increased SOD activity.

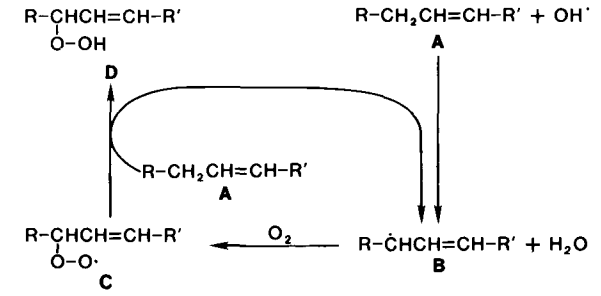

FIGURE 14. The mechanism of free radical-catalyzed breakdown of unsaturated lipids in cell membranes.

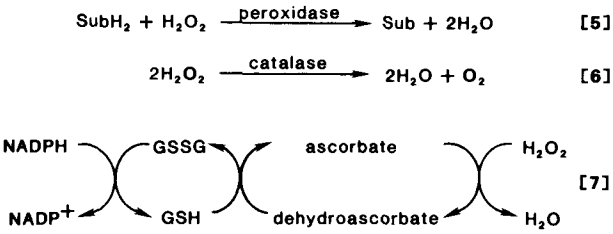

FIGURE 15. Natural mechanisms in plants for the elimination of hydrogen peroxide generated by superoxide dismutase in chloroplasts.

The primary effect of paraquat in light is in the chloroplast. Illumination of chloroplasts produced H_2O_2 and the addition of catalase liberated O_2.[107] Paraquat-induced H_2O_2 was detected cytochemically along the stroma lamellae, grana stack, and in the thylakoids.[108] Paraquat caused less ultrastructural damage to chloroplasts when diuron was applied together with paraquat or when H_2O_2 was precipitated with cerium chloride.[108] Resistant cultivars of perennial ryegrass (*Lolium perenne* L.) contained higher concentrations of SOD in the chloroplasts and higher levels of extrachloroplastic SOD, catalase and peroxidase than the susceptible cultivars.[102] Therefore, detoxication of secondary reactive oxygen species induced by paraquat radicals in the chloroplasts appears to be an important protective and selective mechanism against injury by bipyridylium herbicides.

E. Diclofop-Methyl

Diclofop-methyl is in a new class of herbicides that was developed originally for the control of wild oat (*Avena fatua L.*). Compounds in this class have no intrinsic auxin-like activity like the phenoxy alkanoic acid herbicides but appear to act by interfering with the action of an endogenous auxin, IAA.[9] Diclofop-methyl and several analogues contain a diphenylether moiety, but their activity spectrum and modes of action are quite different from the other diphenylether herbicides.[9]

Diclofop-methyl selectively controls wild oat in wheat. Diclofop-methyl is hydrolyzed rapidly to the free acid, diclofop, in both resistant wheat and susceptible wild oat.[29] The methyl ester is more effective than the acid in inhibiting IAA-induced cell elongation but the acid is more effective than the ester in inhibiting root growth in susceptible oat.[109] The acid was more effective in inhibiting IAA-induced proton extrusion but the ester and acid were nearly equally effective in inhibiting leaf growth when they were injected into susceptible oat stems.[110,111] Differences in tissue penetration between the ester and acid may account for some of the differences in activity, but this is not true for all assays.[110] Oat

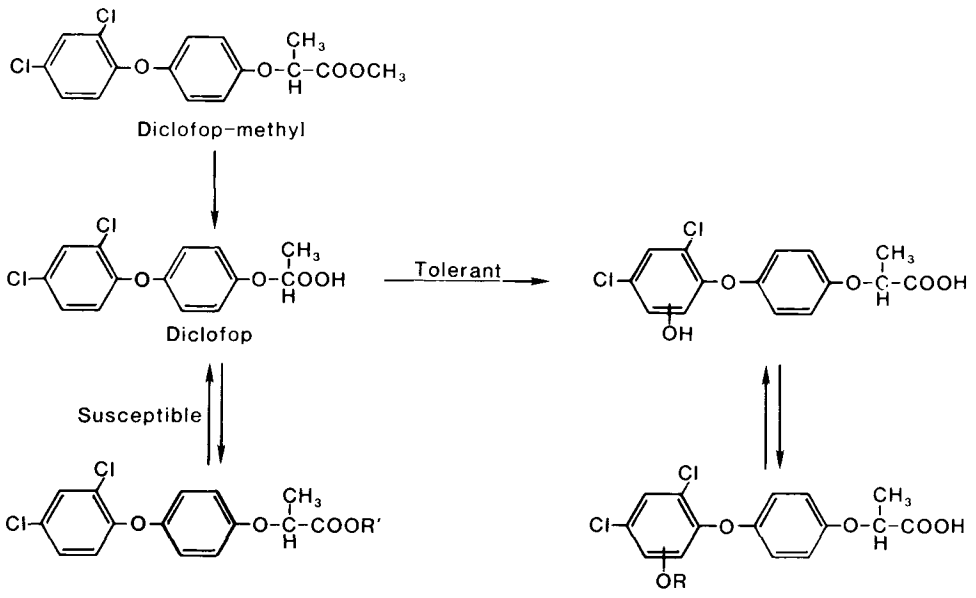

FIGURE 16. The metabolism of diclofop-methyl in tolerant and susceptible plants.

coleoptiles with epidermis present absorbed and retained nearly 80% of the applied ester and 13% of the acid in their tissues.[110] Unpeeled coleoptiles are used for determining IAA-induced cell elongation. When the epidermis was removed, oat coleoptiles absorbed and retained 64% of the applied ester and 51% of the acid.[110] Peeled coleoptiles are used for measuring IAA-induced acidification. Therefore, the hydrolysis of diclofop-methyl to diclofop may not be just an activation mechanism as postulated for benzoylprop-ethyl, another wild oat herbicide.[50]

Very little diclofop remains in both susceptible and resistant species (Figure 16).[29] In susceptible species, conjugation to an ester conjugate occurs rapidly, whereas in tolerant species diclofop is aryl-hydroxylated followed by conjugation to a phenolic conjugate.[29] Both ester and phenolic conjugations appear to be detoxication reactions that yield polar water-soluble metabolites. The predominance of the ester conjugate in susceptible species suggests that this metabolite may be an inactive form of the herbicide that will readily generate active diclofop upon its hydrolysis in vivo.[29] This would be analogous to the free and ester conjugates of IAA.[57,68] However, aryl hydroxylation is an irreversible reaction. Therefore, phenolic conjugation and hydrolysis will not affect the active diclofop pool. The predominance of this pathway in tolerant species suggests that aryl hydroxylation of diclofop may be a detoxication reaction. If not, the conjugation of the hydroxylated metabolite certainly results in a detoxified form of the herbicide. The dynamics of this pathway and its effects on the physiological response of tissues were demonstrated in the assay of IAA-induced proton extrusion in susceptible and tolerant tissues.[110]

Besides metabolic detoxication, intracellular compartmentation of phytotoxic acid herbicides appears to influence greatly the biological activity of these compounds. Compartmentalization of the acid herbicides, flamprop,[112] 2,4-D,[113] diclofop,[110] and their water-soluble conjugates was indicated by efflux analysis and physiological responses. Two separate flamprop pools were detected within the tissues by efflux analysis. These pools were probably sequestered within compartments with membranes of different permeabilities to flamprop.[112] An active diclofop pool appeared to exist in both tolerant and susceptible tissues. The size of the diclofop pool probably influenced the degree of inhibition and rate of recovery

of IAA-induced proton excretion.[110] The size of the active diclofop pool seemed to be smaller when tissues were treated with the more lipophilic methyl ester than with the acid as indicated by the acidification response of peeled coleoptile tissues. This was despite the fact that slightly more ester was absorbed than the acid before hydrolysis of the ester to its acid, diclofop.[110] If two different pools of diclofop are formed, similarly to that of flamprop, the nonpolar diclofop-methyl may penetrate, hydrolyze, and be sequestered in a diclofop pool that may not be as readily available for biological activity as the second diclofop pool formed predominantly by direct absorption of the more polar acid. Therefore, when absorption between the ester and acid is not a limiting factor the acid may be expected to be more active than its ester.[110]

Flamprop is applied both as the isopropyl and the methyl esters for weed control (Figure 7).[114,115] Their bases for detoxication and selectivity are alike but the methyl ester is a more effective herbicide than the isopropyl ester.[114,115] The acid, flamprop, is believed to be the active molecule for both herbicides and the metabolism between the two esters do not appear to differ significantly.[115] The rate of hydrolysis is slightly faster for the methyl ester than the isopropyl ester, but this difference does not appear to account for the difference in biological activity between the two esters. If the active flamprop pool size is influenced by the slight differences in chemical properties between the methyl and isopropyl esters, then the biological activities between these two esters may be different as confirmed by their application to whole plants.[114,115] Therefore, intracellular compartmentation may influence greatly the activity of acid herbicides. However, compartmentation may be effective only as a temporary protective mechanism that may delay the onset of herbicidal injury or increase the threshold concentration for injury especially in susceptible species. This is in contrast to permanent detoxication by metabolic biotransformation reactions.

It has been generally assumed that esterification of acid herbicides is simply to facilitate penetration and uptake of the compounds from plant surfaces. Once absorbed, ester hydrolysis frees the active acid, which elicits the toxic herbicidal action. However, esters may have physiological significance other than to facilitate absorption as indicated by the differential activity between esters and their acids in both in vivo and in vitro assays.[109,110,116]

VI. CONCLUSION

Metabolism or biotransformation of herbicides, resulting in detoxication, is a major factor in herbicide resistance and selectivity in plants. Much is known about herbicide metabolism in plants, their biotransformation products, and the alteration of biological activity due to metabolism. However, information on the enzymes involved in herbicide metabolism, their properties and the reaction mechanisms is lacking. Knowledge about the transport of herbicides and their metabolites across plant cell membranes and their intracellular compartmentation is also lacking. A more biorational approach to the development of new herbicide compounds will be possible with increased knowledge in the above-mentioned areas together with knowledge on the mode of action of herbicides. Synergists and antagonists may be developed to interact specifically with enzymes or herbicidal compounds to modulate the activity spectrum and efficacy of new and existing compounds.

Basic information on herbicide metabolism and detoxication may lead to a better understanding of the molecular basis for pesticide interactions that are becoming increasingly important with the application of pesticide combinations in the field. Antidotes such as R-25788 and naphthalic anhydride,[91] may be developed through a biorational approach and herbicide antagonisms and synergisms observed in field applications may be predicted rather than discovered empirically. The enzymatic mechanisms for herbicide detoxication which are primarily responsible for herbicide resistance and selectivity are genetically controlled characteristics. With the rapid development of genetic engineering and biotechnology, the

incorporation of resistant genes into susceptible species will broaden the use spectrum of herbicides.

REFERENCES

1. **Baldwin, B. C.,** Xenobiotic metabolism in plants, in *Drug Metabolism: From Microbe to Man*, Parke, D. V. and Smith, R. L., Eds., Taylor and Francis, London, 1977, 191.
2. **Casida, J. E. and Lykken, L.,** Metabolism of organic pesticide chemicals in higher plants, *Annu. Rev. Plant Physiol.*, 20, 607, 1969.
3. **Kearney, P. C. and Kaufman, D. D., Eds.,** *Herbicides: Chemistry, Degradation and Mode of Action*, Vol. 1 and 2, 2nd ed., Marcel Dekker, New York, 1975.
4. **Lamoureux, G. L. and Frear, D. S.,** Pesticide metabolism in higher plants, *in vitro* enzyme studies, in *Xenobiotic Metabolism, In Vitro Methods*, Paulson, G. D., Frear, D. S., and Marks, E. P., Eds., ACS Symp. Ser. 97, American Chemical Society, Washington, D.C., 1979, 77.
5. **Menzer, R. E.,** Biological oxidation and conjugation of pesticide chemicals, *Residue Rev.*, 48, 79, 1973.
6. **Sanderman, H., Diesperger, H., and Scheel, D.,** Metabolism of xenobiotics by plant cell cultures, in *Plant Tissue Culture and Its Biotechnological Application*, Barz, W., Reinhard, E., and Zenk, M. H., Eds., Springer-Verlag, New York, 1977, 178.
7. **Shimabukuro, R. H., Lamoureux, G. L., and Frear, D. S.,** Pesticide metabolism in higher plants: reactions and mechanisms, in *Biodegradation of Pesticides*, Matsumura, F. and Krishna Murti, C. R., Eds., Plenum Press, New York, 1982, 21.
8. **Hatzios, K. K. and Penner, D.,** *Metabolism of Herbicides in Higher Plants*, Burgess Publishing Co., Minneapolis, 1982.
9. **Fedtke, C.,** *Biochemistry and Physiology of Herbicide Action*, Springer-Verlag, Berlin, 1982.
10. **Frear, D. S. and Menn, J. J.,** Pesticide transport and transformation of pesticides, in *Symposium on Environmental Transport and Transformation of Pesticides*, EPA 600/9-78-003, U.S. Environmental Protection Agency, Athens, Ga., 1978, 126.
11. **Williams, R. T.,** *Detoxication Mechanisms: The Metabolism and Detoxication of Drugs, Toxic Substances and Other Organic Compounds*, Chapman and Hall, London, 1959.
12. **Frear, D. S., Swanson, H. R., and Tanaka, F. S.,** Herbicide metabolism in higher plants, in *Recent Advances in Phytochemistry*, Vol. 5, Runeckles, V. C. and Tso, T. S., Eds., Academic Press, New York, 1972, 225.
13. **Kaufman, D. D., Still, G. G., Paulson, G. D., and Bandal, S. K., Eds.,** *Bound and Conjugated Residues*, ACS Symp. Ser. 29, American Chemical Society, Washington, D.C., 1976.
14. **Gysin, W. and Knusli, E.,** Chemistry and herbicidal properties of triazine derivations, in *Advances in Pest Control Research*, Vol. III, Metcalf, R. L., Ed., John Wiley & Sons, New York, 1960, 289.
15. **Hamilton, R. H. and Moreland, D. E.,** Simazine: degradation by corn seedlings, *Science*, 135, 373, 1962.
16. **Roth, W. and Knusli, E.,** Beitrag zur kenntnis der resistanzphanomene einzelner pflanzen gegenuber dem phytotoxischen wirkstoff simazin, *Experientia*, 17, 312, 1961.
17. **Castelfranco, P., Foy, C. L., and Deutsch, D. B.,** Non-enzymatic detoxification of 2-chloro-4,6-bis(ethylamino)-*s*-triazine (simazine) by extracts of *Zea mays*, *Weeds*, 9, 580, 1961.
18. **Shimabukuro, R. H., Lamoureux, G. L., and Frear, D. S.,** Glutathione conjugation: a mechanism of herbicide detoxication and selectivity in plants, in *Chemistry and Action of Herbicide Antidotes*, Pallos, F. M. and Casida, J. E., Eds., Academic Press, New York, 1978, 133.
19. **Hamilton, R. H., Hurter, J., Hall, J. K., and Ercegovich, C. D.,** Metabolism of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid by bean plants, *J. Agric. Food Chem.*, 19, 480, 1971.
20. **Fleecker, J. and Steen, R.,** Hydroxylation of 2,4-D in several weed species, *Weed Sci.*, 19, 507, 1971.
21. **Makeev, A. M., Makoviechuk, A. I. U., and Chkanikov, D. C.,** Microsomal hydroxylation of 2,4-dichlorophenoxyacetic acid herbicide in plants, cucumbers and peas, *Dokl. Akad. Nauk. SSSR*, 233, 1222, 1977.
22. **Pillmoor, J. B. and Gaunt, J. K.,** The behavior and mode of action of the phenoxyactic acids in plants, in *Progress in Plant Biochemistry*, Vol. 1, Hutson, D. H. and Roberts, T. R., Eds., John Wiley & Sons, New York, 1981, 147.

23. **Gurroff, G., Daly, J. W., Jerina, D. M., Renson, J., Witkop, B., and Udenfriend, S.,** Hydroxylation-induced migration: the NIH shift, *Science*, 157, 1524, 1967.
24. **Jerina, D. M. and Daly, J. W.,** Arene oxides: a new aspect of drug metabolism, *Science*, 185, 573, 1974.
25. **Still, G. G. and Mansager, E. R.,** Soybean shoot metabolism of isopropyl-3-chlorocarbanilate: ortho and para aryl hydroxylation, *Pestic. Biochem. Physiol.*, 3, 87, 1973.
26. **Frear, D. S. and Swanson, H. R.,** Metabolism of cisanilide (cis-2,5-dimethyl-1-pyrrolidine carboxanilide) by excised leaves and cell suspension cultures of carrot and cotton, *Pestic. Biochem. Physiol.*, 5, 73, 1975.
27. **Mine, A., Miyakado, M., and Matsunaka, S.,** The mechanism of bentazon selectivity, *Pestic. Biochem. Physiol.*, 5, 566, 1975.
28. **Gorbach, S. G., Kuenzler, K., and Asshauer, J.,** On the metabolism of HOE-23408 OH in wheat, *J. Agric. Food Chem.*, 25, 507, 1977.
29. **Shimabukuro, R. H., Walsh, W. C., and Hoerauf, R. A.,** Metabolism and selectivity of diclofop-methyl in wild oat and wheat, *J. Agric. Food Chem.*, 27, 615, 1979.
30. **Frear, D. S.,** Pesticide conjugates-glycosides, in *Bound and Conjugated Pesticide Residues*, Kaufman, D. D., Still, G. G., Paulson, G. D., and Bandal, S. K., Eds., ACS Symp. Ser. 29, American Chemical Society, Washington, D.C., 1976, 35.
31. **Frear, D. S., Swanson, H. R., and Tanaka, F. S.,** *N*-demethylation of substituted 3-(phenyl)-1-methylureas: Isolation and characterization of a microsomal mixed-function oxidase from cotton, *Phytochemistry*, 8, 2157, 1969.
32. **Shimabukuro, R. H., Kadunce, R. E., and Frear, D. S.,** Dealkylation of atrazine in mature pea plants, *J. Agric. Food Chem.*, 14, 392, 1966.
33. **Hodgson, R. H. and Hoffer, B. L.,** Diphenamid metabolism in pepper and an ozone effect. II. Herbicide metabolite characterization, *Weed Sci.*, 25, 331, 1977.
34. **Marquis, L. Y., Shimabukuro, R. H., Stolzenberg, G. E., Feil, V. J., and Zaylskie, R. G.,** Metabolism and selectivity of fluchloralin in soybean roots, *J. Agric. Food Chem.*, 27, 1148, 1979.
35. **Tanaka, F. S., Swanson, H. R., and Frear, D. S.,** An unstable hydroxymethyl intermediate formed in the metabolism of 3-(4-chlorophenyl)-1-methylurea in cotton, *Phytochemistry*, 11, 2701, 1972.
36. **Esser, H. O., Dupuis, G., Ebert, E., Marco, G., and Vogel, C.,** *s*-Triazines, in *Herbicides: Chemistry, Degradation and Mode of Action*, Vol. 1, Kearney, P. C. and Kaufman, D. D., Eds., Marcel Dekker, New York, 1975, 129.
37. **Chang, F. Y. and Vanden Born, W. H.,** Translocation and metabolism of dicamba in tartary buckwheat, *Weed Sci.*, 19, 107, 1971.
38. **Niki, Y., Kuwatsuka, S., and Yokomichi, I.,** Absorption, translocation and metabolism of chlomethoxynil (X-52) in plants, *Agric. Biol. Chem.*, 40, 683, 1976.
39. **Lamoureux, G. L. and Rusness, D. G.,** Malonylcysteine conjugates as end-products of glutathione conjugate metabolism in plants, in *IUPAC Pesticide Chemistry: Human Welfare and the Environment*, Miyamoto, J., Ed., Pergamon Press, New York, 1983, 295.
40. **Casida, J. E., Gray, R. A., and Tilles, H.,** Thiocarbamate sulfoxides: Potent, selective and biodegradable herbicides, *Science*, 184, 573, 1974.
41. **Lay, M-M., Hubbel, J. P., and Casida, J. E.,** Dichloroactamide antidotes for thiocarbamate herbicides: mode of action, *Science*, 189, 287, 1975.
42. **Eastin, E. F.,** Fate of fluoridifen in resistant peanut seedlings, *Weed Sci.*, 19, 261, 1971.
43. **Lamoureux, G. L. and Rusness, D. G.,** Catabolism of glutathione conjugates of pesticides in higher plants, in: *Sulfur in Pesticide Action and Metabolism*, Rosen, J. D., Magee, P. S. and Casica, J. E., Eds., ACS Symp. Ser. 158, American Chemical Society, Washington, D.C., 1981, 133.
44. **Rusness, D. G. and Lamoureux, G. L.,** Pentachloronitrobenzene metabolism in peanut. 2. Characterization of chloroform-soluble metabolites produced in vivo, *J. Agric. Food Chem.*, 28, 1070, 1980.
45. **Frear, D. S.,** The benzoic acid herbicides, in *Herbicides: Chemistry, Degradation, and Mode of Action*, Vol. 2, Kearney, P. C. and Kaufman, D. D., Eds., Marcel Dekker, New York, 1975, 541.
46. **Niki, Y. and Kuwatsuka, S.,** Degradation of diphenyl ether herbicides in soils, *Soil Sci. Plant Nutr.*, 22, 223, 1976.
47. **Shimabukuro, R. H., Lamoureux, G. L., Swanson, H. R., Walsh, W. C., Stafford, L. E., and Frear, D. S.,** Metabolism of substituted diphenylether herbicides in plants. II. Identification of a new fluorodifen metabolite, *S*-(2-nitro-4-trifluoromethyl)-glutathione in peanut, *Pestic. Biochem. Physiol.*, 3, 483, 1973.
48. **Frear, D. S. and Swanson, H. R.,** Metabolism of substituted diphenylether herbicides in plants. I. Enzymatic cleavage of fluorodifen in peas (*Pisum sativum* L.), *Pestic. Biochem. Physiol.*, 3, 473, 1973.
49. **Loos, M. A.,** Phenoxyalkanoic acids, in *Herbicides: Chemistry, Degradation and Mode of Action*, Vol. 1, Kearney, P. C. and Kaufman, D. D., Eds., Marcel Dekker, New York, 1975, 1.
50. **Jeffcoat, B. and Harries, W. N.,** Selectivity and mode of action of ethyl (±)-2-(*N*-3-benzoyl-3,4-dichloroanilino)propionate in the control of *Avena fatua* in cereals, *Pestic. Sci.*, 4, 891, 1973.

51. **Fedtke, C. and Schmidt, R. R.,** Chlorofenprop-methyl: Its hydrolysis *in vivo* and *in vitro* and a new principle for selective herbicidal action, *Weed Res.*, 17, 233, 1977.
52. **Beynon, K. I., Stoydin, G., and Wright, A. N.,** The breakdown of the triazine herbicide cyanazine in soils and maize, *Pestic. Sci.*, 3, 293, 1972.
53. **Buckland, J. L., Collins, R. F., and Pullin, E. M.,** Metabolism of bromoxynil octanoate in growing wheat, *Pestic. Sci.*, 4, 149, 1973.
54. **Dorough, W.,** Biological activity of pesticide conjugates, in *Bound and Conjugated Pesticide Residues*, Kaufman, D. D., Still, G. G., Paulson, G. D., and Bandal, S. K., Eds., ACS Symp. Ser. 29, American Chemical Society, Washington, D.C., 1976, 11.
55. **Mumma, R. O., Khalifa, S., and Hamilton, R. H.,** Spectroscopic identification of metabolites of carbaryl in plants, *J. Agric. Food Chem.*, 19, 445, 1971.
56. **Frear, D. S., Swanson, H. R., Mansager, E. R., and Wien, R. G.,** Chloramben metabolism in plants: Isolation and identification of glucose ester, *J. Agric. Food Chen.*, 26, 1347, 1978.
57. **Pengelly, W. L., Hall, P. J., Schulze, A., and Bandurski, R. S.,** Distribution of free and ester indole-3-acetic acid in the cortex and stele of the *Zea mays* mesocotyl, *Plant Physiol.*, 69, 1304, 1982.
58. **Still, G. G. and Mansager, E. R.,** Aryl hydroxylation of isopropyl-3-chloro-carbanilate by soybean plants, *Phytochemistry*, 11, 515, 1972.
59. **Still, G. G.,** Metabolism of 3,4-dichloropropionanilide in plants: the metabolic fate of the 3,4-dichloroaniline moiety, *Science*, 59, 992, 1968.
60. **Hodgson, R. H., Frear, D. S., Swanson, H. R., and Regan, L. A.,** Alteration of diphenamid metabolism in tomato by ozone, *Weed Sci.*, 21, 542, 1973.
61. **Shimabukuro, R. H., Walsh, W. C., Stolzenberg, G. E., and Olson, P. A.,** Metabolism of fluorodifen in peanuts, *Weed Sci. Soc. Amer., Abstr.*, 171, Weed Society of America, Champaign, Ill., 1975.
62. **Dutton, A. J., Roberts, T. R., and Wright, A. N.,** Characterization of acidic conjugates of flamprop in wheat, *Chemosphere*, 3, 195, 1976.
63. **Mumma, R. O. and Hamilton, R. H.,** Amino acid conjugates, in *Bound and Conjugated Pesticide Residues*, Kaufman, D. D., Still, G. G., Paulson, G. D. and Bandal, S. K., Eds., ACS Symp. Ser. 29, American Chemical Society, Washington, D.C., 1976, 68.
64. **Feung, C. S., Mumma, R. O., and Hamilton, R. H.,** Metabolism of 2,4-dichlorophenoxyacetic acid. VI. Biological properties of amino acid conjugates, *J. Agric. Food Chem.*, 22, 307, 1974.
65. **Davidonis, G. H., Hamilton, R. H., Vellejo, R. P., Buly, R., and Mumma, R. O.,** Biological properties of D-amino acid conjugates of 2,4-D, *Plant Physiol.*, 70, 357, 1982.
66. **Thomas, E. W., Loughman, B. C., and Powell, R. G.,** Metabolic fate of some chlorinated phenoxyacetic acids in the stem tissue of *Avena sativa*, *Nature (London)*, 204, 286, 1964.
67. **Zenk, M. H.,** 1-*O*-(indole-3-acetyl)-β-D-glucose, a new compound in the metabolism of indole-3-acetic acid in plants, *Nature (London)*, 191, 493, 1961.
68. **Hangarter, R. P. and Good, N. E.,** Evidence that IAA conjugates are slow-release sources of free IAA in plant tissues, *Plant Physiol.*, 68, 1424, 1981.
69. **Lamoureux, G. L., Shimabukuro, R. H., Swanson, H. R., and Frear, D. S.,** Metabolism of 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine) in excised sorghum leaf sections, *J. Agric. Food Chem.*, 18, 81, 1970.
70. **Boyland, E. and Chasseaud, L. F.,** The role of glutathione and glutathione *S*-transferases in mercapturic acid biosynthesis, *Adv. Enzymol.*, 32, 173, 1969.
71. **Lamoureux, G. L., Stafford, L. E., Shimabukuro, R. H., and Zaylski, R. G.,** Atrazine metabolism in sorghum: Catabolism of the glutathione conjugate of atrazine, *J. Agric. Food Chem.*, 21, 1020, 1973.
72. **Lamoureux, G. L., Stafford, L. E., and Tanaka, F. S.,** Metabolism of 2-chloro-*N*-isopropylacetanilide (Propachlor) in the leaves of corn, sorghum, surgarcane, and barley, *J. Agric. Food Chem.*, 19, 346, 1971.
73. **Leavitt, J. R. C. and Penner, D.,** In vitro conjugation of glutathione and other thiols with acetanilide herbicides and EPTC sulfoxide and the action of the herbicide antidote, R-25788, *J. Agric. Food Chem.*, 27, 533, 1979.
74. **Lamoureux, G. L., Stafford, L. E., and Shimabukuro, R. H.,** Conjugation of 2-chloro-4,6-bis(alkylamino)-*s*-triazine in higher plants, *J. Agric. Food Chem.*, 20, 1004, 1972.
75. **Guddewar, M. B. and Dauterman, W. C.,** Studies on a glutathione *S*-transferase preparation from mouse liver which conjugates chloro-*s*-triazine herbicides, *Pestic. Biochem. Physiol.*, 12, 1, 1979.
76. **Crayford, J. V. and Huston, D. H.,** The metabolism of the herbicide, 2-chloro-4-(ethylamino)-6-(1-cyano-1-methylethylamino)-*s*-triazine, in the rat, *Pestic. Biochem. Physiol.*, 2, 295, 1972.
77. **Lamoureux, G. L. and Davison, K. L.,** Mercapturic acid formation in the metabolism of propachlor, CDAA, and fluoridifen in the rat, *Pestic. Biochem. Physiol.*, 5, 497, 1975.
78. **Collett, G. F. and Pont, V.,** Le role de la cysteine dans la detoxification dun herbicide, *C.R. Acad. Sci. Paris Ser. D*, 286, 681, 1978.

79. **Still, G. G. and Rusness, D. G.,** *S*-cysteinyl-hydroxychlorpropham: Formation of the *S*-cysteinyl conjugate of isopropyl-3'-chloro-4'-hydroxycarbanilate in oat (*Avena sativa* L.), *Pestic. Biochem. Physiol.*, 7, 210, 1977.
80. **Rusness, D. G. and Still, G. G.,** Effect of isopropyl-3-chlorocarbanilate and isopropyl-3-chlorohydroxycarbanilate analogs upon oxidative phosphorylation in plant mitochondria, *Pestic. Biochem. Physiol.*, 4, 24, 1974.
81. **Shimabukuro, R. H.,** Atrazine metabolism and herbicidal selectivity, *Plant Physiol.*, 42, 1269, 1967.
82. **Shimabukuro, R. H. and Swanson, H. R.,** Atrazine metabolism, selectivity, and mode of action, *J. Agric. Food Chem.*, 17, 199, 1969.
83. **Shimabukuro, R. H., Walsh, W. C., Lamoureux, G. L., and Stafford, L. E.,** Atrazine metabolism in sorghum: chloroform-soluble intermediates in the *N*-dealkylation and glutathione conjugation pathways, *J. Agric. Food Chem.*, 21, 1031, 1973.
84. **Frear, D. S. and Swanson, H. R.,** Biosynthesis of *S*-(4-ethylamino-6-isopropyl amino-2-*s*-triazino) glutathione: Partial purification and properties of a glutathione *S*-transferase from corn, *Phytochemistry*, 9, 2123, 1970.
85. **Jensen, K. I. N., Stephenson, G. R., and Hunt, L. A.,** Detoxification of atrazine in three gramineae subfamilies, *Weed Sci.*, 25, 212, 1977.
86. **Jachetta, J. J. and Radosevich, S. R.,** Enhanced degradation of atrazine by corn (*Zea mays*), *Weed Sci.*, 29, 37, 1981.
87. **Still, G. G. and Herrett, R. A.,** Methylcarbamates, carbanilates, and acylanilides, in *Herbicides: Chemistry, Degradation and Mode of Action*, Vol. 2, Kearney, P. C. and Kaufman, D. D., Eds., Marcel Dekker, New York, 1975, 609.
88. **Davis, D. G., Hoerauf, R. A., Dusbabek, K. E., and Dougall, D. K.,** Isopropyl *m*-chlorocarbanilate and its hydroxylated metabolites: their effects on cell suspensions and cell division in soybean and carrot, *Physiol. Plant.*, 40, 15, 1977.
89. **Fang, S. C.,** Thiocarbamates, in *Herbicides: Chemistry, Degradation, and Mode of Action*, Vol. 1, Kearney, P. C. and Kaufmann, D. D., Eds., Marcel Dekker, New York, 1975, 323.
90. **Hubell, J. P. and Casida, J. E.,** Metabolic fate of the *N,N*-dialkylcarbamoyl moiety of thiocarbamate herbicides in rats and corn, *J. Agric. Food Chem.*, 25, 404, 1977.
91. **Pallos, F. M. and Casida, J. E., Eds.,** *Chemistry and Action of Herbicide Antidotes*, Academic Press, New York, 1978.
92. **Lay, M-M. and Casida, J. E.,** Involvement of glutathione and glutathione *S*-transferases in the action of dichloroacetamide antidotes for thiocarbamate herbicides, in *Chemistry and Action of Herbicide Antidotes*, Pallos, F. M. and Casida, J. E., Eds., Academic Press, New York, 1978, 151.
93. **Lay, M-M. and Casida, J. E.,** Dichloroacetamide antidotes enhance thiocarbamate sulfoxide detoxification by elevating corn root glutathione content and glutathione *S*-transferase activity, *Pestic. Biochem. Physiol.*, 6, 442, 1976.
94. **Carringer, R. D., Rieck, C. E., and Bush, L. P.,** Metabolism of EPTC in corn (*Zea mays*), *Weed Sci.*, 26, 157, 1978.
95. **Carringer, R. D., Rieck, C. E., and Bush, L. P.,** Effect of R-25788 on EPTC metabolism in corn (*Zea mays*), *Weed Sci.*, 26, 167, 1978.
96. **Ezra, G. and Gressel, J.,** Rapid effects of a thiocarbamate herbicide and its dichloroacetamide protectant on macromolecular synthesis and glutathione levels in maize cell cultures, *Pest. Biochem. Physiol.*, 17, 48, 1982.
97. **Stephenson, G. R., Bunce, N. J., Makowski, R. I., and Curry, J. C.,** Structure-activity relationships for *S*-ethyl *N,N*-dipropylthiocarbamate (EPTC) antidotes in corn, *J. Agric. Food Chem.*, 26, 137, 1978.
98. **Stephenson, G. R., Bunce, N. J., Makowski, R. I., Bergsma, M. D., and Curry, J. C.,** Structure-activity relationships for antidotes to thiocarbamate herbicides in corn, *J. Agric. Food Chem.*, 27, 543, 1979.
99. **Lay, M-M., Adams, C. A., and Casida, J. E.,** *N,N*-diallyl 2,2-dichloroacetamide elevates corn root glutathione *S*-transferase activity and sulfate utilization, *5th Intl. Congr. Pestic. Chem. (IUPAC)*, Kyoto, Abst. IVe-4, International Union of Pure and Applied Chemistry, Oxford, 1982.
100. **Calderbank, A. and Slade, P.,** Diquat and paraquat, in *Herbicide: Chemistry, Degradation and Mode of Action*, Vol. 2, Kearney, P. C. and Kaufman, D. D., Eds., Marcel Dekker, New York, 1975, 501.
101. **Harvey, B. M. R., Muldoon, J., and Harper, D. B.,** Mechanism of paraquat tolerance in perennial ryegrass. I. Uptake, metabolism and translocation of paraquat, *Plant Cell Environ.*, 1, 203, 1978.
102. **Harper, D. B. and Harvey, B. M. R.,** Mechanism of paraquat tolerance in perennial ryegrass. II. Role of superoxide dismutase, catalase and peroxidase, *Plant Cell Environ.*, 1, 211, 1978.
103. **Halliwell, B.,** The toxic effects of oxygen on plant tissues, in *Superoxide Dismutase*, Oberley, L. W., Ed., CRC Press, Boca Raton, Fla., 1982.

104. **Dodge, A. D.,** The role of light and oxygen in the action of photosynthetic inhibitor herbicides, in *Biochemical Responses Induced by Herbicides*, Moreland, D. E., St.John, J. B., and Hess, F. D., Eds., ACS Symp. Ser. 181, American Chemical Society, Washington, D.C., 1982, 57.
105. **Farrington, J. A., Ebert, M., Land, E. J., and Fletcher, K.,** Bipyridylium quartenary salts and related compounds. V. Pulse radiolysis studies of the reaction of paraquat radical with oxygen. Implications for the mode of bipyridyl herbicides, *Biochim. Biophys. Acta*, 314, 372, 1973.
106. **McCord, J. M. and Fridovich, I.,** Superoxide dismutase. An enzymic function for erythrocuprein (haemocuprein), *J. Biol. Chem.*, 244, 6049, 1969.
107. **Dodge, A. D.,** The mode of action of the bipyridylium herbicides, paraquat and diquat, *Endeavor*, 30, 130, 1971.
108. **Vaughn, K. C. and Duke, S. O.,** *In situ* localization of the sites of paraquat action, *Plant Cell Environ.*, 6, 13, 1983.
109. **Shimabukuro, M. A., Shimabukuro, R. H., Nord, W. S., and Hoerauf, R. A.,** Physiological effects of methyl 2-[4(2,4-dichlorophenoxy)phenoxy]propanoate on oat, wild oat, and wheat, *Pestic. Biochem. Physiol.*, 8, 199, 1978.
110. **Shimabukuro, M. A., Shimabukuro, R. H., and Walsh, W. C.,** The antagonism of IAA-induced hydrogen ion extrusion and coleptile growth by diclofop-methyl, *Physiol. Plant.*, 56, 444, 1982.
111. **Donald, W. W. and Shimabukuro, R. H.,** Selectivity of diclofop-methyl between wheat and wild oat: growth and herbicide metabolism, *Physiol. Plant.*, 49, 459, 1980.
112. **Pillmoor, J. B., Roberts, T. R., and Gaunt, J. K.,** Compartmentation and mobility of flamprop and its metabolites in leaf slices of oats, *J. Exp. Bot.*, 33, 919, 1982.
113. **Davidonis, G. H., Hamilton, R. H., and Mumma, R. O.,** Evidence for compartmentalization of conjugates of 2,4-dichlorophenoxyacetic acid in soybean callus tissue, *Plant Physiol.*, 70, 939, 1982.
114. **Jeffcoat, B. and Harries, W. H.,** Selectivity and mode of action of flamprop-isopropyl, isopropyl(±)-2-[*N*-(3-chloro-4-fluorophenyl)benzamido]propionate, in the control of *Avena fatua* in barley, *Pestic. Sci.*, 6, 283, 1975.
115. **Jeffcoat, B., Harries, W. N., and Thomas, D. B.,** Factors favoring the choice of flamprop-methyl, methyl(±-2-[*N*-(3-chloro-4-fluorophenyl)benzamido]-propionate, for the control of *Avena* species in wheat, *Pestic. Sci.*, 8, 1, 1977.
116. **Andreev, G. K. and Amrhein, N.,** Mechanism of action of the herbicide 2-chloro-3(4-chlorophenyl)propionate and its methyl ester: interaction with cell responses mediated by auxin, *Physiol. Plant.*, 37, 175, 1976.

Appendix

COMMON AND CHEMICAL NAMES OF HERBICIDES, INSECTICIDES, AND PLANT GROWTH REGULATORS USED IN TEXT

Acifluorfen	5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoic acid
Alachlor	2-chloro-2′,6′-diethyl-*N*-(methoxymethyl)acetanilide
Ametryn(e)	2-ethylamino-4-(isopropylamino)-6-(methylthio)-*s*-triazine
Amitrole	3-amino-*s*-triazole
Asulam	methyl sulfanilylcarbamate
Atraton	4-ethylamino-6-isopropylamino-2-methoxy-1,3,5-triazine
Atrazine	2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine
Barban	4-chloro-2-butynyl *m*-chlorocarbanilate
Bentazon	3-isopropyl-1*H*-2,1,3-benzothiadiazin-4(3*H*)-one2,2-dioxide
Benzadox	(benzamidooxy)acetic acid
Benzoylprop-ethyl	ethyl(±)-2-(*N*-benzoyl-3,4-dichloroanilino)propionate
Benzthiazuron	*N*-(benzothiazol-2-l)-*N*-methylurea
Bifenox	methyl 5-(2,4-dichlorophenoxy)-2-nitrobenzoate
Bromacil	5-bromo-3-*sec*-butyl-6-methyluracil
Bromofenoxim (C-9122)	3,5-dibromo-4-hydroxybenzaldehyde *O*-(2,4-dinitrophenyl)oxime
Bromo-nitrothymol	2-bromo-4- nitrothymol
Bromoxynil	3,5-dibromo-4-hydroxybenzonitrile
Bromoxysone	3,5(dibromo)-4-hydroxyphenylmethylsulfone
Butachlor	*N*-(butoxymethyl)-2-chloro-2′,6′-diethylacetanilide
Buthidazole	3-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-4-hydroxy-1-methyl-2-imidazolidinone
Buthiuron	*N*′-(5-butylsulfonyl1-1,3,4-thiadiazol-2-yl)-*N*,*N*′-dimethylurea
Buturon	*N*′-(4-chlorophenyl)-*N*-(1-methylprop-2-ynyl)-*N*-methylurea
Butylate	*S*-ethyl diisobutylthiocarbamate
Carbaryl	1-napthyl methylcarbamate
CDAA	*N*-*N*-diallyl-2-chloroacetamide
Chloramben	3-amino-2,5-dichlorobenzoic acid
Chloranocryl	*N*-(3,4-dichlorophenyl) methacrylamide(dicryl)
Chlorbromuron	3-(4-bromo-3-chlorophenyl)-1-methoxy-1-methylurea
Chlorbufan	1-methyl-2-propynyl(3-chlorophenyl)carbamate
Chlorfenprop-methyl	methyl 2-chloro-3-(4-chlorophenyl)propionate
Chloroxuron	3-[*p*-(*p*-chlorophenoxy)phenyl]-1,1-dimethylurea
Chlorpropham	isopropyl *m*-chlorocarbanilate
Chlorsulfuron	2-chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triaxin-2-yl)amino-carbonyl] benzenesulfonamide
Chlortoluron	*N*′,(3-chloro-4-methylphenyl)-*N*,*N*-dimethylurea
Cisanilide	*cis*-2,5-dimethyl-*N*-phenyl-1-pyrrolidinecarboximide
Clomethoxynil	2,4-dichlorophenyl-3′-methoxy-4′-nitrophenyl ether
Cyanazine	2-[[4-chloro-6-(ethylamino)-*s*-triazin-2- yl]amino]-2-methylpropionitrile
Cycluron	3-cyclooctyl-1,1-dimethylurea
Cyprazine	2-chloro-4-(cyclopropylamino)-6-(isopropylamino)-*s*-triazine
Cypromid	3,4-dichlorocyclopropanecarboxanilide

D_5	1-(*n*-pentyl)-1-(methyl)-3-(3,4-dichlorophenyl)urea
Dalapon	2,2-dichloropropionic acid
DCPTU	1-(methyl)-1-(1,2,3-thiadiazolyl)-3-(3,4-dichlorophenyl)urea
DDT	1,1,1-trichloro-2,2-bis (*p*-chlorophenyl)ethane
Defenuron	*N*-methyl-*N'*-phenylurea
Desmedipham	Defenuron *N*-methyl-*N'*-phenylurea ethyl *m*-hydroxycarbanilate carbanilate(ester)
Diallate	*S*-(2,3-dichloroallyl)diisopropylthiocarbamate
Dicamba	3,6-dichloro-*o*-anisic acid
Dichlobenil	2,6-dichlorobenzonitrile
Dichlone	2,3-dichloro-1,4-naphthoquinone
Dichlormate	3,4-dichlorobenzyl methylcarbamate
Diclofop-methyl	methyl 2-[4-(2,4-dichlorophenoxy)phenoxy]propanoate
Diclomid (R-25788)	*N,N*-diallyl-2,2-dichloroacetamide
Dieldrin	1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-endo-exo-1,4:5,8-dimethanonaphthalene
Difenzoquat	1,2-dimethyl-3,5-diphenyl-1*H*-pyrazolium
Difunon(e) (EMD-IT 5914)	5-(dimethyl-aminomethylene)-2-oxo-4-phenyl-2,5-dihydrofurane-carbonitrile-(3)
Dimefuron	4-[2-chloro-4-(3,3-dimethylureido)phenyl]-2-*t*-butyl-1,3,4-oxadiazolin-5-one
Dinitramine	N^4,N^4-diethyl-α,α,α-trifluoro-3,5-dinitrotoluene-2,4-diamine
Dinoben	2,5-dichloro-3-nitrobenzoic acid
Dinoseb (DNBP)	2-*sec*-butyl-4,6-dinitrophenol
Dinoterb	4,6-dinitro-2-*t*-butylphenol
Diphenamid	*N,N*-dimethyl-2,2-diphenylacetamide
Diquat	6,7-dihydrodipyrido[1,2-α:2',1'-*c*]pyrazinediium ion
Diuron (DCMU)	3-(3,4-dichlorophenyl)-1,1-dimethylurea
DNOC	4,6-dinitro-*o*-cresol
DSMA	disodium methanearsonate
DTP	1,3-dimethyl-4-(2,4-dichlorobenzoyl)-5-hydroxypyrazole
Endothall	7-oxabicyclo[2,2,1]heptane-2,3-dicarboxylic acid
EPTC	*S*-ethyl dipropylthiocarbamate
Ethidimuron	*N'*-(5-ethylsulfonyl-1,3,4-thiadiazol-2-yl)-*N,N'*dimethylurea
Ethofumesate	(±)-2-ethoxy-2,3-dihydro-3,3-dimethyl-5-benzofuranyl methanesulfonate
Fenuron	1,1-dimethyl-3-phenylurea
Flamprop-methyl	methyl *N*-benzoyl-*N*-(3-chloro-4-fluorophenyl)-2-aminopropionate
Flamprop-isopropyl	isopropyl(±)-2-[*N*-(3-chloro-4-fluorophenyl)benzamido] propionate
Fluchloralin	*N*-(2-chloroethyl)-2,6-dinitro-*N*-propyl-4-(trifluoromethyl)-aniline
Fluometuron	1,1-dimethyl-3-(α,α,α-trifluoro-*m*-tolyl)urea
Fluorodifen	*p*-nitrophenyl α,α,α-trifluoro-2-nitro-*p*-tolyl ether
Fluridone	1-methyl-3-phenyl-5-[3- (trifluoromethyl)phenyl]-4(1*H*)-pyridinone
Glyphosate	*N*-(phosphonomethyl)glycine
Glyphosine	*N,N*-bis(phosphonomethyl)glycine
Haloxydine	3,5-dichloro-2,6-difluoro-4-hydroxypyridine
Ioxynil	4-hydroxy-3,5-diiodobenzonitrile
Ioxysone	3,5(diiodo)-4-hydroxyphenylmethylsulfone
Isocarbamid	*N*-isobutylimidazolidin-2-one-1-carboxamide
Isocil	5-bromo-3-isopropyl-6-methyluracil

Isomethiozin	4-(isobutylideneamino)-3-methylthio-6-*t*-butyl-1,2,4-triazin-5-one
Isoproturon	*N'*-(4-isopropylphenyl)-*N,N*-dimethylurea
J739	2,4-dimethyl-6-phenylmethoxy-pyrimidin
J852	2-isopropylamino-4-methyl-6-isobutyloxy-pyrimidin
Karbutilate	*tert*-butylcarbamic acid ester with 3-(*m*-hydroxyphenyl)-1,1-dimethylurea
Karsil	*N*-(3,4-dichlorophenyl)-2-methylpentanamide
Lenacil	3-cyclohexyl-6,7-dihydro-1*H*-cyclopentapyrimidine-2,4 (3*H*,5*H*)-dione
Lindane	1α,2α,3β,4α,5α,6β-hexachlorocyclohexane
Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea
MBPU	1-(α-methylbenzyl)-3-(3,4-dichlorophenyl)urea
MCPA	[(4-chloro-*o*-tolyl)oxy]acetic acid
MCPB	4-[(4-chloro-*o*-tolyl)oxy]butyric acid
MDMP	2-(4-methyl-2,6-dinitroanilino)-*N*-methyl propionamide
Mefluidide	*N*-[2,4-dimethyl-5-[[trifluoromethyl)sulfonyl]amino]phenyl]-acetamide
Metamitron	4-amino-3-methyl-6-phenyl-1,2,4-triazin-5(4*H*)-one
Metflurazon(e) (San 6706)	4-chloro-5-(dimethylamino)-2-(α,α,α,-trifluoro-*m*-tolyl)-3 (2*H*)-pyridazinone
Methabenzthiazuron	*N*-(benzothiazol-2-yl)-*N,N'*-dimethylurea
Methazole	2-(3,4-dichlorophenyl)-4-methyl-1,2,4-oxadiazolidine-3,5-dione
Metobromuron	*N'*-(4-bromophenyl)-*N*-methoxy-*N*-methylurea
Metolachlor	2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide
Metoxuron	*N'*-(3-chloro-4-methoxyphenyl)-*N,N*-dimethylurea
Metribuzin	4-amino-6-*tert*-butyl-3-(methylthio)-*as*-triazin-5(4*H*)-one
MH	1,2-dihydro-3,6-pyridazinedione
Molinate	*S*-ethyl hexahydro-1*H*-azepine-1-carbothioate
Monolinuron	3-(*p*-chlorophenyl)-1-methoxy-1-methylurea
Monuron	3-(*p*-chlorophenyl)-1,1-dimethylurea
Morfamquat	1',1'-bis-(3,5-dimethylmorpholinocarbonylmethyl)4,4'-bipyridylium ion
MSMA	monosodium methanearsonate
Napropamide	2-(α-naphthoxy)-*N,N*-diethylpropionamide
Naptalam (NPA)	*N*-1-naphthylphthalamic acid
Neburon	1-butyl-3-(3,4-dichlorophenyl)-1-methylurea
Nitralin	4-(methylsulfonyl)-2,6-dinitro-*N,N*-dipropylaniline
Nitrofen	2,4-dichlorophenyl *p*-nitrophenyl ether
Norea (Noruron)	3-(hexahydro-4,7-methanoindan-5-yl)-1,1-dimethylurea
Norflurazon (San 9789)	4-chloro-5-(methylamino)-2-(α,α,α-trifluoro-*m*-tolyl)-3 (2*H*)-pyridazinone
Oryzalin	3,5-dinitro-N^4,N^4-dipropylsulfanilamide
Oxyfluorfen	2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)-benzene
Paraquat	1,1'-dimethyl-4,4'-bipyridinium ion
PCNB	pentachloronitrobenzene
PCP	pentachlorophenol
Pebulate	*S*-propyl butylethylthiocarbamate
Pentanochlor	*N*-(3-chloro-4-methylphenyl)-2-methylpentanamide
Perfluidone	1,1,1-trifluoro-*N*-[2-methyl-4-(phenylsulfonyl)phenyl]-methanesulfonamide
Phenisopham	3-isopropoxycarbonylaminophenyl *N*-ethyl-*N*-phenylcarbamate
Phenmedipham	methyl *m*-hydroxycarbanilate *m*-methylcarbanilate

Picloram	4-amino-3,5,6-trichloropicolinic acid
Prometon	2,4-bis(isopropylamino)-6-methoxy-*s*-triazine
Prometryn	2,4-bis(isopropylamino)-6-(methylthio)-*s*-triazine
Pronamide	3,5-dichloro(*N*-1,1-dimethyl-2-propynyl)benzamide
Propachlor	2-chloro-*N*-isopropylacetanilide
Propanil	3′,4′-dichloropropionanilide
Propazine	2-chloro-,6-bis(isopropylamino)-*s*-triazine
Propham	isopropyl carbanilate
Pyrazon (pyramin)	5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone
Pyriclor	2,3,5-trichloro-4-pyridinol
San 9785 (BAS 13338)	4-chloro-5-(dimethylamino)-2-phenyl-3(2*H*)-pyridazinone
Sethoxydim	2-[1-(ethoxyimino)butyl]-5-[2-(ethylthio)1-(ethoxyimino)propyl]-3-hydroxy-2-cy clohexen-1-one
Siduron	1-(2-methylcyclohexyl)-3-phenylurea
Simazine	2-chloro-4,6-bis(ethylamino)-*s*-triazine
Simeton	*N*,*N*′-diethyl-6-methoxy-1,3-5-triazine-2,4-diamine
Simetryn	4,6-bisethylamino-2-methylthio-1,3,5,-triazine
Sulfometuron	2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]benzoic acid
Swep	methyl *N*-(3,4-dichlorophenyl)carbamate
TCA	trichloroacetic acid
Tebuthiuron	*N*-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-y]]-*N*,*N*′-dimethylurea
Terbacil	3-*tert*-butyl-5-chloro-6-methyluracil
Terbutryn	2-(*tert*-butylamino)-4-(ethylamino-6-(methylthio)-*s*-triazine
Tetrafluron	*N*,*N*-dimethyl-*N*′-[3-(1,1,2,2-tetrafluoroethoxy)phenyl]urea
Thiochlormethyl	3-2-chloro-3-fluorodichlormethylthio-1,1-dimethylurea
Trifluralin	α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine
UKJ72J	2-ethylamino,4-amino,5-thiomethyl,6-chloropyrimidine
Vernolate	*S*-propyl dipropylthiocarbamate
2,4-D	(2,4-dichlorophenoxy)acetic acid
2,4-DB	4-(2,4-dichlorophenoxy)butyric acid
2,4,5-T	(2,4,5-trichlorophenoxy)acetic acid

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