STUDIES OF HERBICIDE PHOTOLOYYSIS IN AQUEOUS MEDIA AND ON CORN LEAVES

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Rama Rao Venkatesh, M. S.

The Ohio State University
1996

Dissertation Committee:

Dr. S. Kent Harrison, Adviser
Dr. Paul Henderlong
Dr. Mark Loux
Dr. Mark Bennett

Approved by

S. Kent Harrison
Adviser
Agronomy Graduate Program
Dedicated to my parents
ACKNOWLEDGMENT

I would like to express my deepest appreciation to my adviser, Dr. S. K. Harrison. He has been a constant source of encouragement and provided valuable insights throughout the course of my research. He has guided me not only during my work on the dissertation, but also through my entire graduate program. His trust and confidence in my abilities meant a lot to me, both personally and professionally. I personally acknowledge the financial support provided by him for my graduate program.

I wish to thank Drs. Paul Henderlong, Mark Loux, and Mark Bennett for having served on my dissertation committee and also for their constructive criticisms and helpful comments about this study.

I would like to thank faculty, staff, and graduate students of the Weed Science Group at The Ohio State University for their support and friendship. I particularly recognize Christopher Lences, Harold Brown, and Todd Lucas for their help. I wish to acknowledge the statistical advise provided by J. M. Harrison, University of Florida.

Finally, to my parents who deserve the most credit. Without their support and encouragement, I never would have been in graduate school in the first place. Thanks to my family and friends for their support and trust in me. I thank Rekha for her patience, understanding, and willingness to endure my idiosyncratic behavior as a graduate student.
VITA

1982 ......................... B.Sc. (Agriculture)
University of Agricultural Sciences
Bangalore, India

1985 ......................... M.Sc. (Agronomy)
University of Agricultural Sciences
Bangalore, India

1985-89 ...................... Research Associate
University of Agricultural Sciences
Bangalore, India

1989-96 ...................... Graduate Research Associate
Department of Agronomy
The Ohio State University

PUBLICATION

Research Publication


FIELDS OF STUDY

Major Field: Agronomy
TABLE OF CONTENTS

Dedication.............................................................. ii
Acknowledgments ....................................................... iii
Vita................................................................. iv
List of Tables........................................................ vii
List of Figures ........................................................ x

Chapters:

1. Literature Review .................................................. 1

2. Photolysis of Aqueous Chlorimuron and Imazaquin in the Presence of Phenolic Acids and Riboflavin
   Abstract ......................................................... 39
   Introduction ....................................................... 41
   Materials and Methods ........................................... 44
   Results and Discussion ......................................... 53

3. Riboflavin Sensitized Photolysis of 2,4-D in Aqueous Media
   Abstract ......................................................... 61
   Introduction ....................................................... 63
   Materials and Methods ........................................... 66
   Results and Discussion ......................................... 74
4. Photolytic Degradation of 2,4-D on the Corn Leaf Surface

Abstract ................................................................. 94
Introduction ............................................................. 96
Materials and Methods ............................................... 101
Results and Discussion ................................................ 108

5. Summary .............................................................. 130

List of References ..................................................... 133
LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1. Photolysis of aqueous chlorimuron and imazaquin alone or in the presence of acetone, riboflavin, or each of three phenolic acids. Data shown are half-lives ( (t_{1/2}) ), first-order rate constants ( (k_p) ), and coefficients of determination ( (r^2) ) for the linear regression of log percent herbicide remaining on exposure time</td>
<td>54</td>
</tr>
<tr>
<td>Table 2.2. Photolytic interaction of riboflavin with chlorimuron and imazaquin. Data shown are half-lives ( (t_{1/2}) ), first-order rate constants ( (k_p) ), and coefficients of determination ( (r^2) ) for the linear regression of log percent herbicide and riboflavin remaining on exposure time, and quantum yields ( (\phi) ) of direct and indirect ( (i) ) photolysis</td>
<td>57</td>
</tr>
<tr>
<td>Table 3.1. Effects of light regime, riboflavin concentration, and pH on 2,4-D photolysis in aqueous solution. Data shown are half-lives ( (t_{1/2}) ), first-order rate constants ( (k_p) ), and coefficients of determination ( (r^2) ) for the linear regression of log percent herbicide remaining on exposure time</td>
<td>77</td>
</tr>
<tr>
<td>Table 3.2. Photolysis of lumichrome and lumiflavin in the presence and absence of 2,4-D in aqueous solution. Data shown are half-lives ( (t_{1/2}) ), first-order rate constants ( (k_p) ), and coefficients of determination ( (r^2) ) for the linear regression of log percent lumichrome or lumiflavin remaining on exposure time</td>
<td>90</td>
</tr>
</tbody>
</table>
Table 3.3. Photolysis of riboflavin in the presence and absence of 2,4-D in aqueous solution. Data shown are half-lives ($t_{1/2}$), first-order rate constants ($k_p$), and coefficients of determination ($r^2$) for the linear regression of log percent riboflavin remaining on exposure time ........................................... 92

Table 4.1. Effects of light regime, sensitizer, and harvest interval on the total recovery of $^{14}$C from corn ... 110

Table 4.2. Effects of light regime and harvest interval on the total recovery of $^3$H from corn ...................... 113

Table 4.3. Effects of light regime, sensitizer, and harvest interval on surface residues and distribution of $^{14}$C-2,4-D applied to corn leaves ......................... 115

Table 4.4. Effects of light regime, sensitizer, and harvest interval on surface residues and distribution of $^{14}$C applied to glass ................................. 118

Table 4.5. Effects of light regime and harvest interval on surface residues and distribution of $^3$H applied to corn leaves ....................... 120

Table 4.6. Effects of light regime and harvest interval on surface residues and distribution of $^3$H applied to glass ................................. 122

Table 4.7. Effects of light regime, sensitizer, and harvest interval on the absorption of $^{14}$C applied to corn ................................. 123

Table 4.8. Effects of light regime, sensitizer, and harvest interval on the distribution of $^{14}$C within the treated leaf of corn ......................... 125

Table 4.9. Effects of light regime and harvest interval on the absorption of $^3$H applied to corn leaves ................................. 127

Table 4.10. Effects of light regime and harvest interval on the distribution of $^3$H within the treated leaf of corn ................................. 128
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1. A schematic representation of the fate of an excited molecule</td>
<td>7</td>
</tr>
<tr>
<td>Figure 2.1. Structures and chemical names of chlorimuron and imazaquin</td>
<td>45</td>
</tr>
<tr>
<td>Figure 2.2. Structures and chemical names of sensitizers used in this study</td>
<td>46</td>
</tr>
<tr>
<td>Figure 2.3. Irradiance spectrum of the UV source (A), and UV-visible absorption spectra of the compounds investigated in this study (B, C, and D)</td>
<td>47</td>
</tr>
<tr>
<td>Figure 2.4. Aqueous riboflavin photolysis and photoproduct (lumichrome) formation in the presence of chlorimuron (A), imazaquin (B), and in pure aqueous solution (C). Data points shown are the means of six replications ± SE</td>
<td>58</td>
</tr>
<tr>
<td>Figure 3.1. Structures and chemical names of 2,4-D and trifluralin</td>
<td>67</td>
</tr>
<tr>
<td>Figure 3.2. Structures and chemical names of sensitizers used in this study</td>
<td>68</td>
</tr>
<tr>
<td>Figure 3.3. Irradiance spectrum of the UV source (A), and UV-visible absorption spectra of the compounds investigated in this study (B, C, D, E, and F)</td>
<td>75</td>
</tr>
<tr>
<td>Figure 3.4. Effects of light regime and pH on lumichrome formation from riboflavin photolysis. Data points shown are means of six replications ± SE. Data points for which error bars are missing have low SE</td>
<td>81</td>
</tr>
</tbody>
</table>
Figure 3.5. Effects of light regime and riboflavin concentration on 2,4-dichlorophenol formation. Data points shown are means of six replications ± SE. Data points for which error bars are missing have low SE .................................83

Figure 3.6. Effects of light regime and pH on 2,4-dichlorophenol formation. Data points shown are means of six replications ± SE. Data points for which error bars are missing have low SE ........84

Figure 3.7. Effects of light regime and lumiflavin on 2,4-dichlorophenol formation. Data points shown are means of six replications ± SE. Data points for which error bars are missing have low SE .......85

Figure 4.1. Irradiance spectrum of the UV source (A) and UV-visible absorption spectrum of the compounds investigated in this study (B and C)..... 109
CHAPTER 1

LITERATURE REVIEW

In recent years there has been great public concern regarding environmental pollution by herbicides. Possible sources of pollution include off-target application of herbicides directly to water bodies, runoff or leaching from the site of application, and volatilization from plant, water, and soil surfaces. Herbicides may also exist in the atmosphere as particulates adsorbed to wind-borne soil particles (Cessna and Muir, 1991).

Herbicides applied to soil or plant surfaces may be subjected to several transfer and degradation processes (Weber et al., 1973). Transfer processes such as adsorption, leaching, and volatilization result only in a change in the physical environment or movement of the active ingredient, whereas degradation processes result in the breaking of chemical bonds and a change in the composition of the herbicide molecule. These degradation processes include biodegradation, chemical decomposition, and photodecomposition. Biodegradation is an enzymatically regulated metabolism/detoxification carried out by soil organisms and higher plants. Chemical decomposition occurs as a result of abiotic chemical reactions in soil or water that result in herbicide degradation, e.g. acid-catalyzed hydrolysis. Photodecomposition is due
to the direct or indirect absorption of energy generated by sunlight, particularly ultraviolet radiation. In order to undergo photodecomposition, a herbicide must directly absorb sunlight of sufficient energy to break covalent bonds (direct photolysis) or remain in close proximity with another compound which can absorb light and transfer the bond-breaking energy to the pollutant (indirect photolysis). The review of literature presented here is focussed mainly on the photodecomposition of herbicides by ultraviolet (UV) light.

Theoretical aspects of Photochemistry. The science of photochemistry is defined as the study of chemical changes which are brought about by light interacting with matter (Roberts et al., 1984). In conventional studies of photochemistry, the photochemical reactions are initiated by monochromatic or polychromatic light and the wavelength ranges from 200 to 700 nm. In environmental photochemistry, the source of radiation of greatest interest is natural sunlight and/or artificially generated light in the wavelength region of 290 to 800 nm. Recently there has been increasing emphasis on studies in environmental photochemistry because both natural and anthropogenic chemicals present in natural waters may undergo a series of photochemical reactions after absorption of sunlight (Leifer, 1988). Environmental photochemistry often involves determining rate constants and half-lives of pollutants in natural water bodies exposed to sunlight. An understanding of the fundamental laws and processes of the photochemistry is essential to understand the kinetics of photodecomposition of a chemical in the environment.

The Nature of light. Light is electromagnetic in nature and consists of a discrete packets of energy called quanta. A quantum of electromagnetic radiation is also referred to as a photon and represents a discrete unit
of radiation (Roberts et al., 1984). The energy (E) present in each photon at a given wavelength is calculated using the formula:

$$E = \frac{hc}{\lambda}$$

(1)

where $h$ is Planck's constant ($9.534 \times 10^{-14}$ kcal s mol$^{-1}$), $c$ is the speed of light ($3 \times 10^{17}$ nm s$^{-1}$), and $\lambda$ is the wavelength in nanometers (Kagan, 1993). From the above relationship, it is clear that as the wavelength decreases, the energy of a single photon increases.

The electromagnetic spectrum is divided into several regions. The human eye is sensitive to electromagnetic radiation in the wavelength range of 400 to 800 nm and the range referred to as visible light. Electromagnetic radiation in the 800 to 6000 nm is referred to as infrared and is invisible to the human eye. The ultraviolet (UV) region is just below the visible spectrum and can be divided into four wavebands: vacuum UV (<200 nm), UV-C (200-280 nm), UV-B (280-315 nm), and UV-A (315-400 nm). Wavelengths below 290 nm generally do not reach the earth's surface due to their absorption by oxygen, ozone, and other gases present in the earth's atmosphere (Tevini, 1993). Ultraviolet radiation (297-400) comprises nine percent of the total energy of sunlight reaching the earth's surface. Total solar UV irradiance decreased from 1980 through mid-1985, but remained without change until mid-1987. Of late there has been an increase in the total solar UV irradiance (Lean, 1989). From the environmental photochemistry point of view, the spectral range of interest is from 290 to 800 nm and in the environment this wavelength is composed of both UV and visible light. The corresponding energy in this wavelength range (290 to 800 nm) ranges
from 98.6 to 35.8 kcal mol$^{-1}$ or 415 to 150 kJ mol$^{-1}$ (Leifer, 1988). Energy within this range is sufficient to break many types of chemical bonds. Wavelengths above 800 nm do not possess sufficient energy to break the chemical bonds and are of no interest from the environmental photochemistry point of view.

**Light absorption and energy states of molecules.** Most molecules consist of unlike atoms. Atoms have a massive, positively charged nucleus surrounded by a cloud of negatively charged electrons arranged in orbits. Nuclei consist of neutrons and positively charged protons.

Photochemical reactions occur in response to the absorption of light by a molecule. This results in the breaking and sometimes reforming of chemical bonds. The photochemistry of most molecules is regulated by the specific group or chromophore that is responsible for the absorption of light (Roberts et al., 1984). Chromophores refer to the specific arrangements of atoms leading to absorption of photons at specific wavelengths within the emission spectrum of the light source (Kagan, 1993). A given absorption band of a compound is directly related to the nature of the chromophore group.

Light absorption by a molecule can initiate various intramolecular processes. The absorbed energy can contribute to vibrational, rotational, or electronic excitation in the molecule. Molecules in their vibrational and rotational excited states can lose their excitation energy and reach the ground state faster than the rate of a chemical reaction. In environmental photochemistry, the focus is on electronic excitation because it is associated with the transition of electrons.
from their normal low-energy orbitals to orbitals of higher energy and
the excitation energy thus acquired by the molecule is of such magnitude
that it will be sufficient to break chemical bonds.

A given electron in a molecular orbital can be denoted by an arrow
pointing either up (↑) or down (↓) indicating the two possible spin
states. According to the Pauli Exclusion Principle, no more than two
electrons can occupy a given molecular orbital at the same time
(Horspool and Armesto, 1992; Kagan, 1993). As a result, two electrons
that occupy a molecular orbital must have opposite spins (↑↓). The non-
excited state of a molecule is generally when the highest occupied
molecular orbital (the one farthest from the nucleus) is in the (↑↑)
configuration, referred to as the singlet ground state (two electrons
with opposite spins in the same orbital, paired, and antiparallel),
represented by the symbol S₀. Generally the lowest energy state of a
molecule is referred to as the ground state (state of minimum electronic
energy) while the more energetic states are called excited states, where
one of the electrons is promoted to the next higher orbital upon
absorption of light energy.

The absorption of light results in excitation of electrons. When
photochemical excitation occurs, one of the two electrons in the highest
occupied molecular orbital is promoted into the next higher orbital and
usually its spin is conserved; thus the molecule will have two electrons
with opposite spins but in different orbitals. This state of the
molecule is called the singlet excited state (two electrons with
opposite spins in different orbitals, paired, and antiparallel (↑↓)) and
represented by the symbol S₁. When the excited electron in the highest
occupied molecular orbital undergoes spin inversion so that its spin is in the same direction as its former pair member, then the molecule is referred to as being in the excited triplet state represented by the symbol $T_1$ [two electrons with the same spin in different orbitals, parallel, and not paired (II) described later in more detail]. An excited state molecule is high in energy, and will have a different electron distribution and physical geometry than its ground state counterpart. Therefore, a molecule in its excited state may involve itself in numerous photophysical and photochemical reactions compared to the ground state molecule. The average life-time of the excited singlet state is $10^{-9}$ to $10^{-5}$ s.

**Fate of excited molecules.** The possible fates of an excited singlet state molecule are presented in the Jablonski diagram (Figure 1.1). A Jablonski diagram is a schematic representation of the energy levels and photophysical processes which can occur in the excited molecule (Cowan and Drisko, 1976). The electronic states are represented by the symbols $S_0$, $S_1$, and $S_2$ which correspond to ground state, first, and second excited states, respectively. The first and second excited triplet states are represented by $T_1$ and $T_2$, respectively. The excited molecule ($S_1$ and $T_1$) can undergo several photophysical processes, some resulting in photochemical reactions, which result in deactivation of the excited state. Both the excited singlet and triplet states (explained later) possess energy in the range of 40 to 200 kcal mol$^{-1}$ and are therefore quite labile. Photophysical processes result in physical changes in the
Figure 1.1. A schematic representation of the fate of an excited molecule (Kagan, 1993).
molecule, including change in electron density distribution, molecular geometry etc., (Roberts et al., 1984). Photophysical processes are divided into two types: non-radiative and radiative decay processes.

Non-radiative Processes. Nonradiative processes are those that do not involve emission of light. There are two types of nonradiative transition: intersystem crossing and internal conversion.

Intersystem crossing. Intersystem crossing occurs when an excited molecule goes from singlet excited state \((S_1)\) to its triplet excited state \((T_1)\). The energy transfer from \(S_1\) to \(T_1\) can occur by a process of electronic-, vibrational-, rotational-, and translational- energy transfer (Turro, 1967). While of somewhat lower energy, the life-time of the excited triplet state is 20,000 times longer than the life-time of the singlet state because spin inversion must take place for the triplet state to return to the singlet ground state, and this transition is forbidden and therefore has only a low probability of occurring (Kagan, 1993). The probability of singlet-triplet intersystem crossing is much higher compared to the reverse reaction. The average life-time of the excited triplet state is \(10^{-5}\) to \(10^{-3}\) s.

Excited triplet state molecules may return to the ground state by losing energy as heat or light, by degrading, or by transferring excitation energy to another molecule by a process known as photosensitization (Herkstroeter, 1964). Most sensitized photochemical reactions involve excited triplet state sensitizers. During the process of photosensitization an excited triplet state molecule (donor) will transfer its excitation energy to a molecule in the ground state (acceptor or quencher), which thereby becomes electronically excited.
The entire process of photosensitization is a bimolecular interaction and is more likely to occur with a long-lived electronically excited energy donor which exists in triplet state rather than a short-lived donor in singlet state. Intersystem crossing is the main source for the production of excited triplets and photosensitization usually refers to triplet energy transfer from a sensitizer to an acceptor unless specified otherwise (Turro, 1967).

**Internal conversion.** Internal conversion occurs between molecules which are in the same state of multiplicity. The transition will thus be between singlet to singlet (S\textsubscript{1} to S\textsubscript{0}), triplet to triplet (T\textsubscript{2} to T\textsubscript{1}), but not singlet to triplet (S\textsubscript{1} to T\textsubscript{1}). During this process energy could be lost in the form of heat and the excited molecule will return to the ground state.

**Radiative Processes.** In contrast to nonradiative processes, radiative processes result in emission of light. Radiative processes can be divided into two types: phosphorescence and fluorescence.

**Phosphorescence.** is defined as the emission of light between states of the different multiplicity. During this process, a triplet state (T\textsubscript{1}) molecule will return to the singlet ground state (S\textsubscript{0}). Phosphorescence results in a spin change as the excited electron returns to complete the ground state electron pair and the molecule will lose energy in the form of light. The light emitted due to phosphorescence has a longer wavelength compared to initial incident light and also light emitted due to fluorescence. This is because the energy difference between the triplet state and the singlet ground state is small. As explained earlier there is an inverse relationship between the wavelength and the
energy of light. Phosphorescence occurs only when a molecule is in its triplet state. The life-time of phosphorescence varies from $10^{-5}$ to $10^{-3}$ s and is much longer-lived compared to fluorescence life-time.

**Fluorescence** is defined as the emission of light between states of the same multiplicity. During this process the singlet excited state ($S_1$) molecule will return to the singlet ground state ($S_0$) with emission of light. Since the excited electron already has a spin opposite to its former co-occupant of the ground state pair, fluorescence will not result in spin change. As a result, the life-time of fluorescence varies from $10^{-9}$ to $10^{-3}$ s and its decay (and thus fluorescence) is short-lived compared to the triplet state ($T_1$) and phosphorescence.

In summary, the electronically excited molecule may undergo radiative or non-radiative decay processes or participate in photochemical reactions. Photochemical reactions are unique in character compared to other chemical reactions because they can be initiated upon absorption of either monochromatic or polychromatic light which excites atoms or molecules without exciting the surrounding medium. The structure of ground state and excited state atoms or molecules are generally different and these states exhibit different chemical behavior (Leifer, 1988). Major photochemical pathways include direct and indirect photolysis, photooxidation, photoreduction, photohydrolysis, and rearrangements (Miller and Herbert, 1986). It is the photochemical pathways that are of greatest interest in environmental photochemistry, since they are ultimately responsible for photodegradation of xenobiotics in natural environments. Of those pathways, indirect photolysis is of special interest since it may significantly enhance
degradation of xenobiotics which are not capable of undergoing direct photolysis.

Laws of Photochemistry. The first law of photochemistry states that a substance must first absorb radiation before a photochemical change in the molecule can occur (Grotthus-Draper Law). The second law of photochemistry (Stark-Einstein Law) states that one photon or quantum of light absorbed can activate only one molecule in the first step of a reaction sequence. Photochemical changes may be in the form of homolysis to free radicals, intermolecular rearrangements, and electron transfer reactions, etc. The third law of photochemistry is the Beer-Lambert Law. The Beer-Lambert Law states in essence that the fraction of incident radiation absorbed is proportional to the number of absorbing molecules in its path (Salisbury and Ross, 1992). The Beer-Lambert law can also be stated mathematically as:

$$A = \varepsilon cl$$  \hspace{1cm} (2)

where $A$=absorbance, $\varepsilon$=the extinction coefficient, $c$= concentration of the solute, and $l$=cell pathlength in centimeters (cell refers to special quartz cell). The extinction coefficient is often termed as the molar extinction coefficient when the concentration of the solute is expressed as mol L$^{-1}$. The extinction coefficient is dependent on the characteristic of the absorbing molecule, the solvent in which the molecule is dissolved, and the wavelength of light (Salisbury and Ross, 1992). The Grotthus-Draper law combined with the Beer-Lambert law provides fundamental information about the nature of light absorption by a chemical whereas the Stark-Einstein law may be used to quantify the efficiency with which absorbed light transforms the chemical into products of a photoreaction (Leifer, 1988).
Quantum Yields. The ratio of the number of molecules undergoing photochemical reaction to the total number of photons absorbed by the medium is termed quantum yield. Quantum yield is thus an indicator of the efficiency of conversion of absorbed light energy into photochemical reactions that transform the absorbing molecule. A quantum yield of 1 indicates a 100% efficient conversion of absorbed light energy to photochemical reactions. For many organic molecules in dilute concentration in aqueous media, quantum yield is less than 0.01 indicating that the vast majority of absorbed energy is not utilized for photochemical reactions (Draper, 1985). The number of quanta absorbed by a given medium can be measured by an actinometer, which is a reaction system for which the quantum yield is known (Kan, 1966). Quantum yield can be defined for processes which are either photophysical (phosphorescence, fluorescence, or intersystem crossing) or photochemical (direct or indirect photolysis).

Photochemical processes can be divided into two general categories: direct and indirect photolysis.

Direct Photolysis. Substances that undergo direct photolysis absorb UV radiation directly and undergo transformation. A requirement for direct photolysis is that the UV absorption spectrum of the compound overlap with the UV emission spectrum of the radiation source. The various wavelength ranges at which absorption takes place are determined from the absorption spectra, which are plots of the wavelength against the amount of radiation absorbed on passing through a substance (Kan, 1966). The emission spectrum is the electromagnetic spectrum of the radiation source. The role of direct photolysis in environmental photochemistry is often less than that of indirect photolysis because natural waters
contain dissolved organic compounds, free radicals, and other short-lived intermediates which can act as sensitizers in the process of indirect photolysis (Cessna and Muir, 1991), described below. Direct photolysis cannot occur for some synthetic pesticides because they do not absorb UV wavelengths greater than 290 nm; 2,4-D [(2,4-dichlorophenoxy)acetic acid)] is one such compound.

Indirect Photolysis. The basic mechanism by which pesticides undergo indirect photolysis is via a process called sensitization. For sensitization to occur a donor compound (i.e., sensitizer) in the medium must absorb radiation energy directly and then transfer excitation energy to an acceptor molecule (e.g., a pesticide). The acceptor will then undergo photolysis as if it had acquired the radiation energy directly. This overall process is known as photosensitization. The light-dependent reactions of photosynthesis are an example of indirect photoreaction following the direct absorption of quanta by chlorophyll.

The most important feature of sensitized reactions is that incident light from the light source has to be in the wavelength range absorbed by the sensitizer. The absorption spectrum of the acceptor need not match the emission spectrum of the light source. There are a number of mechanisms for the transfer of (electronic) energy from the sensitizer to an acceptor molecule. The principal mechanisms are radiative and non-radiative transfer. Non-radiative transfer includes short-range (collision) and long-range (vibrational) energy transfer (Choudhry et al., 1979). Indirect photolysis is dependent upon the wavelength of the radiation, the reaction media, the UV absorption characteristics of the substrate and the photosensitizer, and the excitation energy relationship between the sensitizer and substrate (Davidson, 1979).
The entire reaction of indirect photolysis starts with the absorption of light ($hv$) by the sensitizer ($S$) shown below in reaction (3). After absorption of light the sensitizer reaches an excited singlet state which may convert to an excited triplet state via intersystem crossing. The next step involves energy transfer or transfer of electrons or hydrogen atoms from the excited sensitizer molecule to the acceptor molecule ($A$) either directly [reaction-(4)] or via a second system component ($X$) which then reacts with the acceptor [reaction (5) and (6)].

$$S + hv = S'$$  \hspace{1cm} (3)

$$S' + A = S + A'$$ \hspace{1cm} (4)

$$S + X = S + X'$$ \hspace{1cm} (5)

$$X' + A = A' + X$$ \hspace{1cm} (6)

The first excited triplet state ($T_1$) of the sensitizer must be energetically higher than that of the acceptor molecule for energy transfer to occur. A sensitizer will be more effective in inducing indirect photolysis if the energy difference between the singlet excited state ($S_1$) and the first excited triplet state ($T_1$) is small (Choudhry et al., 1979). In addition, the photostability of the sensitizer should be such that it remains intact (i.e., does not photodegrade) during the process of light absorption and energy transfer. After transferring the energy to the acceptor molecule, the photosensitizer can return to its ground state and participate in additional photoreactions. Some sensitizers are relatively unstable after absorption of energy and may
be converted into a new compound having a low-lying triplet state. These new compounds may act as quenchers and stop the indirect photolysis reaction completely (Choudhry et al., 1979; Roof 1982).

Effective photosensitizers absorb energy at longer wavelengths than the acceptor, have a high efficiency of intersystem crossing, and energy transfer to the acceptor. The process of intersystem crossing varies with each substance and its environment, and to a large extent depends on the life-time of the excited singlet state. The longer the life-time of the excited singlet state, the higher the probability of intersystem crossing and consequently more triplets will be produced (Kan, 1966). The efficiency of energy transfer is dependent upon the degree of similarity between the positions and intensities of the bands in the emission spectrum of the donor and absorption spectrum of the acceptor; the greater the similarity the higher the percentage of the emitted radiation being re-absorbed (Choudhry et al., 1979). The efficiency of energy transfer in a particular system is also dependent on the concentration of the energy-accepting molecule (acceptor or quencher), the rate constant for the quenching reaction, and the life-time of the excited state of the sensitizer in the absence of acceptor or quencher. In general, triplet states are much longer-lived than singlet states and so they are quenched readily. Molecular oxygen is a very effective triplet state quencher (Horspool and Armesto, 1992).

Photolysis of phenoxyalkanoic acids. One of the outcomes of modern weed control technology has been the introduction of numerous organic herbicides. The introduction of 2,4-D [(2,4-dichlorophenoxy)acetic acid] and other related phenoxy herbicides as selective weed killers at the
end of World War II revolutionized weed management (Loos, 1975; McEwen and Stephenson, 1979). It has been estimated that more than half of the herbicides used in crops for controlling weeds are applied to corn and that 90% of all corn planted is treated with at least one herbicide (Pimentel and Levitan, 1986). The application of phenoxy herbicides accounts for 20% of the total organic herbicides used in North America (McEwen and Stephenson, 1979).

The members of the phenoxyalkanoic acid herbicides contain a phenoxy ring with a chlorine atom attached at the 4th position of the benzene ring and an aliphatic acid group attached to the number 1 position via an ether linkage. Some of the aliphatic acids showing high herbicidal activity are acetic, butyric, and propionic acid. One of the important members of the phenoxyalkanoic herbicide family is 2,4-D. It is the single most widely used phenoxy herbicide and is used for controlling weeds in small grains, corn, grain sorghum, and turf. The other uses of 2,4-D include controlling woody plants in forest management systems, noncropland sites and for aquatic weed control (Ross and Lembí, 1985). Phenoxy herbicides are more active on weeds when applied to the foliage rather than to the soil. They are typically formulated either as aliphatic esters or amine salts. These formulations undergo transformation to form 2,4-D acid before entering the symplast of living cells (McEwen and Stephenson, 1979). Phenoxy acids dissipate rapidly in water due to chemical hydrolysis, photodegradation, and biodegradation (Muir, 1991).

**Direct photolysis of 2,4-D.** Studies on direct photolysis of 2,4-D have involved different formulations of 2,4-D and several types of light sources. Aly and Faust (1964) exposed 0.26mM solutions of the sodium
salt, isopropyl, and butyl esters of 2,4-D in distilled water to a
mercury discharge lamp. The lamp emitted light of short UV wavelengths
with primary spectral emission at 254 nm. The major product of
photodecomposition was 2,4-dichlorophenol and its formation was pH-
dependent. The rate of photolysis was faster at pH 9 than pH 7 or 4.

Crosby and Tutass (1966) exposed 440 mg L\(^{-1}\) of 2,4-D in water to
artificial light (254 nm) and sunlight. They observed the following
decomposition products in sequence: 2,4-dichlorophenol, chlorocatechol,
chlorophenoxyacetic acid, benezenetriol, and finally polymeric humic
acids. The photoproducts observed under artificial light were similar to
those produced upon exposure to sunlight. When aqueous 2,4,5-
trichlorophenoxyacetic acid (2,4,5-T) was exposed to sunlight and UV
light in the wavelength region of 300-450 nm, the sequence of
decomposition products was: trichlorophenol, dichlorophenoxyacetic acid,
dichlororesorcinol, chlororesorcinol, dichlorophenol, and a dark
polymeric product (Crosby and Wong, 1973). There was an eleven-fold
increase in the rate of 2,4,5-T photolysis when acetone and riboflavin
were included in the solution.

A study was conducted by Zepp et al., (1975) to identify the
products of 2,4-D ester photolysis in hexane and in water following
exposure to a 450-W medium-pressure mercury vapor lamp. The
photoproducts in the hexane reaction medium were monochlorophenoxyacetic
acid esters in hexane, where as chlorohydroxyphenoxyacetic acid esters and
2,4-dichlorophenol were formed in water. The minimum sunlight
photolysis half-life of the butoxyethyl ester was 12 d in water.

Most of the earlier studies conducted on direct 2,4-D photolysis
involved the use of short wavelength, high energy UV radiation (<300
nm). The rate of 2,4-D photolysis and the formation of photoproducts was generally much slower under sunlight than when exposed to artificial light sources emitting light below <300 nm. The first major product of direct photolysis in aqueous media appears to be chlorinated phenols. These factors coupled with the initial reports of acetone and riboflavin sensitized 2,4-D photolysis first suggested that indirect photolysis is an important pathway of 2,4-D degradation in the environment.

**Indirect Photolysis of 2,4-D.** Hansen and Buchholtz (1952) first reported that 2,4-D was inactivated in presence of riboflavin and light as measured by corn root bioassay. Subsequent work has shown that riboflavin serves as a potent photosensitizer of 2,4-D upon exposure to light. In addition to riboflavin, there are some surfactants which apparently sensitized 2,4-D photolysis. The presence of anionic and nonionic surfactants increased the rate of 2,4-D photolysis in water (Hautala, 1978). In another study by Hee et al., (1979), exposed a commercial formulation containing mixed butyl esters of 2,4-D to 300 and 350 nm light in aqueous and hexane solutions. The major photoproduct was (p-chlorophenoxy)acetic esters in both media. The formation of this photoproduct in aqueous solution was attributed to presence of surfactant micelles resulting from surfactants contained in the formulation. Harrison and Wax (1986) looked at the effects of additives on the photodegradation of several herbicides in aqueous solution. The addition of an emulsifier mixture, petroleum oil concentrate (POC), or soybean oil concentrate (SOC) increased the rate of 2,4-D photodegradation due to sensitization when exposed to UV light.

From these studies, it is clear that surfactants can increase the rate of 2,4-D photolysis in addition to other sensitizers such as
riboflavin and acetone. The exact mechanisms (is it a sensitization or solubilization effect?) are not clearly understood. Surfactants promote herbicide photolysis in aqueous solution by acting as a sensitizer as well as increasing the solubility of the herbicide. For surfactants to promote herbicide photolysis in aqueous solution, the herbicide must meet the following requirements: the herbicide must have low water solubility, chloro substituent(s) on the aromatic ring of their structure, and triplet energies of the herbicide should be lower than that of the added surfactant (Tanaka et al., 1981). In aqueous photolysis, when these conditions are not met the addition of a surfactant will either have no effect or might suppress herbicide photolysis. A surfactant will act as a sensitizer only when it is capable of absorbing UV radiation on its own and transfer the energy to the herbicide. If the triplet energy status of the surfactant is less than that of the pesticide, the surfactant may protect the pesticide from undergoing photolysis (Tanaka, 1989). Additives like petroleum oil concentrate (POC) and soybean oil concentrate (SOC) increased the photodegradation rate of bentazon while oxysorbic, an alkyl-substituted surfactant, did not increase bentazon photolysis (Harrison and Wax, 1986). The authors attributed the enhanced photodegradation of bentazon to photosensitization reactions initiated by the aryl component of the additives. They further suggested that oxysorbic may have served as a protectant of bentazon in the presence of UV light.

Surfactants increase the solubility of some herbicides in aqueous solution. The increased solubilization of the herbicide is due to the formation of micelles, in which the herbicide is partitioned into the hydrophobic interior of surfactant micelles where some photonucleophilic
reaons involving the medium are more favorable than in the surrounding aqueous phase (Tanaka et al., 1981). Solubilization effects have been attributed to the lower bond dissociation energy of carbon-hydrogen (C-H) bonds within the hydrocarbon interior of micelles as opposed to the higher bond dissociation energy of covalent oxygen-hydrogen (O-H) bonds in the surrounding aqueous environment (Calvert and Pitts, 1966). The presence of a sensitizer along with a surfactant in a solution might hasten the rate of photolysis because of both sensitization and solubilization effect (Harrison and Wax, 1986). Harrison and Wax reported that the rate of 2,4-D photolysis was enhanced in aqueous solution in presence of acetophenone (sensitizer) + oxysorbic (nonionic surfactant) compared to treatments which had oxysorbic alone.

The pKa value of 2,4-D is 2.87 (Cessna and Grover, 1978). Normally the pH of natural waters is greater than 5, so 2,4-D exists in anionic form in natural aquatic systems. The absorption maximum of the anionic form is at 290 nm (Cessna and Muir, 1991). The chances of 2,4-D acid undergoing hydrolysis, direct photolysis, or biolysis is lower than compared to ester, amine, or salt formulations (McEwen and Stephenson, 1979). These characters make 2,4-D a prime candidate for indirect photolysis in aqueous solution.

**Fate of 2,4-D on leaf surfaces.** The most important plant organs involved in the interception of postemergent herbicide sprays are the leaves. The first part of the leaf that comes in contact with the herbicide spray droplet is the cuticle. The cuticle is the outermost and primary barrier for the uptake of foliar-applied agricultural chemicals.

**Structure of the cuticle.** The plant cuticle serves as the interface between plant and the environment. The cuticle not only provides
protection from pathogens and prevents water loss from the tissue, but also acts as a barrier to the entry of foliar-applied chemicals. The cuticle is a non-living lipophilic membrane which is noncellular in nature. It covers trichomes, pore walls of stomata, guard, and epidermal cells lining the substomatal chamber (Norris and Bukovac, 1968). The structure and composition of the cuticle varies among species and also within the same species (Holloway, 1982).

The principle structural component of the cuticle is the cutin matrix which is a highly cross-linked polymer of hydroxylated fatty acids (Kolattukudy, 1981). The cuticle is covered by an outermost layer of wax called epicuticular wax, which may be amorphous, semicrystalline, or crystalline (Eglinton and Hamilton, 1967; Baker and Parsons, 1971; Baker, 1982). The wax is made up of a variety of long-chain, even-numbered-(C_{22}-C_{24}) primary alcohols, acetates, aldehydes, and fatty acids, and their hydroxy-and oxy derivatives, and odd-numbered-carbon-(C_{17}-C_{35}) hydrocarbons, secondary alcohols, ketones, ketols, and β-diketols (Baker, 1982). Another major component of the cutin is the hexadecanoic acid and its positional isomers (Baker et al., 1982).

Physicochemical properties like the pKa of a herbicide and pH of the spray solution may also influence the process of herbicide penetration through the plant cuticle. The pKa of a herbicide influences the degree of ionization of the compound and in turn its polarity and subsequent penetration through the cuticle. The nonionized species moves more readily through the cuticle compared to the ionized species due to its lower polarity, and therefore its greater solubility in the nonpolar wax and cuticular components. The degree of ionization also depends on
the pH of the spray solution. Most agricultural chemicals are applied using water as a carrier. The pKa of 2,4-D is 2.54 and normally the pH of the tank mix ranges from pH 5 to 8, thus 2,4-D in contact with the cuticle in a spray droplet is typically in its ionized form. Consequently, 2,4-D in its ionized form resists cuticular penetration and can remain in the cuticular layer. In addition, hexadecanoic acid—a constituent of the cutin polymer can form covalent bonding with 2,4-D. In Rubber (Ficus elastica L.) leaf cuticles, the 2,4-D binding rate was a function of leaf age and was directly proportional to the amount of hexadecanoic acid present in the cutin polymer (Schonherr and Riederer, 1989).

From the ecotoxicological point of view, herbicide adsorption or bonding in the cuticle are very important as they may occur whether the plant is dead or alive. Most of crop plants contain epoxy alkanolic acids (hexadecanoic acid) and there is a potential for covalent bonding with some herbicides to occur. This bonding results in the formation of so called "bound" residues. These bound residues can be released from the cutin by fungi and microorganisms which produce cutinases that break bonds between the cutin polymer and xenobiotics. Under temperate conditions, natural and agricultural plant communities will have 180 to 1,500 kg of cuticular material in the living parts of plants growing on one hectare (Riederer and Schonherr, 1984). The amount of xenobiotics present in the plant material in some fields can exceed that held by sorption in the cuticle (Holloway and Deas, 1973).

Corn and other monocots avoid 2,4-D injury by limiting its translocation and by metabolic deactivation (Slife et al., 1962). The possible detoxification reactions include conjugation, oxidation,
hydroxylation, and binding to the lignin component of the cell wall (Hutber et al., 1978; Cohen and Bandurski, 1982; Reinecke and Bandurski, 1987). In addition, a considerable amount of 2,4-D applied to grasses may remain unabsorbed and accumulate in the plant cuticle. About 30% of the 2,4-D applied to corn remained on the leaf surface 72 h after treatment (Chkanikov et al., 1971)

Photolysis of 2,4-D on leaf surfaces. There are no reports of 2,4-D photolysis on leaf surfaces. A few of the leaf surface studies conducted with other agricultural chemicals have been presented here as evidence that photolysis of pesticides on the leaf surface can occur. Matsuo and Casida (1970) studied the photodegradation of dinobuton (a dinitrophenol) and dinoseb (an herbicidal ester of dinitrophenol) on snapbean leaves. The two chemicals were applied to the upper surface of the primary leaf of snapbean seedlings. After application of the chemicals, the plants were exposed to sunlight for three consecutive days-6 h on the first day, 10 h on the second day, and 4 h on the third day. In between sunlight exposures, the plants were stored in darkness for about 14 h or less. The amount of dinobuton recovered from the surface of the treated leaf irrespective of the time of exposure varied from 71 to 73% of the total \(^{14}\text{C}\)-dinobuton applied. Interestingly, some of the dinobuton (an acaricide) was photolyzed into dinoseb (a common herbicide) on the leaf surface. Dinobuton was present only in the surface wash and was not detected inside the treated leaf because the leaf extract was subjected to hydrolysis which degrades dinobuton. The dinoseb produced by dinobuton photolysis was found in approximately equal amounts on the leaf surface and in the penetrated fraction. A
fraction of dinoseb also underwent photolysis and was converted into more persistent and polar compounds.

Ivie and Casida (1971) reported increased degradation of chlorinated cyclodienes and other chemicals on bean leaves upon exposure to sunlight in the presence of rotenone as a sensitizer. Rotenone at a concentration of 10 mg L⁻¹ converted half of the applied dieldrin to photodieldrin on bean leaves, compared to 5% in the absence of rotenone. In the same experiment spinach chloroplasts were effective as sensitizers and increased the rate of N-methylcarbamate photolysis in aqueous solution. The reason for the increased degradation by spinach chloroplasts was attributed to the presence of chlorophyll. Based on these results there exists a possibility of chlorophylls and other natural plant products acting as sensitizers in the photodecomposition of pesticides. Research has not been conducted thus far to determine and explain the role of natural photosensitizers in the photolysis of herbicides on leaf surfaces.

Radiolabeled dinobuton was applied to bean leaves at a concentration of 2.5-3 μg cm⁻² and exposed to sunlight in the presence of rotenone—a known sensitizer (Bandal and Casida, 1972). Rotenone at a concentration of 6 μg cm⁻² accelerated the photolysis of dinobuton on bean leaves. After 4 h of exposure to direct sunlight in presence of rotenone, the leaf wash from the treated leaf contained more photoproducts of dinobuton and less parent compound. Under greenhouse conditions, in the absence of rotenone the rate of dinobuton photolysis was slow.

In another study, Liang and Lichtenstein (1976) investigated photodecomposition of the insecticide azinphosmethyl on soils, leaf
surfaces, and on glass as a model surface. They exposed $^{14}$C-azinphosmethyl on the various surfaces to sunlight for a period of 8 h. The amount of noninsecticidal and water soluble photoproduct recovered was 18%, 3%, and 1% of the applied parent compound on glass, corn, and bean leaves, respectively. Another interesting observation was that the granular formulation of azinphosmethyl was not susceptible to photodecomposition when exposed on any of these surfaces but readily underwent photolysis in aqueous solution. Photolysis of the granular formulation in aqueous solution was attributed to removal of the protective "coating" from the insecticide active ingredient making it more accessible to light.

Status of research on photolysis of herbicides and their residues on leaf surfaces. There are very few published studies on the photolysis of herbicides on leaf surfaces. In some instances, it may be difficult to distinguish photolysis from other transfer or transformation pathways. It is also possible that some pathways, for example plant and microbial metabolism, produce the same end products (Bandal and Casida, 1972; Ivie et al., 1973; Draper and Casida, 1983; Johnsen and Martin, 1983). The presence of natural sensitizers on the leaf surface, whether produced by the plant or from elsewhere, may also influence the process of photodegradation. Model surfaces have been used in an attempt to control these inherent variabilities present on leaf surface and also for ease in isolation and identification of photoproducts.

One of the most common model surface used in herbicide photolytic studies has been silica gel thin-layer chromatography plates (Nilles and Zabik, 1974; Nilles and Zabik, 1975; Pliammer, 1978; Parochetti and Dec, 1978; Herrmann et al., 1985). In those studies the herbicide was
typically applied to a thin-layer chromatography plate by first dissolving it in a solvent like acetone, hexane, etc. After the solvent evaporated the herbicide formed a thin film on the surface and was then exposed to a light source or sunlight. Data from such studies were often extrapolated to estimate the herbicides likelihood of undergoing photolysis on soil or the waxy surface of a leaf (Cessna and Muir, 1991).

There are several disadvantages associated with using model surfaces for studying herbicide photolysis. The rate of herbicide photodegradation and the photoproducts formed in the process can be strongly influenced by the nature of the medium in which the reaction occurs. Therefore, herbicide photolysis reactions on model surfaces may likely not be identical to that occurring on a leaf surface. In addition and particularly with regard to silica gel thin-layer chromatography plates, the film thickness of the droplet deposit may be greater than that of the same droplet applied to a leaf resulting in the photolysis of the upper region of the film while the lower regions of the herbicide remains intact. The physical interaction (i.e. adsorption) between the herbicide molecule and the surrounding medium can also result in a shift or change in the herbicide's light absorption properties, thereby altering the energy required to bring about photodecomposition (Nicholls and Leermakers, 1971; Plimmer, 1972; Gab et al., 1975; Plimmer, 1978). These changes in the pattern of absorption wavelength might therefore differ between model surfaces and leaves. An additional area where research is lacking is the fate of herbicide photoproducts on intact leaves. Distinguishing absorbed photoproducts from biochemical metabolites inside the plant and determining whether plants further
translocate and metabolize absorbed photoproducts is an intriguing, yet neglected area of research in herbicide-plant interactions. Few studies conducted on the photolysis of herbicides on leaf surfaces thus far have not considered the fate of the photoproducts in terms of absorption and translocation in the plant following photolysis on the leaf surface.

Most of the studies on photodecomposition of herbicides have been conducted with the herbicide dissolved in aqueous solution. The studies investigated photolysis of herbicides in distilled water, in natural waters, in the vapor phase, or as thin films on model surfaces in an attempt to mimic the environmental conditions where they may exist. Under natural conditions, the amount of sunlight received in a particular place varies greatly with season, time of day, latitude, thickness of the atmosphere, and the ozone layer, altitude, and cloud cover (Larson and Berenbaum, 1988). These variations in turn will affect the photolytic half-lives of herbicides and other compounds. To control variability, many photolysis studies are conducted under controlled conditions with selected lamps which more or less stimulate sunlight's UV spectrum. The light source most often used in early photochemical studies was a high intensity monochromatic lamp with a strong emission at either 254 or 313 nm which did not resemble the sunlight UV spectrum. The method of exposing chemicals to monochromatic light at 313 nm was followed for the sake of convenience of calculating quantum yields, as most of the molecules studied in early environmental photochemistry absorb light at 313 nm. The light source, the medium in which the herbicide is exposed, and the type of sensitizer used plays a major role in influencing the rate of photolysis and the type of photoproduct formed. Light sources emitting radiation below 290 nm and the use of
sensitizers like benzophenone (Glass, 1975) and organic dyes (Acher and Dunkelblum, 1979; Acher and Saltzman, 1980; Acher et al., 1981; Rejto et al., 1983) results in the formation of photoproducts which do not form when the same compounds are exposed to sunlight in the environment.

Photoproducts of 2,4-D. One of the major products of 2,4-D photolysis in aqueous solution is 2,4-dichlorophenol (2,4-DCP), formed by cleavage of the ether bond of the phenoxy moiety (Crosby and Tutass, 1966). The 2,4-DCP formed in aqueous media does not undergo further photodegradation. The physicochemical properties of 2,4-DCP indicate that it is sparingly soluble in water, so when formed in aqueous media it may separate into a different phase or become adsorbed to suspended particulates or sediments (Isensee, 1991). Studies have indicated that 2,4-DCP bioaccumulates in the liver and kidney of the test animals like sheep, cattle, and is a known carcinogen (Somani and Khalique, 1982). However, the acute oral LD$_{50}$ of 2,4-DCP in mice is 1134 mg kg$^{-1}$ compared to 521 mg kg$^{-1}$ for 2,4-D, so it is half as acutely toxic to mice as 2,4-D (Chkanikov et al., 1976). 2,4-DCP does not undergo direct photolysis in the environment since its UV cutoff for absorption is 295 nm (Cessna and Muir, 1991).

Photoproducts of Riboflavin. Riboflavin, also known as vitamin B$_2$, has a characteristic isoalloxazine ring system which undergoes reduction and oxidation via a ribityl side chain in its structure. The isoalloxazine is also capable of undergoing three different types of photoreactions including photoreduction, photodegradation, and photosensitization. The major photoreaction for riboflavin appears to be photodegradation.
Photoreduction and photosensitization were considered as minor side reactions (Moore and Baylor, 1969). Photoproducts of riboflavin depend on conditions such as the pH of the medium and the presence of oxygen during exposure to the light source (Fife, 1977). In aqueous solution under alkaline condition, the principal photoproduct of riboflavin was lumiflavin whereas as in acidic and neutral condition the major photoproduct was lumichrome.

**General properties of chlorimuron.** Chlorimuron (ethyl 2-(((4-chloro-6-methoxy-2-pyrimidinyl)amino)carbonyl)amino)sulfonyl)benzoate) belongs to sulfonylurea group of herbicides. It is applied as a postemergent herbicide in soybeans and effectively controls many of the problematic weeds in soybeans including common cocklebur (Xanthium strumarium L.), morningglory (Ipomoea sp.), and sicklepod (Cassia obtusifolia L.). Possible dissipation pathways for chlorimuron in the environment include chemical hydrolysis, microbial degradation, photolysis, and volatilization. Photolysis and volatilization play relatively minor roles in chlorimuron dissipation in soil compared to chemical hydrolysis and microbial breakdown (Beyer et al., 1989).

Chlorimuron is a weak acid (pKa=4.2) and pH greatly affects its water solubility. As pH increases, chlorimuron’s water solubility increases because of corresponding increase in the polar anionic form. The rate of hydrolysis of chlorimuron in aqueous media is a function of pH and temperature. Chlorimuron exists in anionic form at neutral and basic pH, and its rate of hydrolysis is relatively slow. Under acidic conditions, the half-life of chlorimuron at 45°C and pH 5 was 0.6 d whereas at pH 8 it was 18 d (Harvey et al., 1985; Friedman et al., 1987). The rate of hydrolysis of chlorimuron increased under highly
alkaline conditions (pH>10) because of a base-catalyzed hydrolysis (Dulka et al., 1985).

**Chlorimuron photolysis.** A number of studies have investigated chlorimuron photolysis under artificial and natural conditions. Direct photolysis of chlorimuron was studied under laboratory conditions in different media like methanol, distilled water, natural creek-water, on the surface of silica gel, and montmorillonite clay by exposing to a mercury high pressure lamp with wavelengths below 290 nm filtered by borosilicate glass filters (Herrmann et al., 1985). The photolytic half-life of chlorimuron ranged from 18 to 92 h in these different media with the shortest half-life of 18 h for natural-creek water and longest half-life of 92 h for methanol. On silica gel and montmorillonite, the half-lives ranged from 60 to 66 h. The increased rate of chlorimuron degradation in natural creek-water was attributed to the presence of photosensitizing agents like humic substances or generation of hydroxy (OH⁻) radicals or the presence of reactive species like alkylperoxy (RO₂⁻) radicals which are capable of inducing indirect photolysis. There was no direct photolysis of chlorimuron in methanol and pure water because chlorimuron can not absorb wavelengths greater than 290 nm. In the absence of indirect photolysis, other types of abiotic degradation processes will contribute for the disappearance of chlorimuron from the environment.

In aqueous photolysis studies, chlorimuron was irradiated for 15 d by continuous exposure to artificially generated light. Chlorimuron had a half-life of 31 to 43 d at pH 9 under the artificial light source. In soil photolysis studies, chlorimuron was sprayed on a thin layer of soil spread on glass plates and exposed to sunlight. The half-life of
chlorimuron on the soil thin layer plates exposed to sunlight was 20 d compared to an average of 44 d for the dark controls (Beyer et al., 1989).

The rate of chlorimuron photolysis in aqueous media and on glass slides was enhanced in the presence of nonionic surfactants like octoxynol and oxysorbic upon exposure to sunlamps with a primary spectral emission between 300 to 320 nm and 360 to 380 nm (Harrison and Thomas, 1990). Chlorimuron photolysis on glass was complete in the presence of either surfactant after 72 h exposure, whereas an average of only 27% loss occurred for corresponding treatments irradiated in water. This was attributed to the reduction in far UV (300 to 320 nm) transmittance by the glass vials in which the aqueous chlorimuron solution was exposed and/or due to lack of oxygen in the system. Octoxynol enhanced the rate of chlorimuron photolysis in aqueous solution to a greater extent than oxysorbic, by a combination of solubilization and sensitization effects. The sensitization effect of octoxynol was due to the presence of an aryl moiety in its structure which absorbs UV light and is capable of transferring excitation energy to chlorimuron. On the contrary, oxysorbic contains no double bonds and can not absorb UV light. Consequently oxysorbic can not act as a photosensitizer. As a result, the authors concluded that oxysorbic promotes photolysis of chlorimuron in aqueous solution primarily via its solubilization effect.

Two nonionic surfactants altered photolysis rates of chlorimuron in aqueous solutions and on glass slides exposed to sunlight (Thomas and Harrison, 1990). In aqueous solution, chlorimuron half-lives were 5, 3, and 2 d with no surfactant, oxysorbic, and octoxynol, respectively. The
half-life of chlorimuron on glass slides varied from 9 to 12 d and was not greatly altered by the presence of surfactants. Greater enhancement of herbicide photolysis in water by octoxynol over that observed with oxysorbic was attributed to solubilization as well as sensitization effect. Another interesting observation in this study was that, chlorimuron in solutions containing riboflavin had a half-life of 1 d compared to 6 d for the control with no riboflavin. Addition of oxysorbic or octoxynol to the chlorimuron plus riboflavin solutions did not alter the rate of chlorimuron photolysis. On glass slides, riboflavin also increased the rate of chlorimuron photolysis, but addition of oxysorbic to this mixture actually increased chlorimuron's photolysis half-life beyond that of the control with chlorimuron alone. The authors concluded that the protective effect of oxysorbic against riboflavin-sensitized chlorimuron photolysis may have been due to riboflavin and/or chlorimuron-sensitized photolysis of oxysorbic, thus allowing chlorimuron to avoid photolytic decomposition.

In conclusion, chlorimuron is capable of undergoing both direct and indirect photolysis, but the rate of photolysis was greatly enhanced in presence of sensitizers.

**General properties of imazaquin.** Imazaquin (2-{4,5-dihydro-4methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl}-3-quinolinecarboxylic acid) belongs to imidazolinone group of herbicides. Several imidazolinone herbicides give excellent weed control at relatively low rates of application (>0.2 kg ha⁻¹). Imazaquin is used for pre- or postemergence control of several broadleaf weeds in soybeans. Excessive persistence in soil and development of resistance in weeds are problems associated with
imidazolinone herbicides (Zimdahl, 1993). Imazaquin has high water solubility, low vapor pressure, and is not subject to losses by volatilization. Imazaquin undergoes slow hydrolytic degradation in aquatic environment, but is degraded in soil primarily by microorganisms (Mangels, 1991a).

Imazaquin photolysis. Basham and Lavy (1987) studied the photolytic dissipation of imazaquin in five soils. The soils had varied amounts of clay and organic matter and were treated with ring labeled $^{14}$C-imazaquin. Photolytic studies of imazaquin on glass slides coated with soil indicated losses up to 56% of the total $^{14}$C-imazaquin applied after continuous exposure to UV light for 168 h. There was significant loss of imazaquin from plain glass and slides coated with soil on exposure to sunlight. They concluded that photodecomposition could be a major mode of dissipation of imazaquin from the soil surface in the field. In another study, McKinnon and Weber (1988) investigated photolysis of several imidazolinone herbicides including imazaquin, imazapyr, imazethapyr, imazamethabenz-methyl, and AC 263,222. The herbicides were exposed in aqueous solution to a oriel ARC lamp for various time intervals up to 16 h. There was degradation of imidazolinones. Bioassay with cotton confirmed that the bioactivity of imidazolinones decreased with exposure to UV light. Studies were conducted on the aqueous photolysis of $^{14}$C-carboxyl-labeled imazaquin in distilled water buffered at pH 5, 7, and 9 (Mangels, 1991a). The light source used was a borosilicate-filtered xenon arc lamp. The rate of degradation of imazaquin followed first-order kinetics and the half-lives in the control, at pH 5, 7, and 9 were 16, 15, 21, and 7 h respectively. The photoproducts were identified as carboxylic acid, dicarboxylic acid,
quinolinecarboxylic acid, and quinoline. In the same study, 15 mg L\(^{-1}\) \(^{14}\)C-quinoline-ring-labeled imazaquin in pH 7 buffer was exposed to the same light source. The half-life of imazaquin followed first-order kinetics and was 15 h under continuous irradiation. In addition to quinolinecarboxylic acid and quinoline, dicarboxylic acid imide was identified as another major photoproduct for ring-labeled imazaquin.

The photolysis of imidazolinone herbicides in soil is generally slow. In general the half-life of imidazolinones in dry soil was approximately 4 mo and no major photoproducts due to photolysis in soil were identified. The photolysis of \(^{14}\)C-carboxyl and \(^{14}\)C-ring-labeled imazaquin on thin-layer soil plates was studied by exposing them to a borosilicate-filtered xenon arc lamp. The half-life of imazaquin was 60 d with continuous irradiation and 120 d with a 12 h light/dark cycle. The photoproducts were not identified (Mangels, 1991b). Curran et al., (1992) reported 100% degradation of aqueous imazaquin at a concentration of 0.08 mM after 48 h of exposure to UV light. Bioassays with wild mustard (Sinapis arvensis L.) plants indicated that the phytotoxicity of imazaquin solution decreased with increasing time of exposure to UV radiation. In the same study, the rate of imazaquin photolysis was higher in sandy soil with high water potential compared to silty clay loam soil at low water potential.

These studies demonstrate that photolysis can be a potentially important mechanism of imazaquin degradation in aquatic systems. The rate of imazaquin degradation in soil by microbes decreases under cool dry conditions. Due to its unusually high biological activity, delayed or reduced degradation of imazaquin in the field creates the possibility of carryover and injury to subsequent sensitive crops grown the
following year. Although photolysis can't occur below the surface of the soil, it may play a role in imazaquin degradation as a result of imazaquin movement with capillary water to the soil surface. In addition, it is possible that photolysis plays a significant role in imazaquin degradation in runoff and surface waters.

Role of organic matter in the photolysis of herbicides in aquatic environments. Organic matter can be divided into two main groups: non-humic and humic substances. Non-humic substances are constituted by unaltered remains of plant, animal tissues, and include substances belonging to the known classes of organic compounds such as the carbohydrates, proteins, fats, waxes, and resins. Humic substances are the result of chemical and biological degradation of organic compounds and represent the most chemically reactive fraction of organic matter in aquatic environments and in soils. The humic fraction of organic matter has various nucleophilic reactive groups, including carboxyl, phenolic, enolic, aliphatic, carbonyl, amino, heterocyclic amino, imino, and sulfhydryl groups (Stevenson, 1972).

A wide array of synthetic organic chemicals end up in aquatic environments because of surface and/or subsurface runoff from agricultural soils and plants. These chemicals may be subject to various transfer and transformation processes including biodegradation, volatilization, hydrolysis, and photolysis (Shelton and Hites, 1978). The dissolved humic substances in natural waters are capable of absorbing sunlight and this results in the formation of electronically excited molecules (Slawinski et al., 1978). These photo-excited molecules can be involved in the photolysis or excitation of other dissolved substrates through the process of electron transfer or other
energy transfer mechanisms. An example of one important molecule which can undergo excitation via energy transfer is singlet oxygen $[^1O_2]$ (Zepp et al., 1978).

The interaction between light, photosensitizers, and oxygen forms the basis for the production of singlet oxygen. Photosensitizers such as dyes (eosin, rose bengal, crystal violet, acridine orange, and methylene blue), pigments (chlorophyll, porphyrins, and flavins), aromatic hydrocarbons (anthracenes and rubrene), humic, and fulvic acids which occur in natural waters are capable of inducing the production of singlet oxygen (Korycka-Dahl and Richardson, 1978; Zepp et al., 1978). One of the major pathways for singlet oxygen generation in the aquatic environment is by absorption of sunlight by humic and fulvic acids which occur in natural waters. After absorption of sunlight, ground-state sensitizer ($S_0$) will reach the first singlet excited state ($S_1$). The life-time of the singlet excited state is very short (order of 10 nanoseconds) to interact with the oxygen that is present in very low concentrations in the environment. The singlet excited state sensitizer might loose energy and return to the ground state via radiationless (thermal) or radiation (fluorescence) decay or might undergo intersystem crossing to form triplet excited state ($T_1$). The long-lived triplet excited state sensitizer will interact with ground-state oxygen ($[^3O_2]$) through energy transfer to form singlet oxygen ($[^1O_2]$). Singlet oxygen is a more powerful oxidant than ground state oxygen and is a short-lived species in aquatic environments (Leifer, 1988), but it is capable of reacting with organic substances (acceptors) in natural waters to form peroxides. The peroxides may then initiate the free
radical oxidation of aquatic pollutants. This indicates that there is a strong possibility of involvement of organic matter in the photolysis of pollutants in natural water bodies. In soil also, humic and fulvic acids can be involved in the photolysis of herbicides. The theory and mechanism of aqueous photolysis by humic and fulvic acids can be also extended to soil because water can function both as a reaction medium as well as a primary reactant for herbicides in soil (Farmer and Aochi, 1987).

The singlet oxygen generation in natural waters is directly related to the concentration of sensitizers like humic and fulvic acids etc. The singlet oxygen formation will be more in the near-surface region of natural waters and decreases with depth (Shao et al., 1994). This is because as the sunlight penetrates into water, the radiation of short-wavelengths are absorbed near the surface region where as radiation of longer-wavelengths penetrates to deeper depths.

With the seemingly continual introduction of new herbicides, there is a need to investigate the photolysis of herbicides in the environment along with the fate and behavior of photoproducts formed. Further studies on the photoproducts will indicate whether they are persistent or toxic compared to the parent compound. There is also a possibility of manipulating the rate of degradation of persistent compounds which don't undergo direct photolysis by selection and use of the appropriate sensitizer(s). Generating information on herbicide photolysis and sensitization in media relevant to those in which they reside in the environment coupled with information on physical properties and other transformation pathways will aid in the development of predictive models to describe herbicide behavior in the environment. Predictive models are
likely to become very useful in the coming years in determining the relative safety of herbicides across different physical conditions and environments.

**Overall objectives.** The overall objective of this research project was to investigate some of the processes and interactions involved in photodegradation as a transformation process in the dissipation of herbicides in the environment. Specific objectives were:

1. To determine the effect of three phenolic acids, riboflavin, and acetone on the photolysis rates of chlorimuron and imazaquin in aqueous solution, and to investigate the photolytic interaction between these herbicides and riboflavin;

2. To determine the effects of riboflavin and pH on 2,4-D photolysis in aqueous media and to investigate the photolytic interactions between photoproducts, 2,4-D, and riboflavin;

3. To determine the effects of light regime and riboflavin on photolysis of 2,4-D and its residues on corn leaves.
CHAPTER 2

Photolysis of Aqueous Chlorimuron and Imazaquin in the Presence of Phenolic Acids and Riboflavin

Abstract. Laboratory experiments were conducted to determine the effect of three phenolic acids, riboflavin, and acetone on aqueous photolysis of chlorimuron and imazaquin. The phenolic acids investigated were caffeic acid (CA), ferulic acid (FA), and p-coumaric acid (PCA). Treatment solutions were contained in quartz vessels and irradiated with 300 to 400 nm UV light in a photoreactor. The extrapolated photolysis half-life of chlorimuron in pure solution was 107 h, compared to a half-life of 0.42 h for pure aqueous imazaquin. Chlorimuron in solutions containing 10 mg L⁻¹ riboflavin, acetone, CA, FA, or PCA exhibited half-lives of 9, 57, 58, 67, and 146 h, respectively. Imazaquin in solutions containing 10 mg L⁻¹ riboflavin, acetone, CA, FA, or PCA had half-lives of 0.70, 0.55, 0.55, 0.48, and 0.55 h, respectively. The presence of PCA in aqueous media delayed chlorimuron photolysis, whereas all other compounds, especially riboflavin, sensitized chlorimuron photolysis. In contrast, imazaquin photolysis was delayed in the presence of the test compounds, with riboflavin having the greatest effect and resulting in a 68% increase in imazaquin half-life over that of imazaquin alone.
Quantum yields for sensitized photolysis of chlorimuron by riboflavin and for riboflavin by imazaquin were 0.1134 and 0.0477, respectively. These results suggest that some soluble and naturally occurring organic compounds may enhance chlorimuron photolysis yet delay imazaquin photolysis in surface waters. Nomenclature: chlorimuron, ethyl 2-[[[[4-chloro-6-methoxy-2-pyrimidinyl]amino]carbonyl]amino]sulfonylebenzoate; imazaquin, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid; Trifluralin, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine.

Additional index words: Herbicide degradation, photodegradation.
INTRODUCTION

The ultraviolet component of sunlight is capable of causing a variety of chemical transformations and plays an important role in the degradation of many pesticides (Crosby, 1969; Crosby, 1976). The relative importance of photolysis as a pathway of herbicide degradation in the environment is often difficult to assess due to competing transfer and transformation processes, as well as by the presence of naturally occurring compounds that can alter rates of herbicide photolysis. However, when combined with molar absorptivity data for the compound of interest, quantum yields of photolysis determined under controlled conditions can be used to model and predict photolytic half-lives of the compound under a variety of environmental conditions (Zepp and Cline, 1977; Zepp, 1982; Miller and Zepp, 1983).

Photochemical transformations of herbicides in the environment depend on the direct or indirect absorption of solar energy. Direct photolysis occurs when a herbicide absorbs wavelengths of sufficient energy to break covalent bonds or otherwise directly transform the herbicide molecule. Indirect photolysis involves the initial absorption of light energy by a secondary chromophore (photosensitizer) present in the medium, followed by either a transfer of excitation energy, or an exchange of electrons or hydrogen atoms to or from an acceptor (i.e. herbicide) in the medium (Cessna and Muir, 1991). A second mechanism of indirect photolysis is possible whereby the herbicide reacts with other
sensitized species in the medium. Photolysis mechanisms and published research on direct and indirect herbicide photolysis have recently been reviewed (Cessna and Muir, 1991).

Humic acids, fulvic acids, and other complex organic constituents in natural waters and soils have been shown to act as sensitizers that induce phototransformations of other organic constituents in the medium (Choudhry, 1984; Faust and Hoigne, 1987). In addition to water-soluble polymeric organic acids, water-soluble monomeric phenolic acids, flavonoids, alkanolic alcohols, esters, acids, and ketones occur in soils and natural waters as organic matter decomposition products (Zepp, 1978; Choudhry, 1984). While research has shown that the addition of well-characterized natural or synthetic sensitizers to dilute aqueous herbicide solutions can drastically increase herbicide photolysis rates (Hansen and Buchholtz, 1952; Whitehead, 1964; Lykken, 1972; Burkhard and Guth, 1976) the photochemical interaction of herbicides with naturally occurring phenolic acids in aqueous solution has not been reported. Given the fact that water-soluble phenolic acids and flavins are ubiquitous, chromophoric, and may be present in natural waters and the soil solution at millimolar concentrations (Zepp, 1978; Choudhry et al., 1979), their effects on herbicide photolysis in aqueous solution warrants further investigation.

The herbicides selected for this study, chlorimuron and imazaquin, are registered for weed control in soybeans. Chlorimuron degradation in soils occurs primarily by acid-catalyzed hydrolysis (Beyer et al., 1988), whereas the major pathway of imazaquin degradation in soils is microbial metabolism (Basham and Lavy, 1987). Several reports have indicated that chlorimuron and imazaquin may persist excessively under
certain environmental conditions and cause injury to nontarget plants (Beyer et al., 1988; Renner et al., 1988; Loux et al., 1989; Mills and Witt, 1989). Basham and Lavy (1987) reported that imazaquin on soil surfaces and glass slides degrades rapidly when exposed to ultraviolet light. In contrast, chlorimuron in pure aqueous solution and on glass slides was not readily photodegraded by sunlight (Thomas and Harrison, 1990). However, the same authors reported that chlorimuron did undergo significant photodegradation in the presence of riboflavin and a surfactant, both of which apparently sensitized chlorimuron photolysis.

The objectives of this study were to determine the effect of three phenolic acids, riboflavin, and acetone on the photolysis rates of chlorimuron and imazaquin in aqueous solution, and to investigate the photolytic interaction between these herbicides and riboflavin.
MATERIALS AND METHODS

Chlorimuron and imazaquin photolysis. Solutions of 50 mg L\(^{-1}\) chlorimuron (99% purity) and imazaquin (99% purity) were prepared in deionized, distilled water alone and in binary mixtures with 10 mg L\(^{-1}\) riboflavin, acetone, caffeic acid (CA), ferulic acid (FA), or p-coumaric acid (PCA). The structure and chemical name of the herbicides and sensitizers used in this study has been presented in Figure 2.1 and 2.2, respectively. The pH of the treatment solutions was adjusted to 7 with 1 M KOH to preclude acid-catalyzed hydrolysis of chlorimuron (Beyer et al., 1988) and to increase water solubility of the herbicides and phenolic acids. Ultraviolet-visible absorption spectra of the treatment solutions and individual compounds investigated in this study were obtained using a diode-array spectrophotometer.

Replicate 4-ml samples were contained in sealed quartz cuvettes and placed randomly in a rotating sample holder, then irradiated in a photoreactor maintained at 30°C and equipped with 16 UV-fluorescent lamps. These particular lamps were chosen because their emission spectrum is well-characterized and limited to environmentally relevant near-UV wavelengths from 310 to 410 nm (Figure 2.3 A). Nonirradiated controls were wrapped in aluminum foil and placed in the photoreactor. Irradiance inside the photoreactor was monitored during each experiment by trifluralin chemical actinometry (Draper, 1985), described later.

Unchanged parent herbicide was determined in aliquots drawn from the imazaquin solutions at 0, 20, 40, 60, 80, 100, and 120 min and from the chlorimuron solutions at 0, 8, 16, 24, 32, 40, and 48 h. Herbicides
Chlorimuron, (ethyl 2-[[[[4-chloro-6-methoxy-2-pyrimidinyl]amino]carbonyl]amino] sulfonyl] benzoate); C_{15}H_{15}ClN_{4}O_{6}S

Imazaquin, [(2-[4,5-dihydro-4-methyl-4-\{-1-methylethyl\}-5-oxo-1H-imidazol-2-yl]-3-quinolinecarboxylic acid); C_{17}H_{17}N_{3}O_{3}]

Trifluralin, ((2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine); C_{13}H_{16}F_{3}N_{3}O_{4})

Figure 2.1. Structures and chemical names of chlorimuron, imazaquin, and trifluralin.
Riboflavin, [(7,8-dimethyl-10-ribitylisoalloxazine); C_{17}H_{20}N_{4}O_{6}]

Acetone, [(2-propanone); CH_{3}COCH_{3}]

Caffeic acid, [(3-(3,4-Dihydroxyphenyl)-2-propenoic acid]; C_{9}H_{8}O_{4}]

Perilic acid, [(3-(4-Hydroxy-3-methoxyphenyl)-2-propenoic acid]; C_{10}H_{10}O_{4}]

P-Coumaric acid, [(3-(4-Hydroxyphenyl)-2-propenoic acid); C_{9}H_{8}O_{3}]

Figure 2.2. Structures and chemical names of the sensitizers used in the study.
Figure 2.3. Irradiance spectrum of the UV source (A), and UV-visible absorption spectra of the compounds investigated in this study (B, C, and D).
were analyzed by High Performance Liquid Chromatography (HPLC) and UV absorbance detection at 240 nm. Samples were assayed on a C-18 reverse-phase column with a mobile phase of 70:30 (v/v) acetonitrile and 0.2% aqueous formate at a flow rate of 1 ml min$^{-1}$. Parent herbicides were detected as a single peak by comparison with analytical standards and parent herbicide remaining was expressed as a percentage of the initial concentration immediately prior to irradiation. Detection limits were 0.5 and 0.1 mg L$^{-1}$ for chlorimuron and imazaquin, respectively.

Treatments were replicated three times in a completely randomized design and all experiments were conducted twice for each herbicide. Data were combined and the dependent variable means for percent herbicide remaining were log-transformed and regressed on exposure time. Actual and extrapolated herbicide photolysis half-lives ($t_{1/2}$) were calculated based on first-order kinetics:

$$
t_{1/2} = \frac{\ln([C]_o/[C]_t)}{k_p} = \frac{0.693}{k_p}
$$

where $C_o$ is the initial concentration of the herbicide, $C_t$ is its concentration after irradiation time $t$, and $k_p$ is the first-order rate constant (equal to the linear regression coefficient) in units of reciprocal time. Statistically significant differences among treatment rate constants were determined as described by Neter et al., (1983) by using indicator variables to represent treatments in a linear regression.
model and conducting pairwise t-tests of the regression coefficients. An α level of 0.005 was used for each comparison to ensure that the overall α level did not exceed 0.05 (Neter et al., 1983).

Herbicide-riboflavin interactions. To investigate the photolytic interaction of the herbicides with riboflavin in more detail, a second study was conducted in which 50 mg L⁻¹ of chlorimuron or imazaquin was irradiated in the presence and absence of 10 mg L⁻¹ riboflavin. Treatments were irradiated over a time-course as described previously, only sampling was done at 1-or 2-min intervals for the first 15 min in solutions containing riboflavin+imazaquin and riboflavin alone. At each sampling time, parent herbicide and riboflavin remaining in the pure solutions and the mixtures were quantified by HPLC and the photolysis half-lives were calculated. Lumichrome, the principal photoproduct of aerobic riboflavin photolysis (Owen and O’Boyle, 1971), was also quantified by HPLC in all samples initially containing riboflavin. Methods for HPLC analysis of herbicides, riboflavin, and lumichrome were identical to those described in the previous section, and the detection limit for riboflavin and lumichrome was 0.2 mg L⁻¹.

The quantum yield of herbicide photolysis is a unitless ratio of the number of herbicide molecules that undergo photolysis to the number of photons absorbed by the system, and determinations of wavelength-averaged quantum yields in polychromatic light have been described by Draper (1985; 1987). Briefly, quantum yields (Φ) for direct photolysis of chlorimuron, imazaquin, and riboflavin were calculated using the formula (Zepp, 1978):

49
\[ \phi = \frac{0.693 \, j}{(2.303) \, t^{1/2} \, \Sigma \epsilon \, Z} \]  

(2)

where \( j \) is the conversion factor \( 6.02 \times 10^{20} \) photons \( \text{mol}^{-1} \) which makes the units of \( \epsilon \), compatible with those of \( Z \), \( t^{1/2} \) is the photolysis half-life of the compound in seconds, and \( \Sigma \epsilon \, Z \), is the rate of light absorption in photons \( \text{mol}^{-1} \, \text{s}^{-1} \), obtained as the integrated overlap of the compound’s average extinction coefficient (\( \epsilon \), in \( \text{L} \, \text{mol}^{-1} \, \text{cm}^{-1} \)) over a given wavelength interval and lamp emission (\( Z \), in photons \( \text{cm}^{-1} \, \text{s}^{-1} \)) over the same interval.

Irradiance must be determined accurately in order to calculate accurate quantum yields. The UV irradiance inside the photoreactor was measured during each experiment using trifluralin-acetonitrile chemical actinometry (Draper, 1985). Light flux (\( Z \)) for UV wavelengths from 310 to 410 nm was determined with the actinometer and corrected values for quantum yields of chlorimuron, imazaquin, and riboflavin were calculated as:

\[ \phi_{310-410} \text{ (Corrected)} = \phi_{310-410} \text{ (measured)} \times \frac{\text{trifluralin } t_{1/2} \text{(observed)}}{\text{trifluralin } t_{1/2} \text{(expected)}} \]  

(3)
where $\phi_{310-410}$ (measured) is the experimentally determined quantum yield of photolysis for the compound of interest, trifluralin $t_{1/2}$ (observed) is the measured trifluralin actinometer half-life, and trifluralin $t_{1/2}$ (expected) is the expected half-life of trifluralin calculated on the basis of an irradiance of 10,280 $\mu$W cm$^{-2}$ in the 310 to 410 nm wavelength region (manufacturer's specifications) and a previously determined $\phi$ value of 0.0052 for trifluralin in an identical system (Draper, 1985).

The quantum yield for indirect (sensitized) photolysis is the ratio of the number of acceptor molecules that undergo transformation to the number of quanta absorbed by the sensitizer. Quantum yields for sensitized photolysis of herbicides and riboflavin in the mixtures were calculated as:

$$\phi_{s\lambda} = Q_{\lambda} [C]$$  \hspace{1cm} (4)

where $\phi_{s\lambda}$ is the quantum yield of indirect photolysis, $[C]$ is the molar concentration of the acceptor compound, $Q_{\lambda}$ is a proportionality constant calculated as:

$$Q_{\lambda} = \frac{k_{sp} j}{2.303 [S] \sum_{\lambda} Z_{\lambda}}$$  \hspace{1cm} (5)

where $k_{sp}$ is the first-order rate constant of sensitized photolysis.
(overall rate constant minus the direct photolysis rate constant). \(\sum_{\lambda} Z_{\lambda} \) is the rate of light absorption obtained as the integrated overlap of the sensitizer's average extinction coefficient \(\xi_{\lambda} \) over a given wavelength interval and lamp emission \(Z_{\lambda} \) over the same interval, and \([S]\) is the molar concentration of the sensitizer (Zepp, 1982). Other terms in equation 5 were described earlier.
RESULTS AND DISCUSSION

Chlorimuron and imazaquin photolysis. There was significant overlap of lamp emission and UV absorption spectra of imazaquin, riboflavin, and the phenolic acids (Figure 2.3), suggesting that these compounds might undergo direct photolysis and/or act as photosensitizers in this experimental system. The UV-cutoff for chlorimuron was about 300 nm according to the spectral data, suggesting that its direct photolysis would occur very slowly, if at all, since the lamps employed in this study emit little light at wavelengths <310 nm. There was no degradation of imazaquin or chlorimuron in the dark controls over the course of each experiment.

Results of the first study generally confirmed predictions based on overlap of lamp UV emission and absorption spectra. Chlorimuron and imazaquin photolysis in the presence or absence of other compounds proceeded by typical first-order kinetics. Coefficients of determination indicated a good fit of the data to the first-order model, and linear regression equations calculated from the means were used to estimate the photolysis rate constants and herbicide half-lives presented in Table 2.1.

The extrapolated photolysis half-life of 50 mg L\textsuperscript{-1} chlorimuron in the absence of other compounds was 107 h (Table 2.1). With the exception of PCA, all other compounds tested apparently sensitized chlorimuron photolysis in aqueous solution. Chlorimuron photolysis was most strongly sensitized by riboflavin, which increased the photolysis rate constant from 0.0028 h\textsuperscript{-1} to 0.0245 h\textsuperscript{-1} and resulted in a ten-fold decrease in
Table 2.1. Photolysis of aqueous chlorimuron and imazaquin alone or in the presence of acetone, riboflavin, or each of three phenolic acids. Data shown are half-lives ($t_{1/2}$), first-order rate constants ($k_p$), and coefficients of determination ($r^2$) for the linear regression of log percent herbicide remaining on exposure time.

<table>
<thead>
<tr>
<th>Herbicide</th>
<th>$t_{1/2}$</th>
<th>$k_p^a$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h</td>
<td>h$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>Chlorimuron:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alone</td>
<td>107</td>
<td>0.0028a</td>
<td>0.97</td>
</tr>
<tr>
<td>+ acetone</td>
<td>57</td>
<td>0.0053b</td>
<td>0.94</td>
</tr>
<tr>
<td>+ riboflavin</td>
<td>9</td>
<td>0.0245c</td>
<td>0.98</td>
</tr>
<tr>
<td>+ caffeic acid</td>
<td>58</td>
<td>0.0046b</td>
<td>0.88</td>
</tr>
<tr>
<td>+ p-coumaric acid</td>
<td>146</td>
<td>0.0020d</td>
<td>0.94</td>
</tr>
<tr>
<td>+ ferulic acid</td>
<td>67</td>
<td>0.0042b</td>
<td>0.97</td>
</tr>
<tr>
<td>Imazaquin:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alone</td>
<td>0.42</td>
<td>0.6600a</td>
<td>0.98</td>
</tr>
<tr>
<td>+ acetone</td>
<td>0.55</td>
<td>0.5076b</td>
<td>0.99</td>
</tr>
<tr>
<td>+ riboflavin</td>
<td>0.70</td>
<td>0.4374c</td>
<td>0.99</td>
</tr>
<tr>
<td>+ caffeic acid</td>
<td>0.55</td>
<td>0.5094b</td>
<td>0.99</td>
</tr>
<tr>
<td>+ p-coumaric acid</td>
<td>0.55</td>
<td>0.5070b</td>
<td>0.99</td>
</tr>
<tr>
<td>+ ferulic acid</td>
<td>0.48</td>
<td>0.5808d</td>
<td>0.99</td>
</tr>
</tbody>
</table>

$^a$Rate constants within each herbicide that are followed by the same letter do not differ significantly at the 0.005 level.
chlorimuron half-life compared to that in pure solution. There was no statistical difference among chlorimuron photolysis rate constants in solutions containing acetone, CA, or FA, and chlorimuron apparently underwent indirect photolysis in those treatments and had an average half-life of 61 h. PCA had the opposite effect on chlorimuron and extended its photolysis half-life beyond that of all other treatments, including chlorimuron in pure solution. Thomas and Harrison (1990) reported that rates of aqueous chlorimuron photolysis were enhanced by riboflavin and two nonionic surfactants, but inducement of delayed chlorimuron photolysis by other compounds in dilute aqueous solution has not been reported previously.

In contrast to chlorimuron, imazaquin readily underwent direct photolysis in aqueous solution (Table 2.1). Also in contrast to the effects observed with chlorimuron, all treatment compounds significantly decreased the rate of imazaquin photolysis in aqueous solution compared to that in pure aqueous solution. Acetone, CA, and FA each reduced the rate of imazaquin photolysis to the same extent, FA had the least effect, and riboflavin resulted in the slowest rate of imazaquin photolysis. The half-life of pure aqueous imazaquin was 0.42 h, whereas imazaquin in solutions containing acetone, riboflavin, CA, PCA, or FA had half-lives of 0.55, 0.70, 0.55, 0.55, and 0.48 h, respectively.

**Herbicide-riboflavin interactions.** In the previous experiment, riboflavin caused the greatest sensitization of chlorimuron photolysis and the greatest inhibition of imazaquin photolysis. Results from the second experiment verified that riboflavin was a strong sensitizer of chlorimuron photolysis in an aqueous mixture of the two compounds ((Table 2.2). The quantum yield of riboflavin-sensitized chlorimuron
photolysis was 0.1134, resulting in a ninefold increase in its net photolysis rate constant from 0.0028 h\(^{-1}\) alone to 0.0252 h\(^{-1}\) in the mixture. Contrary to its effects on chlorimuron, riboflavin reduced the net imazaquin photolysis rate constant by 35\% and its quantum yield of direct photolysis from 0.0058 in pure solution to 0.0033 in the mixture (Table 2.2).

Riboflavin degradation in the presence or absence of imazaquin proceeded by first-order kinetics, and pure riboflavin exhibited a half-life of 0.48 h in aqueous solution, compared to 0.13 h in the presence of imazaquin (Table 2.2). These data suggest direct energy transfer or a related energy-coupled process (e.g. photooxidation via singlet oxygen formation) from photoexcited imazaquin to ground state riboflavin, thus regenerating ground-state imazaquin while concurrently increasing the rate of riboflavin photolysis. The quantum yield for imazaquin-sensitized riboflavin photolysis was 0.0477, indicating that sensitization occurred with approximately half the efficiency of that observed for riboflavin-sensitized chlorimuron photolysis. Consequently, the half-life of imazaquin in the mixture with riboflavin was extended to 0.67 h, compared to 0.38 h in pure solution.

Figure 2.4 illustrates the effect of chlorimuron and imazaquin on stoichiometry of riboflavin photobleaching with the concurrent formation of lumichrome, long known to be the major aerobic photolysis product of riboflavin in neutral or acidic media (Owen and O‘Boyle, 1971). Riboflavin disappearance in the presence of chlorimuron, imazaquin, and in pure solution was highly correlated (r=0.95, 0.93, and 0.89, respectively) with the formation of lumichrome at each sampling time. A
Table 2.2. Photolytic interaction of riboflavin with chlorimuron and imazaquin. Data shown are half-lives ($t_{1/2}$), first-order rate constants ($k_p$), and coefficients of determination ($r^2$) for the linear regression of log percent herbicide and riboflavin remaining on exposure time, and quantum yields ($\phi$) of direct and indirect (i) photolysis.

<table>
<thead>
<tr>
<th>Compound</th>
<th>$t_{1/2}$</th>
<th>$k_p^a$</th>
<th>$r^2$</th>
<th>$\phi$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>h</td>
<td>h$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorimuron:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alone</td>
<td>111</td>
<td>0.0028</td>
<td>0.95</td>
<td>&lt;0.0001b</td>
</tr>
<tr>
<td>+ riboflavin</td>
<td>12</td>
<td>0.0252c</td>
<td>0.99</td>
<td>0.1134 (i)d</td>
</tr>
<tr>
<td>Imazaquin:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alone</td>
<td>0.38</td>
<td>0.7440</td>
<td>0.98</td>
<td>0.0058</td>
</tr>
<tr>
<td>+ riboflavin</td>
<td>0.67</td>
<td>0.4800</td>
<td>0.90</td>
<td>0.0033 (i)</td>
</tr>
<tr>
<td>Riboflavin:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alone</td>
<td>0.48</td>
<td>0.5880</td>
<td>0.87</td>
<td>0.0004</td>
</tr>
<tr>
<td>+ imazaquin</td>
<td>0.13</td>
<td>2.0880</td>
<td>0.96</td>
<td>0.0477 (i)</td>
</tr>
<tr>
<td>+ chlorimuron</td>
<td>0.22</td>
<td>1.0140</td>
<td>0.93</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Rate constants among treatments within each compound were significantly different at the 0.005 level.

$^b$Estimated value based on available lamp spectral data below 310 nm.

$^c$Net rate constants includes both direct and indirect photolysis.

$^d$Quantum yield of indirect photolysis.
Figure 2.4. Aqueous riboflavin photolysis and photoporduct (lumichrome) formation in the presence of chlorimuron (A), imazaquin (B), and in pure aqueous solution (C). Data points shown are the means of six replications ± SE.
number of reactions may be involved in riboflavin photolysis and its sensitization of herbicide photolysis. Lumichrome can be formed directly from riboflavin via decay of the excited singlet state of riboflavin by a photoelimination mechanism following photon absorption (Sato et al., 1982). Alternatively, a radiationless transition of excited singlet state riboflavin produces the longer-lived excited triplet state, which may transfer excitation energy directly to the herbicide or to ground state oxygen, the latter resulting in the formation of singlet oxygen (Owen and O'Boyle, 1971). Although the actual mechanism of herbicide photosensitization cannot be discerned from the data presented here, singlet oxygen formation from triplet state riboflavin cannot be ruled out.

Silber et al. (1976) reported that 2,4-D [(2,4-dichlorophenoxy) acetic acid] sensitized aqueous riboflavin photolysis; however, lumichrome formation was suppressed in the presence of 2,4-D. Those authors suggested that a different pathway for the disappearance of riboflavin exists in the presence of 2,4-D compared to that for riboflavin in pure solution. In contrast, our study indicated that imazaquin simultaneously enhanced riboflavin photolysis and lumichrome formation (Figure 2.4 B and C). Equimolar concentrations of riboflavin and lumichrome were present after approximately 6 min of exposure in solutions containing imazaquin, compared to >15 min in the pure riboflavin solution. Although this suggests that imazaquin somehow just accelerated the normal riboflavin aerobic photolysis pathway, the actual mechanism of enhanced lumichrome formation and sensitized riboflavin
photolysis by imazaquin is unclear, given that lumichrome normally arises as a degradation product of the relatively short-lived lowest excited singlet state of riboflavin (Owen and O’Boyle, 1971).

Data reported here indicate that chlorimuron and imazaquin, though similar in biological activity, possess quite different photochemical properties in aqueous media. Imazaquin readily underwent direct photolysis but also acted as a sensitizer of riboflavin in the medium, thereby prolonging its own half-life and suggesting that it may have the highest triplet energy state of all the compounds investigated in this study. In contrast, chlorimuron was quite photostable in pure aqueous solution, but underwent indirect photolysis via photosensitization by all but one of the compounds evaluated as photosensitizers in this study.

Although photolysis is not a major pathway of degradation for chlorimuron or imazaquin under normal use and field conditions (Basham and Lavy, 1987; Beyer et al., 1988), investigation of secondary degradation pathways aids in further refinement of herbicide environmental fate and transport models and provides basic information on the general behavior of synthetic organics in the environment. The data presented here further illustrate that naturally occurring compounds present in aqueous media may enhance, delay, or have little effect on herbicide photolysis. Additional investigation of the reaction mechanisms and energy relationships among photoexcited donor and sensitized acceptor compounds that are present as solutes in natural waters is required in order to more accurately predict the relative importance of photolysis as a mechanism of herbicide degradation in those environments.
CHAPTER 3

Riboflavin Sensitized Photolysis of 2,4-D in Aqueous Media

Abstract. Experiments were conducted to determine the effects of riboflavin, pH of the medium, and photoproducts on aqueous photolysis of 2,4-D. Treatment solutions were contained in quartz vessels and irradiated with UV (300-400 nm) and visible light in a controlled-environment growth chamber. In pure solution, 2,4-D did not undergo direct photolysis. Riboflavin sensitized 2,4-D photolysis under both UV and visible light. The effect was more pronounced under UV light in presence of riboflavin. Photolysis of 2,4-D in aqueous solution in the presence of riboflavin appears to be concentration-dependent. The extrapolated photolysis half-life of 2,4-D in solution containing 10 mg L\(^{-1}\) of riboflavin was 7 and 11 h under UV and visible light, respectively. In contrast, at 2.5 mg L\(^{-1}\) riboflavin the extrapolated half-lives were 26 h under UV and 39 h under visible light. Photolysis of 2,4-D in aqueous solution in the presence of riboflavin increased under both UV and visible light as pH of the treatment solution was decreased from 7.5 to 4.5. At pH 4.5, the half-life of 2,4-D in presence of riboflavin under UV light was 1 h compared to 3 h under visible light. 2,4-D did not undergo any photolysis at pH 7.5 even in the
presence of riboflavin and UV light. Sensitized photolysis of 2,4-D in aqueous solution under both UV and visible light consistently yielded 2,4-DCP. During the process of 2,4-D sensitization, riboflavin underwent photodegradation forming lumichrome and lumiflavin. There was no photolysis of 2,4-D in treatments containing only lumichrome or 2,4-DCP as sensitizers. Lumiflavin was capable of sensitizing 2,4-D photolysis in aqueous solution under both UV and visible light. Results from this experiment indicate that lumiflavin was a stronger sensitizer of 2,4-D photolysis in aqueous solution than riboflavin except when pH of the riboflavin treatment solution was adjusted to 4.5. Under both UV and visible light, riboflavin underwent rapid photolysis in pure aqueous solution and its photolysis was delayed in the presence of 2,4-D and 2,4-DCP. These results suggest that the concentration of the sensitizer as well as pH of the reaction media are critical for 2,4-D photolysis in natural aquatic systems. Nomenclature: 2,4-D, (2,4-dichlorophenoxy)acetic acid); Trifluralin, 2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine.

Additional index words: Photolysis, pH, 2,4-dichlorophenol (2,4-DCP), riboflavin, lumichrome, lumiflavin.
INTRODUCTION

A wide array of synthetic organic chemicals end up in the aquatic environment because of surface and subsurface runoff from agricultural soils and plants (Shelton and Hites, 1978). They may then be subjected to several degradation and/or transfer processes (Weber et al., 1973). The degradation processes include biodegradation, chemical degradation, and photodecomposition.

Photodecomposition results from the direct or indirect absorption of energy from Ultraviolet (UV) radiation (300-400 nm) by a compound. In order for photodecomposition to occur, a herbicide either has to absorb UV radiation directly (direct photolysis) or remain in contact with another compound (sensitizer) which can absorb light and transfer the excitation energy to the herbicide (acceptor), which then undergoes degradation (indirect photolysis). For an efficient sensitization reaction to occur, the sensitizer should be able to absorb radiation at longer wavelengths compared to the acceptor, it should have high efficiency of intersystem crossing, and the first excited triplet state of the sensitizer should be energetically higher than that of the acceptor. The photoproducts of the sensitizer as well as herbicide may influence the rate of indirect photolysis by acting as quenchers that inhibit the photoreaction (Choudhry et al., 1979) or as additional sensitizers that promote herbicide photolysis (Boval, 1972). The theory and mechanisms of photolysis have been presented in detail in several reviews (Choudhry et al., 1979; Cessna and Muir, 1991).

The single most widely used phenoxy herbicide is 2,4-D. It is used
for controlling broadleaf weeds in small grains, corn, sorghum, and turf (Ross and Lembi, 1985). Physicochemical properties like the pKa value of a herbicide and pH of natural waters may influence the process of photolysis (direct or indirect) of a herbicide in the environment. The pKa value of 2,4-D is 2.87 (Cessna and Grover, 1978), and the pH of the natural waters is typically greater than 5, so 2,4-D exists primarily in the anionic form in natural aquatic systems. The UV absorption maximum of anionic form is at 290 nm (Cessna and Muir, 1991), yet solar wavelengths below 290 nm are almost completely absorbed by the earth's ozone layer and thus do not play a role in direct photolysis of compounds like 2,4-D which do not absorb UV wavelengths >290 nm (Kagan, 1993). These characteristics make 2,4-D a prime candidate for indirect photolysis in the aquatic environment.

It is clear from previous studies that the most common pathway for photolysis of pesticides in the natural aquatic environment appears to be through indirect photolysis. Natural waters contain many naturally occurring dissolved organic materials like riboflavin, chlorophyll, phenolic acids, tryptophan, humic substances etc., which strongly absorb sunlight (Cessna and Muir, 1991; Venkatesh et al., 1993). Humic and fulvic acids absorb sunlight, form excited triplet state species, and can then transfer the energy to other compounds present in the water which results in the production of reactive intermediates. The reactive intermediates formed includes singlet oxygen, alkylperoxy radicals, hydroxy radicals, superoxide anion, hydroperoxy radicals, and triplet excited states of other humic substances (Leifer, 1988). These reactive intermediates may then be involved in the decomposition of organic compounds. 2,4-D has high affinity for water in aquatic systems (Funari
et al., 1995), so there is a strong possibility 2,4-D undergoing indirect photolysis because of the presence of natural sensitizers in natural aquatic environments.

Most of the earlier studies conducted on photolysis of 2,4-D involved use of short-wavelength, high energy UV radiation (<300 nm). Aly and Faust (1964) and Crosby and Tutass (1966) reported direct photolysis of 2,4-D in aqueous solution when samples were exposed to a light source which emitted short wavelength UV light with a primary spectral emission at 254 nm. Others have reported that the rate of indirect photolysis of 2,4-D increased in aqueous media in presence of sensitizers like riboflavin, anionic, and nonionic surfactants when irradiated with wavelengths >300 nm (Hansen and Buchholtz, 1952; Hautala, 1978; Harrison and Wax, 1986). Thus the radiation source, the medium in which the herbicide is exposed, and the type of sensitizer used plays a major role in influencing the rate of herbicide photolysis and the type of photoproducts formed.

The study presented here was conducted by exposing 2,4-D in aqueous solution and in the presence of riboflavin, a known photosensitizer (Crosby, 1976), to a light source which emits polychromatic UV light with a wavelength spectrum which more closely resembles solar wavelengths reaching the earth's surface. The objectives of this study were to: 1) determine the effects of riboflavin and pH on 2,4-D photolysis in aqueous media, and 2) to investigate the photolytic interactions between photoproducts, 2,4-D, and riboflavin.
MATERIALS AND METHODS

Methods common to all experiments. Studies were conducted in a controlled-environment growth chamber equipped with an irradiation source consisting of blacklight blue fluorescent lamps. These lamps deliver an emission spectrum of near-UV radiation from 300 to 400 nm with a peak emission at 356 nm. The growth chamber was partitioned into two compartments with a polycarbonate sheet coated with UV-blocking resin (UV cutoff=400 nm). Each side was fitted with 5 black light blue lamps (40 W lamp⁻¹) spaced 20 cm apart. The growth chamber also contained fluorescent and incandescent lights that provided approximately 300 μE m⁻² s⁻¹ PPF for a 14 h daily photoperiod. Temperature inside the growth chamber ranged from 27 to 28 C and relative humidity ranged from 28 to 30%.

The UV-visible absorption spectra of the individual compounds investigated in this study were obtained using a diode-array spectrophotometer. The structure and chemical name of the herbicides and sensitizers used in this study has been presented in Figure 3.1 and 3.2, respectively. Treatment solutions were prepared using nonradiolabeled materials except where specified otherwise. A sample volume of 4-ml was irradiated for each treatment and samples were contained in sealed quartz test tubes. The treatment samples were randomly distributed horizontally on the top of a box positioned 28 cm below the light source and were exposed to visible and supplemental UV light (UV+VL) in the growth chamber. Visible light-only (VL) controls were placed under the light source and shielded with a thin sheet of coated polycarbonate that
2,4-D, [(2,4-dichlorophenoxy)acetic acid];
\[ C_8H_5Cl_2O_3 \]

Trifluralin, [(2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine]; \[ C_{13}H_{16}F_3N_3O_4 \]

Figure 3.1. Structures and chemical names of 2,4-D and Trifluralin.
Figure 3.2. Structures and chemical names of the sensitizers used in this study.
blocks most of the UV light while allowing transmission of the visible spectrum. Nonirradiated (dark) controls consisted of treatment tubes that were wrapped in aluminum foil and then transferred to the growth chamber. The total UV energy emitted by the light source was measured using trifluralin-acetonitrile chemical actinometry, described later.

The samples were irradiated over a time course of 0, 30, 60, 120, 240, 360, and 480 min. At each harvest interval a 0.5 ml aliquot was drawn from each treatment and analyzed for the parent herbicide (2,4-D), riboflavin, and degradation products by High Performance Liquid Chromatography (HPLC) with UV absorbance detection at 240 nm. Samples were assayed on a C-18 reverse-phase column with a mobile phase of acetonitrile and 0.2% aqueous formate [50:50, (v/v) for 2,4-D, and 2,4-DCP; 20:80, (v/v) for riboflavin, lumichrome, and lumiflavin] with a flow rate of 1 ml min\(^{-1}\). In the experiment where \(^{14}\)C and \(^{3}\)H were used, a radioactivity flow detector outfitted with a stream splitter was used with HPLC to determine the presence of labelled parent compounds and degradation products. The presence of 2,4-D, riboflavin, and suspected degradation products were confirmed by cochromatography with reference standards.

The UV irradiance inside the growth chamber during each experiment was measured using trifluralin-acetonitrile chemical actinometry (Draper, 1987; Venkatesh et al., 1993). The dinitroaniline herbicide trifluralin was used as a chemical actinometer because it absorbs light uniformly between 300 and 400 nm, reacts with a well-defined quantum yield, photodegrades at a convenient rate, and can be analyzed easily and rapidly by HPLC (Draper, 1985; Venkatesh et al., 1993). Trifluralin actinometer was first calibrated in a photoreactor equipped with 16 UV-
fluorescent lamps which emit light in the near-UV wavelength region limited to 310 and 410 nm with a total energy output of 10,280 μW cm⁻² (manufacturer's specifications). Replicate 4 ml samples of 20 mg L⁻¹ trifluralin (96% purity) in acetonitrile were prepared from a trifluralin stock solution of 10 g L⁻¹, samples were contained in sealed quartz tubes, and irradiated over a 30 min time-course in the photoreactor. Aliquots of 0.5 ml were assayed at 5 min intervals by HPLC with UV absorbance detection at 240 nm. Parent trifluralin was assayed using a C-18 reverse-phase column with a mobile phase of 70:30, (v/v), acetonitrile and 0.2% aqueous formate with a flow rate of 1 ml min⁻¹. Parent compound was detected as a single peak and confirmed by comparison with an analytical standard.

Irradiance was monitored with replicate samples of trifluralin as explained earlier, during all subsequent photolysis experiments in the growth chamber. The total UV irradiance in the 300-400 nm region of the light source in the growth chamber was calculated using the formula:

\[
\text{Total UV irradiance (300-400 nm) = } \frac{\text{trifluralin } t_{1/2 \ (\text{experimental})}}{\text{trifluralin } t_{1/2 \ (\text{observed})}} \times 10,280 \ \mu W \ cm^{-2}
\]  

where trifluralin \( t_{1/2 \ (\text{experimental})} \) is the actual half-life of trifluralin irradiated in a photoreactor equipped with 16 UV-lamps that emitted light in the 310-410 nm region. The value of 10,280 μW cm⁻² is
the lamp output according to manufacturer's specifications. Trifluralin $t_{1/2}$ (observed) is the measured trifluralin actinometer half-life in the growth chamber. The procedure for computation of half-life has been explained in the data analysis section.

Experiment 1. Riboflavin concentration effects on 2,4-D photolysis. Aqueous solutions of 100 mg L$^{-1}$ 2,4-D (98% purity) were prepared in deionized, distilled water alone and in mixtures with 2.5, 5, and 10 mg L$^{-1}$ riboflavin (99% purity). The pH of the solutions was adjusted to 6 with 1 M KOH. Stock solutions of $^{14}$C-2,4-D were prepared using uniformly phenyl-ring labeled herbicide (specific activity=743 M bq mmol$^{-1}$) dissolved in 500 μl ethanol and water (1:1, v/v). Tritiated riboflavin stock solutions were prepared from general-labelled riboflavin (specific activity=1221 G bq mmol$^{-1}$) dissolved in 524 μl ethanol and water (1:1, v/v). The 2,4-D+riboflavin treatment solutions were spiked with 20,000 dpm ml$^{-1}$ each of $^{14}$C-2,4-D and $^3$H-riboflavin. The 2,4-D alone treatment solution contained only 20,000 dpm ml$^{-1}$ of $^{14}$C-2,4-D. A total of 4 ml treatment solution containing 26 K bq (2,4-D+riboflavin) or 13 K bq (2,4-D alone) radioactivity was exposed. Radioactivity was quantified by liquid scintillation counting (LSC). Decay correction and dual-label counting were employed for treatments containing $^3$H-riboflavin. Counts were corrected for quenching, background, and dilution and converted to disintegrations per minute (dpm) for analysis.

Experiment 2. Solution pH effects on riboflavin-sensitized 2,4-D photolysis. Treatment solutions of 100 mg L$^{-1}$ 2,4-D were prepared in deionized, distilled water and adjusted to pH 4.5, 6, and 7.5 with 1 N
HCl or 1 M KOH. After adjusting the pH, riboflavin was added at a concentration of 10 mg L⁻¹ to all three treatment solutions. The pH of the treatment solution was monitored periodically and did not vary more than 0.1 pH unit during the experiment.

**Experiment 1. Herbicide-photoproduct and sensitizer-photoproduct interactions.** A series of experiments were conducted to investigate the photolytic interactions among the parent compounds (2,4-D and riboflavin) and some of their major individual photoproducts in aqueous solution. In the first experiment, 100 mg L⁻¹ solutions of 2,4-D containing either 10 mg L⁻¹ 2,4-DCP (99% purity), lumichrome (98% purity), or lumiflavin (99% purity), or no photoproduct were prepared. The pH of the solutions was adjusted to 6 with 1 M KOH. In a second experiment, solutions of 10 mg L⁻¹ riboflavin were irradiated in the presence and absence of 100 mg L⁻¹ 2,4-D, or 10 mg L⁻¹ 2,4-DCP. In the last experiment, solutions of 10 mg L⁻¹ lumichrome and 10 mg L⁻¹ lumiflavin were each irradiated in the presence and absence of 100 mg L⁻¹ 2,4-D.

**Data analysis.** Due to the lack of herbicide or sensitizer degradation in any of the dark controls over the course of all experiments, losses were attributed to photodegradation and are expressed uniformly as percent herbicide loss compared to nonirradiated controls at time zero. Parent herbicide and sensitizers were detected as single peaks. Treatments in all experiments were replicated three times in a completely randomized design and all experiments were conducted twice. Data were combined and the dependent variable means for percent herbicide remaining were log
transformed and regressed on exposure time. Actual and extrapolated herbicide photolysis half-lives ($t_{1/2}$) were calculated based on pseudo-first-order kinetics:

$$t_{1/2} = \frac{\ln([C_o]/[C_t])}{k_p} = \frac{0.693}{k_p} \quad (2)$$

where $C_o$ is the initial concentration of the herbicide, $C_t$ is its concentration after irradiation time $t$ and $k_p$ is the first-order rate constant (equal to the linear regression coefficient) in units of reciprocal time. Statistically significant differences among treatment rate constants (slopes) were determined by comparing their standardized pairwise differences to the 95th percentile of the studentized range distribution. This procedure, which is equivalent to performing Tukey's HSD (Honestly Significant Difference Test) procedure in a one-way ANOVA (Analysis of Variance), controls the experiment wise Type I error rate at 5% (Zar, 1996).
RESULTS AND DISCUSSION

Average half-life of the trifluralin actinometer during all experiments was 5 and 37 h under direct UV light and under coated polycarbonate (CP) respectively. Based on these actinometric half-lives, the average UV light intensity inside the growth chamber was calculated to be 634 and 84 μW cm⁻² under direct UV light and CP, respectively. Thus there was over a seven-fold attenuation (but not total occlusion) of UV light by the CP used in this system. Hereafter and for simplicity the two light regimes-direct UV-visible light and attenuated UV-visible light, will be referred to as UV+VL and VL, respectively.

Investigation of UV-visible absorption spectra revealed a significant overlap between the UV lamp emission spectrum and the absorption spectra of riboflavin and lumiflavin indicating the possibility that these compounds would undergo direct photolysis and/or possibly act as photosensitizers in this experimental system (Figure 3.3). The UV cutoff for 2,4-D, 2,4-DCP, and lumichrome is about 300 nm according to spectral data, indicating a low possibility for those compounds to undergo direct photolysis in this system, since the lamps used in this study emit little if any light at wavelengths <300 nm. Similarly, those compounds could not undergo direct photolysis in the environment due to absorption of solar wavelengths <290 nm by the earth’s ozone layer (Kagan, 1993).

There was no measurable loss of 2,4-D in the dark controls over the course of each experiment. Data for all experiments, when regressed as mean log percent herbicide remaining on hours of exposure, resulted
Figure 3.3. Irradiance spectrum of the UV source (A), and UV-visible absorption spectra of the compounds investigated in this study (B, C, D, E, and F).
in linear relationships, thus indicating that herbicide photolysis proceeded by pseudo-first-order kinetics. It should be emphasized that photolysis half-lives presented herein, whether actual or extrapolated from the regression equations, are applicable only to the specific experimental and environmental conditions under which these experiments were conducted. Further, photolysis half-lives calculated here serve only as basis for treatment comparisons. Coefficients of determination indicated a good fit of the data to the first-order model and linear regression equations calculated from the means were used to estimate the photolysis rate constants and herbicide half-lives presented in the following sections.

Riboflavin concentration and solution pH effects on 2,4-D photolysis.

There was no photolysis of 2,4-D under either light regime in treatments with no riboflavin (Table 3.1). In contrast, 2,4-D was photolyzed in the presence of riboflavin and this apparent sensitized photolysis of 2,4-D was more rapid under UV+VL than under VL. Half-lives of 2,4-D in solutions irradiated with UV+VL ranged from 7 h in solutions containing 10 mg L⁻¹ riboflavin to 26 h in solutions containing 2.5 mg L⁻¹ riboflavin. Under VL the 2,4-D half-life also decreased with increasing riboflavin concentration and ranged from 11 to 39 h. Photolysis rate constants increased significantly from 0.0262 to 0.0963 h⁻¹ in presence of increasing riboflavin concentration under UV+VL, while under VL the rate constants increased from 0.0178 to 0.0655 h⁻¹. Coefficients of determination indicated a good fit of the data to the first-order regression model and ranged from 0.72 to 0.90.

The photolysis of 2,4-D in aqueous solutions containing riboflavin is attributable to photosensitization. Riboflavin absorbs both UV and
Table 3.1. Effects of light regime, riboflavin concentration, and pH on 2,4-D photolysis in aqueous solution. Data shown are half-lives ($t_{1/2}$), first-order rate constants ($k_p$), and coefficients of determination ($r^2$) for the linear regression of log percent herbicide remaining on exposure time.

<table>
<thead>
<tr>
<th>Ribo conc</th>
<th>$t_{1/2}$</th>
<th>$k_p$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Light regime</strong></td>
<td>UV+VL</td>
<td>VL</td>
<td>UV+VL</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ 2.5 ppmw</td>
<td>26</td>
<td>39</td>
<td>0.0262a</td>
</tr>
<tr>
<td>+ 5 ppmw</td>
<td>12</td>
<td>18</td>
<td>0.0580b</td>
</tr>
<tr>
<td>+ 10 ppmw</td>
<td>7</td>
<td>11</td>
<td>0.0963c</td>
</tr>
</tbody>
</table>

**pH effect**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>UV+VL</th>
<th>VL</th>
<th>UV+VL</th>
<th>VL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+R. pH 4.5</td>
<td>1</td>
<td>3</td>
<td>0.6962a</td>
<td>0.2641c</td>
</tr>
<tr>
<td>+R. pH 6</td>
<td>4</td>
<td>15</td>
<td>0.1919b</td>
<td>0.0475d</td>
</tr>
<tr>
<td>+R. pH 7.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Rate constants within an experiment that are followed by the same letter do not differ significantly at the 0.05 level according to Tukey’s HSD.
Abbreviations used: UV+VL=Ultraviolet+visible light, VL=visible light, Ribo and R=Riboflavin, conc=concentration, ‘-’=no photolysis.
visible light and is capable of inducing herbicide photolysis through energy transfer from its photoexcited triplet state to the receptive herbicide molecules (Lykken, 1972; Tanaka et al., 1981). Harrison and Wax (1985) reported that rates of aqueous 2,4-D photolysis were similarly enhanced by the presence of selected adjuvants which may act as sensitizers upon exposure to UV light. However, under similar experimental conditions in that study, there was no apparent sensitization of 2,4-D photolysis by adjuvants in the visible light (UV-attenuated) controls. This is in contrast to riboflavin, which the data here suggests is capable of photosensitizing 2,4-D photolysis in visible as well as in the UV spectrum, albeit at a slower rate. The energy present in most of the visible spectrum is not adequate to cause photolysis of 2,4-D (Roberts et al., 1984) and consequently a lower 2,4-D photolytic rate constant and a higher half-life was observed under VL.

The concentration-dependency of riboflavin sensitization efficiency in an aqueous system is predictable and most likely the result of greater overall presence of the relatively stable riboflavin triplet complexes capable of sensitizing 2,4-D photolysis (Murrov, 1973). Higher concentration of sensitizer results in higher production of triplets (Turro, 1967), and photosensitization reactions usually involve triplet sensitizers (Kagan, 1993). In effect the increased rate of 2,4-D photolysis with higher concentration of riboflavin may also be related to the effectiveness of energy transfer from triplet riboflavin to 2,4-D. The efficiency of energy transfer in a particular system depends on the concentration of the energy-accepting molecule which acts as a quencher (Horspool and Armesto, 1992). In our study the initial concentration of 2,4-D was held constant and was considerably higher
than that of the sensitizer. As a result, the first-order rate constants were directly proportional to the riboflavin concentrations under both UV+VL and VL conditions.

2,4-D photolysis in aqueous solution in presence of riboflavin increased under both UV+VL and VL as pH of the treatment solution was decreased from 7.5 to 4.5 (Table 3.1). At pH 4.5, the half-life of 2,4-D in presence of riboflavin under UV+VL was 1 h compared to 3 h under VL. The net photolysis rate constant was significantly higher at pH 4.5 under UV+VL (0.6962 h⁻¹) compared to all the other treatments. This is in contrast to the findings reported for other herbicides. Acher and Saltzman (1980) reported no riboflavin-sensitized photolysis of bromacil at pH <5, and an increase in pH up to 8 caused an increase in the bromacil degradation rate at 6.5% for each pH unit increase. Watts et al., (1985) similarly reported low rates of riboflavin-sensitized photolysis of bromacil at pH <7, with increasing rates as pH was increased to 9. The same authors, however reported that riboflavin-sensitized photooxidation of terbacil in aqueous solution was much greater at pH <5 compared to the solutions in the pH 5 to 9 range, and that riboflavin-sensitized fluometuron photolysis increased linearly as pH of the solution was decreased unit-wise from 9 to 3.

In studies using actinic UV light in the 220-310 nm range, Chamarro and Esplugas (1993) reported increased rates of direct 2,4-D photolysis as initial pH of the solution decreased from 8.9 to 3.5. They attributed the decrease in 2,4-D photolysis with increasing pH over time to the increased formation of humic acids, possibly resulting in greater quenching of incident radiation. However, those authors presented no evidence that humic substances were in fact formed in the treatment
solutions. We observed no evidence of humic substances (e.g., solution discoloration) in the experiments presented here.

The higher rates of riboflavin-sensitized 2,4-D photolysis observed in our study as initial pH was decreased from 7.5 to 4.5 may be partially attributable to increased stability of the riboflavin molecule under acidic conditions when exposed to UV light. Halwer (1951) reported that lowering the solution pH alters the riboflavin molecule in such a way so as to make it resistant to direct photolysis. Riboflavin has a characteristic isoalloxazine ring system with nitrogen atoms at positions number 1, 3, 9, and 10 (Figure 3.2). For riboflavin to undergo photolysis, it requires the presence of a proton at position 9. At low pH values, the nitrogen at position number 10 becomes protonated producing a positive charge, thus making the nitrogen atom in position 9 unable to acquire a proton of its own. Optimally, an efficient photosensitizer would not photodegrade and would maintain a steady concentration in solution. This is not the case with riboflavin, which readily undergoes direct photolysis both under UV and visible light. However, riboflavin photoproduct formation in our study occurred at slower rates as pH decreased (Figure 3.4), thus lending support to the hypothesis that riboflavin remains intact longer under acidic conditions than under neutral or alkaline conditions and thereby promotes 2,4-D photolysis to a greater extent.

2,4-D did not undergo measurable photolysis in aqueous solution at pH 7.5 in presence of riboflavin under either light regime. As explained earlier, the nitrogen at position 9 becomes protonated under neutral-alkaline conditions which makes riboflavin more susceptible to direct
**Figure 3.4.** Effects of light regime and pH on lumichrome formation from riboflavin photolysis. Data points shown are the means of six replications ± SE. Data points for which error bars are missing have low SE.
photolysis (Halwer, 1951). The rapid loss of riboflavin resulted in virtually no 2,4-D photolysis when the pH of the treatment solution was adjusted to 7.5 (Table 1; Figure 3.4).

Isolation and identification of photoproducts. Analysis of dark controls showed only the presence of parent compounds (2,4-D, and riboflavin). Sensitized photolysis of 2,4-D in aqueous solutions under both UV+VL and VL consistently yielded a compound that co-chromatographed with 2,4-dichlorophenol (2,4-DCP) as the major photoproduct (Figure 3.5, 3.6, and 3.7). The formation of 2,4-DCP as a 2,4-D photoproduct is due to cleavage of the ether bond of 2,4-D (Chamarro and Esplugas, 1993). The production of 2,4-DCP has also been reported as a product of direct photolysis when 2,4-D alone in aqueous solution was exposed to short-wavelength monochromatic UV light (Aly and Faust, 1964; Crosby and Tutass, 1966; Zepp et al., 1975). The production of 2,4-DCP as the major photoproduct of riboflavin-sensitized 2,4-D photolysis has not been reported, to our knowledge. The production of 2,4-DCP from 2,4-D photolysis in aqueous solution is of concern because 2,4-DCP, like 2,4-D, does not undergo direct photolytic degradation in natural sunlight and poses environmental concerns due to its toxicity (Somani and Khalique, 1982).

Formation of 2,4-DCP in solution and its subsequent pattern of degradation was a function of riboflavin concentration in the media (Figure 3.5). Following an initial increase, the amount of 2,4-DCP present when exposed to UV+VL decreased to lower levels at later harvest intervals, presumably due to some flavin-sensitized 2,4-DCP photolysis (Figure 3.5 A). In contrast to the two lowest riboflavin concentrations,
Figure 3.5. Effects of light regime and riboflavin concentration on 2,4-dichlorophenol formation. Data shown are the means of six replications ± SE. Data points for which error bars are missing have low SE.
Figure 3.6. Effects of light regime and pH on 2,4-dichlorophenol formation. Data shown are the means of six replications ± SE. Data points for which error bars are missing have low SE.
Figure 3.7. Effects of light regime and lumiflavin on 2,4-dichlorophenol formation. Data shown are the means of six replications ± SE. Data points for which error bars are missing have low SE.
2,4-DCP formation in treatments containing 10 mg L\(^{-1}\) riboflavin solution were much higher (>240 µM) and showed a slight increase from 120 to 480 min. Apparently this was because there remained sufficient riboflavin and/or lumiflavin in the media by 480 min to continue the 2,4-D sensitization process. At lower riboflavin concentrations under UV+VL, overall 2,4-DCP concentrations never exceeded 60 µM and remained essentially constant after 120 min for the 2.5 mg L\(^{-1}\) riboflavin treatment and after 360 min for the 5 mg L\(^{-1}\) riboflavin treatment. Under the VL treatment, 2,4-DCP formation followed a similar pattern to that observed in the UV+VL treatment (Figure 3.5 B).

2,4-DCP formation was influenced by the pH of the reaction medium (Figure 3.6). At lower pH there was increased formation of 2,4-DCP as a result of increased degradation of 2,4-D. Under UV+VL, 2,4-DCP concentration at pH 4.5 generally increased until 240 min, and relatively high variability among replicates at pH 4.5 may be indicative of other factors influencing 2,4-DCP formation and/or photolysis under acidic conditions. At pH 6 under UV+VL, 2,4-DCP formation was less variable and increased from 30 to 360 min, followed by a decrease at 480 min (Figure 3.6 A). Under VL, at pH 4.5 2,4-DCP concentration in the medium continued to increase throughout the experiment and there was no decline in 2,4-DCP concentration at pH 6, perhaps indicating that light of insufficient energy was available for sensitized 2,4-DCP photolysis compared to UV+VL treatments (Figure 3.6 B).

Two photoproducts were produced from riboflavin photolysis in aqueous solution under both UV+VL and VL. They were lumichrome, a major photoproduct, and lumiflavin, a minor photoproduct. Several pathways exist for the formation of lumichrome and lumiflavin from riboflavin and
has been postulated that the photodegradation products of riboflavin are formed due to hydrogen abstraction from all positions on the ribityl side chain (Fife, 1977).

Lumichrome formation from riboflavin photolysis was influenced by the pH of the reaction mixture in which riboflavin was exposed (Figure 3.4). At pH 4.5 and 6 the production of lumichrome in solutions exposed to UV+VL was lower than that which occurred at pH 7.5 for the first 360 min exposure (Figure 3.4 A). These data support the kinetic data for 2,4-D photolysis (Table 3.1) and indicate that the riboflavin molecule is less photolabile under acidic conditions and is thus able to avoid photolysis and/or sensitize 2,4-D photolysis over a longer period than at higher pH. Lumichrome concentration at pH 7.5 ranged from 18 to 34 μM during the first 360 min of exposure and began to show a sharp decline beginning at 240 min, apparently due to sensitized photolysis by either the remaining riboflavin or some other secondary photopродuct. In the VL-treated solutions, lumichrome formation at pH 7.5 rose to 31 μM after 1 h exposure but declined to an average of 14 μM and remained there for the rest of the experiment (Figure 3.4 B). In contrast to the UV+VL treatment, lumichrome formation at pH 6 increased steadily from 14 μM at 120 min exposure to 25 μM at 480 min, apparently due to a lack of UV-sensitized lumichrome photolysis. At pH 4.5, lumichrome was detected at a concentration of approximately 8 μM only during the first 60 min of exposure.

**Herbicide-photopродuct and sensitizer-photopродuct interactions.** In the experiment to determine the effects of photopродucts on 2,4-D photolysis, there was no photolysis of 2,4-D in treatments containing
only lumichrome or 2,4-DCP as possible sensitizers (data not shown). In contrast, 10 mg L$^{-1}$ lumiflavin enhanced 2,4-D photolysis in aqueous solution to a greater extent than 10 mg L$^{-1}$ riboflavin as observed in previous experiments. The half-life of 2,4-D in presence of lumiflavin was 1 and 2 h under UV+VL and VL, respectively (data not shown), compared to extrapolated photolysis half-lives of 7 and 11 h under UV+VL and VL, respectively, in the presence of riboflavin (Table 3.1). The net photolysis rate constant of 2,4-D in the presence of lumiflavin under UV+VL was 0.6780 h$^{-1}$ ($r^2=0.87$) and was sevenfold higher than the rate constant of 0.0963 h$^{-1}$ obtained in presence of 10 mg L$^{-1}$ riboflavin (Table 1). However, 2,4-D photolysis in aqueous solution adjusted to pH 4.5 and exposed to UV+VL in presence of 10 mg L$^{-1}$ riboflavin had a net photolysis rate constant of 0.6962 h$^{-1}$, which was similar to the net photolysis rate constant obtained by lumiflavin-sensitized 2,4-D photolysis. Lumiflavin-sensitized 2,4-D photolysis under VL was also greater ($k_p=0.5544; r^2=0.98$) than that observed in the presence of riboflavin.

Results from this experiment indicate that lumiflavin was a stronger sensitizer of 2,4-D photolysis in aqueous solution than riboflavin except when pH of the riboflavin treatment solution was adjusted to 4.5. Literature regarding lumiflavin being used as a sensitizer in aqueous photolytic studies of herbicides or pollutants is scant. For a compound to be an efficient sensitizer it should have a longer half-life and should absorb light strongly at longer wavelengths than those of the acceptor. There was a significant overlap of lamp emission and the UV-visible absorption spectrum of lumiflavin, which is similar to that of riboflavin (Figure 3.2). One possible explanation for
the relative effectiveness of lumiflavin as a sensitizer compared to riboflavin is that, under UV+VL and VL in the absence of other compounds, the half-life of lumiflavin was 5 and 6 h respectively (Table 3.2) compared to 1.5 and 2 min respectively, for riboflavin (Table 3.3). The efficiency of a photosensitized reaction will decrease if the sensitizer is lost during the process of sensitization (Herkstroeter et al., 1964), so the relative stability of lumiflavin may have contributed to its effectiveness as a sensitizer.

An additional property of lumiflavin, greater triplet life times may also contribute to its effectiveness as a sensitizer. Photophysical properties like quantum yield of phosphorescence and intersystem crossing of a sensitizer will directly influence the process of sensitization. Under ideal situations, lower quantum yield for phosphorescence coupled with higher quantum yield for intersystem crossing results in higher production of triplets. Riboflavin is considered an efficient sensitizer because it has a low quantum yield of phosphorescence but high quantum yield for intersystem crossing (Moore et al., 1977). Even though lumiflavin can be formed from riboflavin photolysis in aqueous solution, it has photophysical properties very similar to riboflavin (Heelis, 1982). However, lumiflavin has a lower triplet decay rate compared to riboflavin (Naman and Tegner, 1986). The lower triplet decay rate indicates that lumiflavin will have a longer triplet lifetime compared to riboflavin and will therefore have a greater probability of being involved in sensitization reactions.

Under VL, 2,4-DCP formed in the presence of lumiflavin at 480 min was around 240 μM (Figure 3.7). In contrast, 2,4-DCP concentration under UV+VL increased to 130 μM by 60 min but then declined with longer
Table 3.2. Photolysis of lumichrome and lumiflavin in the presence and absence of 2,4-D in aqueous solution. Data shown are half-lives \(t_{1/2}\), first-order rate constants \(k_p\), and coefficients of determination \(r^2\) for the linear regression of log percent lumichrome or lumiflavin remaining on exposure time.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(t_{1/2}) (h)</th>
<th>(k_p) (h^{-1})</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light regime</td>
<td>Light regime</td>
<td>Light regime</td>
<td></td>
</tr>
<tr>
<td>UV+VL</td>
<td>VL</td>
<td>UV+VL</td>
<td>VL</td>
</tr>
<tr>
<td>Lumc alone</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+ 2,4-D</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lumf alone</td>
<td>5</td>
<td>6</td>
<td>0.1478a</td>
</tr>
<tr>
<td></td>
<td>0.1107a</td>
<td>0.96</td>
<td>0.78</td>
</tr>
<tr>
<td>+ 2,4-D</td>
<td>1</td>
<td>5</td>
<td>0.4897b</td>
</tr>
<tr>
<td></td>
<td>0.1260a</td>
<td>0.87</td>
<td>0.98</td>
</tr>
</tbody>
</table>

\(^a\)Rate constants within each light regime that are followed by the same letter do not differ significantly at the 0.05 level according to Tukey’s HSD.
Abbreviations used: UV+VL=Ultraviolet+visible light, VL=visible light, Lumc=lumichrome, Lumf=lumiflavin, 2,4-D=(2,4-dichlorophenoxy)acetic acid, ‘-‘=no photolysis.
exposure times and was not present after 360 min exposure. These data indicate that lumiflavin is also more efficient at sensitizing 2,4-DCP photolysis than riboflavin, particularly when exposed to supplemental UV radiation.

Lumiflavin degradation in aqueous solution in the presence of 2,4-D proceeded by pseudo-first-order kinetics under UV+VL and the half-life was 1 h compared to 5 h in control (Table 3.2). This data indicates that during the process of 2,4-D sensitization, lumiflavin also can apparently undergo enhanced photolysis. Larson et al., (1992) similarly reported that riboflavin undergoes photolytic degradation during the process of sensitizing aromatic compounds.

In the presence of 2,4-D and 2,4-DCP, riboflavin had a half-life of 61 and 66 min under UV+VL and VL respectively, compared to 1.5 min in the absence of other compounds (Table 3.3). The photolytic rate constants did not differ significantly between the treatments containing 2,4-D and 2,4-DCP. The increased half-life of riboflavin in presence 2,4-D and 2,4-DCP is attributable to the quenching of excited state riboflavin by those compounds, thus "protecting" riboflavin against direct photolysis by UV light. Riboflavin has high triplet energy compared to 2,4-D or 2,4-DCP which results in riboflavin transferring the absorbed energy to those compounds. Tanaka et al., (1986) reported that some herbicides may undergo delayed photolysis because they are capable of transferring excitation energy to surfactants in aqueous solution through the process of photosensitization. They observed that monuron and chlorsulfuron caused sensitized photolysis of a nonionic surfactant, hexaethoxylated 2,6,8-trimethyl-4-nonanol (TMN-6) while glyphosate, 2,4-D, and diquat showed no sensitization effect.
Table 3.3. Photolysis of riboflavin in the presence and absence of 2,4-D in aqueous solution. Data shown are half-lives ($t_{1/2}$), first-order rate constants ($k_p$), and coefficients of determination ($r^2$) for the linear regression of log percent riboflavin remaining on exposure time.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$t_{1/2}$</th>
<th>$k_p$</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light regime</td>
<td>Light regime</td>
<td>Light regime</td>
</tr>
<tr>
<td></td>
<td>UV+VL</td>
<td>VL</td>
<td>UV+VL</td>
</tr>
<tr>
<td>Ribo alone</td>
<td>1.5</td>
<td>2</td>
<td>0.4509a</td>
</tr>
<tr>
<td>+ 2,4-D</td>
<td>61</td>
<td>61</td>
<td>0.6749a</td>
</tr>
<tr>
<td>+ 2,4-DCP</td>
<td>61</td>
<td>66</td>
<td>0.6749a</td>
</tr>
</tbody>
</table>

*Rate constants within in each light regime that are followed by the same letter do not differ significantly at the 0.05 level according to Tukey’s HSD.

Abbreviations used: UV+VL=Ultraviolet+visible light, VL=visible light, Ribo=riboflavin, 2,4-D=(2,4-dichlorophenoxy)acetic acid, 2,4-DCP=2,4-dichlorophenol, ‘-’=no photolysis.
The laboratory studies on the photochemistry of 2,4-D in aqueous solution presented here have significance with regard to herbicide persistence parameters. The findings confirm that direct photodecomposition of 2,4-D in aqueous solution under environmental conditions is not possible; however, the UV portion of the sunlight spectrum can still play an important role in the photodecomposition of 2,4-D through photosensitization processes. Riboflavin is an effective sensitizer of 2,4-D photolysis and is a naturally occurring compound low in toxicity. Its effectiveness as a sensitizer of 2,4-D photolysis increases as pH of the media decreases from 7.5 to 4.5. Lumiflavin's effectiveness as a sensitizer at pH 6 is similar to that of riboflavin at pH 4.5. It should be noted, however, that the major photoproduct of both riboflavin- or lumiflavin-sensitized 2,4-D photolysis is 2,4-DCP which may be of equal or greater concern than 2,4-D. However, it is significant that lumiflavin is much more efficient at sensitizing 2,4-DCP photolysis than riboflavin under enhanced UV conditions.

Another important outcome of this study is the role of pH in photochemical reactions. Reducing the pH of the media resulted in the stability of the riboflavin molecule and thus a more rapid and complete photolysis of 2,4-D. While the pH effect on sensitization will vary with the compounds in question, it should nonetheless be a critical consideration in the selection of sensitizers for a given system.
CHAPTER 4

Photolytic Degradation of 2,4-D on the Corn Leaf Surface

Abstract. Studies were conducted in a controlled environment-growth chamber to determine the effects of UV light and riboflavin on 2,4-D and its residues on the corn leaf surface. Alone and in combination with riboflavin, 2,4-D was applied in droplets to the third leaf of corn and then harvested after 0, 3, 10, 24, and 42 h exposure to either UV-enhanced or UV-attenuated light. Riboflavin-sensitized 2,4-D photolysis occurred under both light regimes but a larger percentage of surface residues under visible light was intact 2,4-D. Sensitized 2,4-D photolysis also apparently occurred in the absence of riboflavin. Riboflavin-sensitized photolysis of 2,4-D resulted in 67% greater degradation of surface 2,4-D residues than in the absence of riboflavin during the first 10 h after treatment. There was no difference in 2,4-D photolysis at 24 and 42 h as riboflavin underwent rapid photolysis under both UV and visible light. More than 90% loss of applied riboflavin was observed by 10 h, thus accounting for the lack of differences in photolysis in treatments with and without riboflavin at 24 and 42 h. On glass slides ≥95% of applied 2,4-D was not degraded in the absence of riboflavin, and 60% was degraded after 42 h in the presence of
riboflavin. Riboflavin did not have any effect on the absorbed 2,4-D distribution within the plant. Nomenclature: 2,4-D, (2,4-dichlorophenoxy)acetic acid); Corn, *Zea mays* L.

Additional index words. Photolysis, leaf surface, riboflavin.
INTRODUCTION

2,4-D is one of the most well-known phenoxy herbicides and is widely used in postemergent sprays to control broadleaf weeds in small grains, corn, sorghum, and turf (Ross and Lembi, 1985). The most important plant organs involved in the interception, absorption, and translocation of postemergent herbicide sprays are the leaves and the primary barrier for the uptake of foliar-applied agricultural chemicals is the leaf cuticle. The cuticle is a non-living, lipophilic membrane which is non-cellular in nature. The principle structural component of the cuticle is the cutin matrix, which is comprised of highly cross-linked polymers of hydroxylated fatty acids (Kolattukudy, 1981). The cuticle is covered by an outermost layer of wax called epicuticular wax. The ultra structure of the epicuticular wax and its thickness varies with species, leaf age, and environmental conditions during wax deposition (Baker, 1982).

Physicochemical properties including the pKa of the herbicide and spray solution pH may also influence the process of herbicide penetration through the leaf cuticle. The pKa of 2,4-D (a weak carboxylic acid) is 2.54 and normally the pH of a spray tank mix ranges from 5 to 8, thus 2,4-D in contact with the cuticle within a spray droplet is typically in its ionized form. The nonionized species of 2,4-D moves more readily through the cuticle compared to the ionized species due to its lower polarity and therefore its greater solubility in the non-polar wax and other lipophilic cuticular components (Riederer and Schonherr, 1984). Alternatively, 2,4-D in its polar ionized form resists
cuticular penetration and can accumulate in the outer cuticular layer or surface. The presence of hexadecanoic acid – a constituent of the cutin polymer, can covalently bond with 2,4-D molecule to render it immobile (Riederer and Schonherr, 1986). Plants that avoid 2,4-D injury (e.g., corn) do so by immobilizing 2,4-D in leaf tissues (Slife et al., 1962) and also by metabolism (Hutber et al., 1978; Cohen and Bandurski, 1982; Reinecke and Bandurski, 1987). Chkanikov et al., (1971) reported that about 30% of the 2,4-D applied to corn remained on the leaf surface 72 h after treatment. Thus in addition to immobilization and metabolic deactivation, limitations on absorption may play an additional role in corn tolerance to 2,4-D and result in a significant quantity of dislodgable or weakly bound 2,4-D residue on the leaf surface.

Dislodgable or weakly adsorbed herbicide surface residues increases the probability of off-target herbicide movement and contamination of natural water supplies (Leonard and Knisel, 1988). In addition, bound chemical residues can be released from the cutin by fungi and microorganisms which produce cutinases that break bonds between the cutin polymer and xenobiotics (Kolattukudy et al., 1981). The amount of bound xenobiotic present in the plant material in some fields can exceed that held by sorption in the cuticle (Holloway and Deas, 1973). Many unbound herbicide residues are subject to several possible degradation and transfer processes. Among the degradation processes, photolysis reactions mediated by the UV component of sunlight are capable of causing the greatest number of chemical transformations and may play an important role in the degradation of some herbicides.

Herbicide photolysis may occur directly or indirectly (Cessna and Muir, 1991). Direct photolysis may occur when the herbicide is capable
of absorbing UV radiation. In the environment direct photolysis requires that a herbicide absorb wavelengths >290 nm, since wavelengths <290 nm do not reach the earth's surface (Kagan, 1993). Transformation reactions from direct UV absorption include oxidation, reduction, isomerization, or elimination (Crosby, 1969). Indirect herbicide photolysis occurs when a donor compound (photosensitizer) in the reaction medium absorbs UV radiation and transfers excitational energy to an acceptor molecule (e.g., a reactive intermediate or a herbicide) which then undergoes chemical transformation. Indirect photolysis via sensitization is an important process in the degradation of many herbicides in the environment and is the only photochemical transformation mechanism possible for those herbicides which do not absorb UV light >290 nm (Plimmer, 1970; Miller and Zepp, 1983). One such compound for which indirect photolysis is the only possible mechanism of phototransformation by sunlight is 2,4-D, which does not absorb wavelength >290 nm.

There are very few published studies on the photolysis of pesticide residues on leaf surfaces. In some instances, it may be difficult to distinguish photolysis from other transfer or transformation pathways that occur on plant surfaces. It is also possible that some pathways, for example plant and microbial metabolism, produce the same-end products following transformation (Bandal and Casida, 1972; Ivie et al., 1973; Johnsen and Martin, 1983). The presence of natural sensitizers on the leaf surface, whether produced by the plant or from elsewhere, may also influence the process of photodegradation. Model surfaces have been used in an attempt to control these inherent variabilities present on leaf surfaces and also for ease
in isolation and identification of photoproducts. One of the most commonly used model surfaces in herbicide photolysis studies has been glass and silica gel thin-layer chromatography plates (Nilles and Zabik, 1974; Nilles and Zabik, 1975; Herrmann et al., 1985).

Several disadvantages are associated with using model surfaces for studying herbicide photolysis. The rate of herbicide photodegradation and the photoproducts formed in the process can be strongly influenced by the nature of the medium in which the reaction occurs. Therefore, herbicide photolysis reactions which occur on model surfaces may likely not be identical to those occurring on a leaf surface. For example, the film thickness of the droplet deposit on silica gel may be greater than that of the same droplet applied to a leaf, resulting in the photolysis of the upper region of the film while the lower regions of the herbicide remains intact. The physical interaction (i.e., adsorption) between the herbicide molecule and the reaction surface can also result in a shift or change in the herbicides light absorption properties, thereby altering the energy required to bring about photodecomposition (Slade, 1966; Gab et al., 1975). These changes in the pattern of a herbicide's absorption wavelength might therefore differ between model surfaces and leaves. An additional area where research is lacking is the fate of herbicide photoproducts on intact leaves. Distinguishing absorbed photoproducts from biochemical metabolites inside the plant and determining whether plants further translocate and metabolize absorbed photoproducts is an intriguing yet neglected area of research in herbicide-plant interactions. Studies conducted on the photolysis of herbicides on leaf surfaces thus far have not considered the fate of photoproducts in terms of absorption and translocation in the plant.
following photolysis on the leaf surface.

The nature and extent of 2,4-D photolysis on leaf surfaces has not been reported. A few of the leaf surface studies conducted with other agricultural chemicals have been presented here as evidence that direct and indirect photolysis of pesticides can occur on the leaf surface. Ivie and Casida (1971), reported increased degradation of chlorinated cyclodienes, dinobuton, and dinoseb on bean leaves upon exposure to sunlight in presence of rotenone as a sensitizer. Rotenone at a concentration of 10 mg L\(^{-1}\) resulted in the conversion of 50% of the dieldrin residue on bean leaves to photodieldrin compared to 5% in the absence of rotenone. Photodecomposition of the insecticide azinphosmethyl was reported on soils, leaf surfaces, and glass upon exposure to sunlight (Liang and Lichtenstein, 1976).

The increasing use of postemergence herbicides, the concern for reducing environmental contamination by herbicides, and the extensive use of 2,4-D worldwide justifies this study on 2,4-D photolysis on the leaf surface. The objective of this study was to determine the effects of light regime and riboflavin on photolysis of 2,4-D and its residues on corn leaves.
MATERIALS AND METHODS

Corn (‘Pioneer 3241’) seeds were sown in a silt-loam soil, vermiculite, and peat moss mixture (1:1:1 v/v/v) in 4-cm-diam by 20-cm deep plastic containers. After emergence, seedlings were thinned to one plant per container and grown in a greenhouse equipped with high-pressure sodium lamps that provided approximately 450 μE m\(^{-2}\) s\(^{-1}\) PPF supplemental lighting for 14 h daily. Daytime temperatures ranged from 24 to 28 C and night temperatures ranged from 20 to 23 C. Plants were transferred to a controlled-environment growth chamber 24 h prior to treatment application. The growth chamber had both fluorescent and incandescent lights that provided 300 μE m\(^{-2}\) s\(^{-1}\) PPF for a 14 h daily photoperiod. Daytime temperatures ranged from 27 to 28 C, night temperatures ranged from 22 to 23 C, and relative humidity ranged from 28 to 30% in the growth chamber. Plants were watered regularly and fertilized twice a week with 200 mg L\(^{-1}\) NPK fertilizer solution (20:20:20 [w/v]).

Aqueous stock solutions of 100 mg L\(^{-1}\) 2,4-D (98% purity) were prepared in deionized, distilled water either alone or in mixture with 10 mg L\(^{-1}\) riboflavin (99% purity). The pH of the solution was adjusted to 6 with 1 M KOH. 0.25% (v/v) oxysorbic (20 POE) surfactant was added to both treatment solutions. Stock solutions of \(^{14}\)C-2,4-D were prepared using uniformly phenyl-ring labeled herbicide (specific activity=743 GBq mmol\(^{-1}\)) dissolved in 500 μl ethanol and water (1:1, v/v). Tritiated riboflavin stock solutions were prepared from general-labeled riboflavin (specific activity=1221 GBq mmol\(^{-1}\)) dissolved in 524 μl ethanol and
water (1:1, v/v). Solutions were prepared just prior to application to corn leaves by spiking aliquots of the aqueous stock solutions with radioactivity. The specific radioactivity of the treatment solutions were then quantified by liquid scintillation counting (LSC). The 2,4-D+riboflavin treatment solution contained 100,000 dpm µl⁻¹ each of ¹⁴C-2,4-D and ³H-riboflavin. The 2,4-D alone treatment solution contained 100,000 dpm µl⁻¹ ¹⁴C-2,4-D. Decay correction and dual-label counting were employed for treatments containing ³H-riboflavin. Counts were corrected for quenching, background, and dilution and the resulting data were reported as disintegrations per minute (dpm). Ultraviolet-visible absorption spectra of the compounds investigated in this experiment were obtained using a diode-array spectrophotometer.

Corn plants were 20 cm tall and had four leaves when treated in the growth chamber. Treatment solutions were agitated with a vortex mixer just before application and treatments were applied to leaves with a microsyringe fitted with a repeating dispenser that delivered 0.5 µl droplets. A total of 5 µl treatment solution containing 16 kBq (2,4-D+riboflavin) or 8 kBq (2,4-D alone) radioactivity was applied as 10-0.5 µl droplets to a 2.5 cm⁻² area near the mid-portion of the adaxial surface of the fully expanded third leaf. No droplets were applied on the midvein of the leaf. The UV irradiation source and the arrangement of UV lights in the growth chamber was similar to that used previously in aqueous photolysis experiments, explained in detail in chapter 3. Twenty-four hours after applying the herbicide the plants were exposed to visible and supplemental UV light (UV+VL) in the growth chamber. Plants were exposed to UV light only during the 14 h visible light photoperiod each day. Visible light-only (VL) controls were placed under
the light source and shielded with a thin sheet of coated polycarbonate (CP) that blocks most of the UV light while allowing transmission of the visible spectrum. Nonirradiated (dark) controls consisted of plants in which the treated spot was completely shielded with aluminum foil. Plants were harvested after 0, 3, and 10 h of exposure during the first day, then after 10 h exposure on the second day, and at the end of the light period on the third day, resulting in a time-course harvest of plants following 0, 3, 10, 24, and 42 h exposure to light (photoperiod) over the 3 days. Trifluralin-acetonitrile actinometry was used to quantify the UV flux on shielded versus non-shielded plants (Draper, 1985; Venkatesh et al., 1993).

Upon harvest, the treated area of the treated leaf was washed by consecutive immersions in 10 ml distilled water adjusted to pH 8 to remove the nonabsorbed herbicide and riboflavin. The treated area of the treated leaf was then allowed to dry for 5 minutes at room temperature. A thick layer of viscous cellulose acetate solution (5%, [w/v] cellulose acetate in acetone) was applied to the surface of the treated area of the treated leaf using a small paint brush and allowed to dry. The dried cellulose acetate strip was then peeled away to remove 2,4-D and riboflavin deposits remaining after the aqueous wash that had partitioned into the epicuticular wax (Silcox and Holloway, 1986). The film was added directly into 10 ml of the scintillation cocktail for LSC.

After removal of nonabsorbed residues, absorbed radioactivity was quantified by sectioning of the treated plants into four parts: the treated leaf, leaves above the treated leaf, leaves below the treated leaf, and stem portion. The plant parts were homogenized in methanol and
filtered through #1 filter paper. The filter paper containing the residue was rinsed with 3 aliquots of 1-ml methanol and dried at room temperature (30 C), then oxidized by combustion for quantification of non-extractable $^3$H and $^{14}$C. The tritiated water vapor and $^{14}$CO$_2$ evolved as a result of combustion were trapped in respective $^3$H and $^{14}$C trapping solution/cocktail and quantified by LSC.

**Extraction of parent compounds and degradation products.** The pH of the pooled aqueous leaf washes were adjusted to 2 with 1 N HCl. After adjusting the pH, a 1-ml aliquot of the leaf wash was added to 10 ml of liquid scintillation cocktail for radioassay. The remaining leaf wash was subjected to solid phase extraction (SPE), described later.

The various plant parts were homogenized in methanol, and the extract was transferred to a flask and the solvent was removed by rotary evaporation at 37 C. The residue in the flask was resuspended in 10 ml pH 8 water and then was adjusted to 2 with 1 N HCl before SPE, described later. A 1-ml aliquot was radioassayed by LSC. After removing the residues, the flask was washed with 15 ml methanol to help remove additional residues adhering to the glass. The methanol wash was transferred to a vial and radioactivity in a 1-ml aliquot was quantified by LSC. All dpm values were then converted to percent of total $^{14}$C-2,4-D and $^3$H-riboflavin recovered for each plant.

**Solid Phase Extraction.** A SPE system was used for the extraction of $^{14}$C-2,4-D, $^3$H-riboflavin, and degradation products from the leaf wash as well as the plant extract. The C-18 reverse-phase cartridge columns were preconditioned by passing 3 ml methanol followed by 3 ml water adjusted to pH 2 with 1 N HCl through the column. After conditioning, leaf washes
or the plant extracts were passed through the cartridge then eluted with four successive 1-ml aliquots of methanol and retained for further analysis by HPLC. One ml of the extracted waste solution was radioassayed to ensure that all radioactivity was retained by the column.

**Instrumentation for analysis.** After preparing the leaf wash and plant extracts by SPE, the samples were analyzed for the parent herbicide (2,4-D), riboflavin, and degradation products by High Pressure Liquid Chromatography (HPLC) with in-line UV absorbance detection at 240 nm and a β-radioactive flow detector. Samples were assayed on a C-8 reverse-phase column with a mobile phase of acetonitrile and 0.2% aqueous formate (50:50, v/v). The presence of 2,4-D and riboflavin were confirmed by cochromatography with reference standards.

In this study, the effects of light regime, sensitizer, and harvest interval on 2,4-D photolysis was measured by the amount and distribution of parent and degradation products present in the leaf wash and plant extracts by using the radioactivity detector. The parent as well as degradation products were expressed as percent of total activity in each sample.

The peaks identified as major degradation products of $^3$H and $^{14}$C photolysis were not present in the parent solution and dark controls. This clearly suggests that these peaks were not due to any impurities present in the parent solution and it did not undergo any degradation during storage.

An examination of the detailed photolysis reactions is beyond the scope of this study. Many unstable intermediates are generated in the course of photoalteration and metabolism of 2,4-D and riboflavin.
forming terminal residues that have not been characterized. The molecular structure of the various suspected degradation products separated by HPLC and radioactivity detector is not known. We are continuing our investigation on the characterization of reaction mechanisms and degradation products.

**Study of 2,4-D photolysis on glass as a model surface.** In separate experiments, 2,4-D photolysis was investigated in presence of riboflavin on glass slides for comparison with results obtained on the leaf surface. Aqueous treatment solutions of 100 mg L\(^{-1}\) of 2,4-D were prepared as described for the corn leaf experiments, only 2,4-D+riboflavin treatments were spiked with 2,650 and 12,000 dpm \(\mu\)L\(^{-1}\) each of \(^3\)H-riboflavin and \(^{14}\)C-2,4-D. The 2,4-D alone treatment was spiked with 2,700 dpm \(\mu\)L\(^{-1}\) of \(^{14}\)C-2,4-D.

The treatment solutions were deposited on a pre-cleaned glass 7-by 2-cm microscope slide using a microsyringe fitted with a repeating dispenser that delivered 0.5 \(\mu\)L droplets. Treatment solutions were agitated with a vortex mixer just before application and a total of 65 \(\mu\)L containing 15 kBq (2,4-D+riboflavin) or 3 kBq (2,4-D alone) radioactivity was applied as 130-0.5 \(\mu\)L droplets uniformly over the entire upper surface of the slide which eventually coalesced within the total surface area of 28 cm\(^2\). The slides were then allowed to dry at room temperature (30 C) prior to irradiation. The treated slides were then exposed to UV light in the growth chamber with the appropriate controls as described earlier for the leaf surface experiments. The glass slides were randomly distributed on the top of a box positioned 28 cm directly underneath the light source, and irradiated over a time course of 0, 1, 3, 7, and 11 h.
At harvest, the slides were placed in a covered glass petri dish (10-cm diam by 2-cm height) and gently shaken for 5 min with 20 ml distilled water adjusted to pH 8. The shaking was followed by gentle scrubbing of the slide with a rubber spatula to help dislodge any adsorbed residues that were not removed by shaking. After scrubbing, the rubber spatula was washed with 2 ml distilled water adjusted to pH 8 to help remove additional residues adhering to it. The pH of the wash was then adjusted to 2 with 1 N HCl. A 1-ml aliquot of the wash was retained for radioassay. The remaining rinsate was put through SPE, the parent compounds and degradation products were assayed as described earlier.

**Data analysis.** Experiments were conducted in a randomized complete block design with 3 replications. Treatments were arranged factorially with light regime, sensitizer, and harvest interval as factors. Each experiment was repeated and the data were combined for analysis (Steel and Torrie, 1980). Treatment means were separated at the 5% level of significance by Fisher's protected least significance (LSD).
RESULTS AND DISCUSSION

The UV light source inside the growth chamber emitted the major portion of its radiation in the UV region from 300 to 400 nm (peaking at 356 nm), and the average trifluralin actinometer half-life inside the growth chamber was 6 h under direct UV light and 103 h under the coated polycarbonate (CP) sheet. Based on these actinometric half-lives, the UV light intensity inside the growth chamber was calculated to be 550 and 30 µW cm⁻² under direct UV light and CP respectively. Thus, there was a significant attenuation (but not total occlusion) of UV light by the CP. Hereafter and for simplicity the two light regimes, direct UV+visible light and attenuated UV+visible light, will be referred to as UV+VL and VL, respectively.

Preliminary investigation revealed a significant overlap of UV lamp emission and the UV-visible absorption spectrum of riboflavin, suggesting that riboflavin might undergo direct photolysis and/or act as photosensitizer in this experimental system (Figure 4.1). The UV cutoff for 2,4-D is about 300 nm according to spectral data, indicating a low possibility for it to undergo direct photolysis in this system, since the lamps employed emit little if any light at wavelengths <300 nm (Figure 4.1). Similarly, 2,4-D does not undergo direct photolysis in the environment due to absorption of solar wavelengths <290 nm by the earth's ozone layer (Kagan, 1993).

The total ¹⁴C recovered from corn as a percent of that applied was influenced by the light regime and presence or absence of riboflavin (Table 4.1). Averaged over other factors, significantly less ¹⁴C was
Figure 4.1 Irradiance spectrum of the UV source (A) and UV-visible absorption spectrum of the compounds investigated in this study (B and C).
Table 4.1. Effects of light regime, sensitizer, and harvest interval on the total recovery of \(^{14}\text{C}\) from corn\(^a\).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Sensitizer (S)</th>
<th>(\pm R)</th>
<th>(-R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV+VL</td>
<td>71a</td>
<td>82a</td>
<td></td>
</tr>
<tr>
<td>VL</td>
<td>77b</td>
<td>84b</td>
<td></td>
</tr>
</tbody>
</table>

LSD (0.05) LR x S                      1.6

HI', h

<table>
<thead>
<tr>
<th></th>
<th>(\pm R)</th>
<th>(-R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>78a</td>
<td>80a</td>
</tr>
<tr>
<td>10</td>
<td>78a</td>
<td>80a</td>
</tr>
<tr>
<td>24</td>
<td>76a</td>
<td>80a</td>
</tr>
<tr>
<td>42</td>
<td>76a</td>
<td>78a</td>
</tr>
</tbody>
</table>

LSD (0.05) S x HI                      NS

\(X^b\)                                         76A     81B

\(^a\)Means within a column and factor followed by the same lower case letter are not significantly different (\(P=0.05\)) according to Fisher's LSD.

\(^b\)Main effect means within a row and factor followed by the same capital letter are not significantly different (\(P=0.05\)) according to Fisher's LSD.

Abbreviations used: \(\pm R\)=treatments with riboflavin, \(-R\)=treatments without riboflavin, LR=light regime, UV+VL=Ultraviolet+visible light, VL=visible light, HI=harvest interval, NS=F values are not significant.

\(\dagger\) Plants were exposed to UV+VL and VL 24 h after applying the treatments.
recovered in treatments containing riboflavin compared to those without riboflavin. Within each sensitizer treatment, $^{14}C$ recovery was somewhat higher under VL than under UV+VL especially in the treatments containing riboflavin. The $^{14}C$ recovered did not differ significantly among harvest intervals regardless of treatment.

Total recovery of $^{14}C$ from treated corn plants averaged about 79% of that applied. However, there was an average of 90% recovery from the dark control plants observed over the 4-day course of the experiments. The 10% $^{14}C$ loss from the dark controls may be attributable in part to volatilization, thermal degradation, surface biodegradation and/or experimental error due to handling losses during extraction. The additional 5 to 20% of applied $^{14}C$ lost from exposed plants may then be partially attributable to an enhancement of one or more of the various processes which occurred in the dark controls and/or the formation of relatively non-polar $^{14}C$-photoproducts that volatilized from the leaf surface. The 84% average recovery of $^{14}C$ in plants under VL in the absence of riboflavin was close to recovery in the dark controls, suggesting that loss of volatile photoproducts may have been the primary source of the additional $^{14}C$ loss in UV+VL-exposed plants.

The lack of an effect of harvest interval on total $^{14}C$ recovery suggests that these losses of $^{14}C$ from the leaf surface occurred very early upon initial exposure, perhaps before the 2,4-D residue on the leaf surface was adsorbed and/or fully partitioned into the epicuticular wax. Photolysis and loss of surface residues of chlorinated cyclodienes [(insecticides); (Ivie and Casida, 1971)], DNP (herbicide) and dinobuton (acaricide) on bean leaves (Bandal and Casida, 1972), and azinphosmethyl (insecticide) on corn leaves (Liang and Lichtenstein,
1976) has been reported.

Light regime did not differentially affect recovery of applied $^3$H from corn when averaged over other factors (Table 4.2). There was no measurable loss of applied $^3$H in the dark control over the 4-day course of the experiment, but $^3$H recovery in exposed plants ranged from 73 to 90% of applied and generally declined with increasing harvest interval time, presumably due to volatilization of $^3$H-riboflavin degradation products.

Sensitizer, light regime, or harvest interval did not influence the total recovery of $^{14}$C as a percent of that applied to glass (data not shown), and recovery of applied $^{14}$C ranged from 90 to 95%. A loss of 5 to 7% of the applied $^{14}$C occurred in the dark control over the entire course of the experiment under both UV+VL and VL. An additional 3 to 5% of applied $^{14}$C not recovered from glass slides in the presence of riboflavin under UV+VL might be due to limited photolysis of 2,4-D and subsequent volatilization of photoproducts.

Riboflavin recovery from the glass surface also followed the same pattern as that of $^{14}$C and was not influenced significantly by light regime or harvest interval (data not shown). There was no measurable loss of $^3$H in the dark control or under VL during the course of the experiment. A loss of 5 to 10% $^3$H in presence of UV+VL was observed. The rapid loss of $^3$H from the leaf surface and to a lesser extent from glass may be due to volatilization of one or more $^3$H-riboflavin photoproducts from the leaf surface.

Preliminary experiments indicated that the leaf wash technique used in this study consistently recovered 90 to 95% of the applied, nonabsorbed $^{14}$C and $^3$H from the corn leaf surface. Statistical analyses

112
Table 4.2. Effects of light regime and harvest interval on the total recovery of $^3$H from corn$^a$.

<table>
<thead>
<tr>
<th>Factor (HI', h)</th>
<th>Light regime (LR)</th>
<th>UV+VL</th>
<th>VL</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td></td>
<td>90a</td>
<td>82a</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>87a</td>
<td>83a</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>77a</td>
<td>79a</td>
</tr>
<tr>
<td>42</td>
<td></td>
<td>73a</td>
<td>77a</td>
</tr>
</tbody>
</table>

LSD (0.05) LR × HI | NS
X$^b$ | 82A | 80A

$^a$Means within a column and factor followed by the same lower case letter are not significantly different (P=0.05) according to Fisher's LSD.

$^b$Main effect means within a row and factor followed by the same capital letter are not significantly different (P=0.05) according to Fisher's LSD.

Abbreviations used: HI=harvest interval, UV=Ultraviolet-visible light, VL=visible light. NS=F values are not significant.

† Plants were exposed to UV+VL and VL 24 h after applying the treatments.
revealed a significant interaction between light regime and sensitizer and between sensitizer and harvest interval for nonabsorbed $^{14}$C in the leaf wash (Table 4.3). In the presence of riboflavin, an average of 18% of the total recovered $^{14}$C was contained in the leaf wash and there was no difference between light regimes. Similarly, 20% of the recovered $^{14}$C was in the leaf wash of plants treated with $^{14}$C-2,4-D alone and exposed to UV+VL. In contrast, an average of 29% of the recovered $^{14}$C was contained in the leaf wash of the 2,4-D alone treatment exposed to VL, a value similar to that reported by Gauvrit and Gaillardon (1991) for corn plants grown under normal conditions in the growth chamber. The amount of nonabsorbed $^{14}$C in treatments containing riboflavin ranged from 27% of the total $^{14}$C recovered at 3 h to 9% at 42 h. This compares with a range of 40% at 3 h to 13% at 42 h in plants treated with $^{14}$C-2,4-D alone, and the significant difference with and without riboflavin treatments occurred only at the 3 and 10 h harvest intervals. These data indicate that while disappearance of nonabsorbed $^{14}$C was more rapid in the presence of riboflavin during the first 10 h, nonabsorbed $^{14}$C residues on the leaves were approximately the same for treatments with and without riboflavin after 24 h.

The distribution of $^{14}$C-2,4-D and $^{14}$C-degradation products in the leaf wash was also influenced by light regime, sensitizer, and harvest interval. Averaged over other factors, the percentage of nonabsorbed $^{14}$C as parent $^{14}$C-2,4-D in the presence of riboflavin under UV+VL was 17% compared to 42% in the absence of riboflavin (Table 4.3). Similarly under VL, 35% of surface $^{14}$C residues was $^{14}$C-2,4-D in the presence of riboflavin compared to 53% without riboflavin. These data show that riboflavin effectively sensitized photolysis of 2,4-D residues on the
Table 4.3. Effects of light regime, sensitizer, and harvest interval on surface residues and distribution of $^{14}$C-2,4-D applied to corn leaves$^a$.

<table>
<thead>
<tr>
<th>Nonabsorbed $^{14}$C (leaf wash)</th>
<th>Remaining as parent $^{14}$C-2,4-D</th>
<th>Sensitizer (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of recovered $^{14}$C</td>
<td>% of nonabsorbed $^{14}$C</td>
<td></td>
</tr>
<tr>
<td>Factor</td>
<td>LR $^+$</td>
<td>-R</td>
</tr>
<tr>
<td>UV+VL</td>
<td>17a</td>
<td>20a</td>
</tr>
<tr>
<td>VL</td>
<td>19a</td>
<td>29b</td>
</tr>
<tr>
<td>LSD (0.05) LR x S</td>
<td>5.1</td>
<td></td>
</tr>
</tbody>
</table>

| HI $^T$, h                        | 3                             | 27a           | 40a            | 55a           | 64a           |
|                                  | 10                            | 22a           | 33a            | 41a           | 63a           |
|                                  | 24                            | 12b           | 18b            | 15b           | 26b           |
|                                  | 42                            | 9b            | 13b            | 13b           | 16B           |
| LSD (0.05) S x HI                | 7.2                          |               | 17.3           |
| $\bar{x}^b$                      | 18A                          | 26B           | 29A            | 44B           |

$^a$Means within a column and factor followed by the same lower case letter are not significantly different (P=0.05) according to Fisher's LSD.

$^b$Main effect means within a row and factor followed by the same capital letter are not significantly different (P=0.05) according to Fisher's LSD.

Abbreviations used: +R=treatments with riboflavin, -R=treatments without riboflavin, LR=light regime, UV+VL=ultraviolet-visible light, VL=visible light, HI=harvest interval.

$^T$Plants were exposed to UV+VL and VL 24 h after applying the treatments.
corn leaf surface and this effect increased with supplemental UV irradiance. While most of the nonabsorbed $^{14}$C recovered in the 2,4-D alone treatment remained as parent $^{14}$C-2,4-D compared to the riboflavin treatment, it is surprising that an average of 56% of the nonabsorbed $^{14}$C-2,4-D was degraded to other $^{14}$C-products in the absence of riboflavin. These products were not observed in the dark controls, where $^{14}$C-2,4-D was the only radiolabeled compound detected in the leaf wash.

These data suggest that natural compounds may act as in situ sensitizers on the leaf surface. Corn epicuticular wax is composed predominantly of primary alcohols (60 to 70%), aldehydes (20%), esters (15%), and alkanes (1%) (Bianchi et al., 1979; Stevens and Baker, 1987). Although it is a speculation, some primary alcohols, aldehydes, and or esters present in the epicuticular wax of corn might be capable of sensitizing photolysis of 2,4-D residues, but this remains to be investigated. The hydrocarbon-rich environment of the epicuticular wax may also produce the so-called "solubilization effect" whereby 2,4-D undergoes photoreduction in which hydrogen is abstracted from a hydrocarbon medium (Calvert and Pitts, 1966; Tanaka et al., 1981; Harrison and Wax, 1985). Interestingly, the solubilization effect via hydrogen abstraction has been reported as one of the major mechanisms involved in the photolysis of other halogenated aromatic compounds dissolved in organic solvents, including primary alcohols (Plimmer, 1970). Other possible reasons for the greater photolysis of 2,4-D on corn leaves in the absence of riboflavin may involve a shift in the absorption spectrum of 2,4-D to longer wavelengths (>290nm) after adsorption to the leaf surface. The shift in the absorption spectrum might result in 2,4-D absorbing light by itself and undergoing
photochemical degradation. Similar shift in absorption spectrum after adsorption on to the leaf surface and silica gel has been reported for paraquat, polychlorinated aromatics, and DDT (Slade, 1966; Gab et al., 1975).

The nonabsorbed $^{14}$C-2,4-D decreased with increasing harvest interval in both the presence and absence of riboflavin (Table 4.3). Riboflavin-sensitized photolysis of 2,4-D resulted in significantly greater degradation of surface 2,4-D residues than in the absence of riboflavin at 10 h after treatment, but there was no statistical difference at 24 and 42 h. Averaged over harvest intervals, only 29% of the nonabsorbed radioactivity was $^{14}$C-2,4-D in treatments containing riboflavin compared to 44% in the absence of riboflavin.

The cellulose acetate film stripping technique was used to determine the $^{14}$C and $^3$H partitioned into the epicuticular wax. Only trace amounts of radioactivity were found to be present in the epicuticular wax by this method. This suggests that the accumulation of 2,4-D and riboflavin residue inside the epicuticular wax was minimal and that the major portion of the nonabsorbed residues are on the surface of the wax. If this is the case, then the possible reaction mechanisms for 2,4-D photolysis in the absence of an added sensitizer described earlier would be operative mainly at the herbicide-wax interface.

Glass was used as a model surface to compare photolysis on leaf surface because it is inert in nature and lacks natural compounds present on the leaf surface that might act as sensitizers. There was >94% recovery of $^{14}$C applied to glass across all treatments (Table 4.4). Sensitized photolysis of $^{14}$C-2,4-D was observed in presence of riboflavin under both UV+VL and VL. On the contrary, 2,4-D remained
Table 4.4. Effects of light regime, sensitizer, and harvest interval on surface residues and distribution of $^{14}$C applied to glass$^a$.

<table>
<thead>
<tr>
<th>Surface wash (% of applied $^{14}$C)</th>
<th>Remaining as parent $^{14}$C-2,4-D (% of applied $^{14}$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor</td>
<td>Sensitizer (S)</td>
</tr>
<tr>
<td>LR</td>
<td>$^+_R$</td>
</tr>
<tr>
<td>UV+VL</td>
<td>94a</td>
</tr>
<tr>
<td>VL</td>
<td>100b</td>
</tr>
<tr>
<td>UV</td>
<td>100a</td>
</tr>
<tr>
<td>VL</td>
<td>56a</td>
</tr>
<tr>
<td>LSD (0.05) LR x S</td>
<td>1.6</td>
</tr>
<tr>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

| HI, h                                 |                                                           |
| 3                                    | 98a                                                      |
| 10                                   | 98a                                                      |
| 24                                   | 97b                                                      |
| 42                                   | 95a                                                      |
| LSD (0.05) S x HI                     | NS                                                       |
| NS                                   |                                                           |

$^{a}$Means within a column and factor followed by the same lower case letter are not significantly different (P=0.05) according to Fisher's LSD.  
$^b$Main effect means within a row and factor followed by the same capital letter are not significantly different (P=0.05) according to Fisher's LSD.  
Abbreviations used: $^+_R$=treatments with riboflavin, $^-R$=treatments without riboflavin, LR=light regime, UV+VL=ultraviolet+visible light, VL=visible light, HI=harvest interval, NS=F values are not significant.
intact on glass with little or no photolytic degradation in the absence of riboflavin under both light regimes. This is in stark contrast to the leaf surface which supplies an apparently reactive surface that is directly involved in the photochemical reaction(s) of foliar residues of 2,4-D. An average of 38% of the radioactivity applied to glass and exposed to UV+VL in the presence of riboflavin was $^{14}$C-2,4-D compared to 56% under VL. This confirms that riboflavin is capable of sensitizing solid-phase 2,4-D photolysis under UV+VL as well as VL. Again in contrast to results from the leaf surface, the rate of riboflavin-sensitized 2,4-D photolysis on glass was generally slower. The differences in the rate of photolysis of 2,4-D between leaf and glass surface might be due to a) the differences in physical state and or the chemical nature of the reaction medium on which they are deposited, and b) surface adsorption of herbicide molecule to the components of glass that will make them unavailable for direct or indirect photolysis (Hautala, 1978). These results also indicate that photolytic half-life determinations are applicable only to the specific conditions under which the experiment was conducted.

The nonabsorbed $^3$H removed by the leaf wash decreased with time and at the same rate under both UV+VL and VL (Table 4.5). However, light regime had a significant effect on the initial rate of $^3$H-riboflavin degradation on the leaf surface, with only 7% of the recovered $^3$H as $^3$H-riboflavin under UV+VL after 3 h compared to 92% under VL. Intact riboflavin decreased to 10% or less of the nonabsorbed $^3$H under both light regimes by 10 h, and essentially all of the nonabsorbed riboflavin was photodegraded by 42 h. The rapid loss of riboflavin from the leaf surface probably accounts for the lack of a sensitizer effect on $^{14}$C-
Table 4.5. Effects of light regime and harvest interval on surface residues and distribution of $^3$H applied to corn leaves$^a$.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Light regime (LR)</th>
<th>Nonabsorbed $^3$H (leaf wash) (% of recovered $^3$H)</th>
<th>Remaining as parent $^3$H-Ribo (% of nonabsorbed $^3$H)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV+VL</td>
<td>VL</td>
<td>UV+VL</td>
</tr>
<tr>
<td>3</td>
<td>80a</td>
<td>80a</td>
<td>7a</td>
</tr>
<tr>
<td>10</td>
<td>75a</td>
<td>75b</td>
<td>6a</td>
</tr>
<tr>
<td>24</td>
<td>73b</td>
<td>66c</td>
<td>1a</td>
</tr>
<tr>
<td>42</td>
<td>62c</td>
<td>63c</td>
<td>0a</td>
</tr>
</tbody>
</table>

LSD (0.05) LR x HI 5.1 15.8

$^a$Means within a column and factor followed by the same lower case letter are not significantly different (P=0.05) according to Fisher's LSD.

$^b$Main effect means within a row and factor followed by the same capital letter are not significantly different (P=0.05) according to Fisher's LSD.

Abbreviations used: Ribo=riboflavin, HI=harvest interval, UV+VL=ultraviolet+visible light, VL=visible light.

† Plants were exposed to UV+VL and VL 24 h after applying the treatments.
2,4-D photolysis at the 24 and 42 h harvest interval (Table 4.3). Under VL, most of the riboflavin applied was still intact at 3 h. As a result, sensitized photolysis of 2,4-D surface residues was somewhat prolonged but occurred at a slower initial rate than observed under UV+VL.

The $^3$H recovered from the glass surface declined over time in the presence of UV+VL compared to VL, apparently due to formation of volatile $^3$H-photoproducts under enhanced UV (Table 4.6). Under VL, more than 50% of the applied $^3$H was parent riboflavin after 42 h exposure compared to 2% under UV. The rate of riboflavin photolysis from glass surface was generally slower compared to riboflavin photolysis on the corn leaf surface. The slower $^3$H-riboflavin degradation on the glass surface was similar to that observed for $^{14}$C-2,4-D and again illustrates the photocatalytic effect of the leaf surface compared to glass.

Absorption and Distribution of $^{14}$C and $^3$H in corn. In the presence of riboflavin, an average of 82% of the recovered $^{14}$C was absorbed and there was no difference between light regimes (Table 4.7). An average of 81% of the recovered $^{14}$C was absorbed in the absence of riboflavin under UV+VL and 71% was absorbed under VL. There was also significantly more $^{14}$C-absorbed overall in the presence of riboflavin under both light regimes during the first 10 h after treatment. Increased total absorption of $^{14}$C with riboflavin treatments within 3 to 10 h after treatment compared to those without riboflavin might be due in part to the combined absorption of $^{14}$C-2,4-D along with riboflavin-generated $^{14}$C-photoproducts that were more readily absorbed than $^{14}$C-2,4-D. In the absence of riboflavin, a lower proportion of $^{14}$C-2,4-D was photolyzed which may account for lower overall absorption of $^{14}$C, since 2,4-D is a
Table 4.6. Effects of light regime and harvest interval on surface residues and distribution of $^3$H applied to glassa.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Light regime (LR)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV+VL</td>
<td>VL</td>
<td>UV+VL</td>
<td>VL</td>
<td></td>
</tr>
<tr>
<td>HI . h.</td>
<td>3</td>
<td>82a</td>
<td>95a</td>
<td>88a</td>
<td>95a</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>78a</td>
<td>92a</td>
<td>45a</td>
<td>64a</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>77a</td>
<td>92a</td>
<td>3a</td>
<td>41a</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>74a</td>
<td>91a</td>
<td>2a</td>
<td>55a</td>
</tr>
</tbody>
</table>

LSD (0.05) LR x HI NS NS

$^bX$ 78A 92B 34A 64B

Means within a column and factor followed by the same lower case letter are not significantly different (P=0.05) according to Fisher's LSD.

Means within a row and factor followed by the same capital letter are not significantly different (P=0.05) according to Fisher's LSD.

Abbreviations used: Ribo = riboflavin, HI = harvest interval, UV+VL = ultraviolet+visible light, VL = visible light, NS = P values are not significant.
Table 4.7. Effects of light regime, sensitizer, and harvest interval on the absorption of $^{14}$C applied to corn$^a$.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Sensitizer (S)</th>
<th>$^{14}$C absorption$^b$ (% of recovered)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR</td>
<td>$^{+}$R</td>
<td>83a</td>
</tr>
<tr>
<td>UV+VL</td>
<td>81a</td>
<td></td>
</tr>
<tr>
<td>VL</td>
<td>81a</td>
<td>71b</td>
</tr>
<tr>
<td>LSD (0.05) LR X S</td>
<td>5.1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HI', h</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>73a</td>
<td>60a</td>
</tr>
<tr>
<td>10</td>
<td>78a</td>
<td>67a</td>
</tr>
<tr>
<td>24</td>
<td>88b</td>
<td>82b</td>
</tr>
<tr>
<td>42</td>
<td>91b</td>
<td>87b</td>
</tr>
<tr>
<td>LSD (0.05) S X HI</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>$\overline{X}^c$</td>
<td>82A</td>
<td>75B</td>
</tr>
</tbody>
</table>

$^a$Means within a column and factor followed by the same lower case letter are not significantly different (p=0.05) according to Fisher's LSD.

$^b$Total absorbed radioactivity refers to the percentage of total recovered $^{14}$C following the treated leaf washes.

$^c$Main effect means within a row and factor followed by the same capital letter are not significantly different (P=0.05) according to Fisher's LSD.

Abbreviations used: $^{+}$R=treatments with riboflavin, -R=treatments without riboflavin, LR=light regime, UV+VL=ultraviolet-visible light, VL=visible light, HI=harvest interval.

$^\dagger$Plants were exposed to UV+VL and VL 24 h after applying the treatments.
relatively polar molecule. At 24 and 42 h, $^{14}$C absorption was similar both in the presence and absence of riboflavin, and previous data (Table 4.5) indicate that very little riboflavin remained intact on plants by these harvest times.

Average of 82 to 94% of the $^{14}$C absorbed by corn leaves remained in the treated leaf over the course of the experiment (Table 4.8). The percentage of absorbed $^{14}$C remaining in the treated leaf was not different between light regimes and there was only slightly more $^{14}$C in leaves treated with riboflavin at the 42 h harvest interval. The percentage of absorbed $^{14}$C in the treated leaf that remained intact as $^{14}$C-2,4-D ranged from an average of 25% at 3 h to 6% at 42 h and was not differentially affected by sensitizer treatments. However, 21% of the absorbed $^{14}$C remained intact as $^{14}$C-2,4-D in treated leaves exposed to VL compared to 9% in UV+VL-treated leaves when averaged over time. This indicates that a portion of the other $^{14}$C products were not only constituted by the products from plant metabolism of 2,4-D but also products from photolysis. These data confirm the established fact that corn tolerates 2,4-D by limiting its translocation and rapid metabolic detoxification (Hutber et al., 1978; Cohen and Bandurski, 1982; Reinecke and Bandurski, 1987).

The distribution of absorbed $^{14}$C in the leaves above and below the treated leaf ranged from 1 to 4% and in the stem portion ranged from 5 to 11% (data not shown). Light regime, sensitizer, and harvest interval did not have any effect on the distribution of absorbed $^{14}$C in these plant parts. The radioactivity levels in most of the extracted fractions of leaves above and below the treated leaf and the stem portion were below the detection limits for the radioactivity detector and data on
Table 4.8. Effects of light regime, sensitizer, and harvest interval on the distribution of $^{14}$C within the treated leaf of corn\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Sensitizer (S)</th>
<th>$^{14}$C in treated leaf (% of absorbed $^{14}$C)</th>
<th>Remaining as parent $^{14}$C-2,4-D (% of absorbed $^{14}$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR</td>
<td>+R</td>
<td>90a</td>
<td>86a</td>
</tr>
<tr>
<td></td>
<td>-R</td>
<td>90a</td>
<td>89a</td>
</tr>
<tr>
<td>UV+VL</td>
<td>+R</td>
<td>90a</td>
<td>86a</td>
</tr>
<tr>
<td></td>
<td>-R</td>
<td>90a</td>
<td>89a</td>
</tr>
<tr>
<td>VL</td>
<td>+R</td>
<td>90a</td>
<td>86a</td>
</tr>
<tr>
<td></td>
<td>-R</td>
<td>90a</td>
<td>89a</td>
</tr>
</tbody>
</table>

LSD (0.05) LR x S NS

<table>
<thead>
<tr>
<th>HI', h</th>
<th>Sensitizer (S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>94a</td>
</tr>
<tr>
<td>10</td>
<td>93a</td>
</tr>
<tr>
<td>24</td>
<td>82b</td>
</tr>
<tr>
<td>42</td>
<td>91b</td>
</tr>
</tbody>
</table>

LSD (0.05) S x HI 4.1 NS

\[ X^b \]

| Sensitizer (S) | 90A | 87B | 13A | 15A |

\textsuperscript{a}Means within a column and factor followed by the same lower case letter are not significantly different (P=0.05) according to Fisher's LSD.

\textsuperscript{b}Main effect means within a row and factor followed by the same capital letter are not significantly different (P=0.05) according to Fisher's LSD.

Abbreviations used: +R=treatments with riboflavin, -R=treatments without riboflavin, LR=light regime, UV+VL=ultraviolet+visible light, VL=visible light, HI=harvest interval, NS=F values are not significant.

\textsuperscript{t}Plants were exposed to UV+VL and VL 24 h after applying the treatments.
distribution of parent $^{14}$C-2,4-D and degradation products was not generated for those plant parts.

Absorption of $^3$H was generally not different among light regimes (Table 4.9). Foliar absorption of $^3$H increased from about 20% of the total recovered $^3$H at 3 h to 38% at 42 h under both the light regimes. The accumulation of absorbed $^3$H within treated leaf of corn was similar to that observed for $^{14}$C (Table 4.10). Within the treated leaf at 3 h in plants exposed to UV+VL, the major portion of the recovered $^3$H was photoproducts other than riboflavin which may have included both riboflavin metabolism products as well as photoproducts. Since there were no difference between light regimes in total $^3$H absorption, there was no apparent difference in the rate of $^3$H-riboflavin and $^3$H photoproduc stud absorption. In contrast, the major portion of the absorbed $^3$H in the treated leaf at 3 h under VL was parent riboflavin. By 10 h after treatment, ≥80% of the absorbed riboflavin was converted into other $^3$H products under both UV+VL and VL, and virtually all riboflavin in the treated leaf was metabolized by 42 h.

Leaves above and below the treated leaf contained 2 to 6% of the absorbed $^3$H across harvest intervals and the absorbed $^3$H in the stem portion ranged from 1 to 12% (data not shown). Light regime and harvest interval did not have any effect on the distribution of $^3$H in these plant parts. The radioactivity levels in the above plant parts was below the detection limits of the radioactivity detector for identification of the parent and degradation products.

Photolysis is one of the important degradation processes that is capable of degrading herbicide residues that remain nonabsorbed on the leaf surface. The study presented here shows that at low concentrations,
Table 4.9  Effects of light regime and harvest interval on the absorption of $^3$H applied to corn leaves$^a$.

<table>
<thead>
<tr>
<th>Factor</th>
<th>$^3$H absorption$^b$ (%) of recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light regime (LR)</td>
</tr>
<tr>
<td></td>
<td>UV+VL</td>
</tr>
<tr>
<td>HI', h</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20a</td>
</tr>
<tr>
<td>10</td>
<td>25a</td>
</tr>
<tr>
<td>24</td>
<td>27b</td>
</tr>
<tr>
<td>42</td>
<td>38c</td>
</tr>
</tbody>
</table>

LSD (0.05) LR X HI  

X$^c$  

28A  

29A

$^a$Means within a column and factor followed by the same lower case letter are not significantly different (p=0.05) according to Fisher's LSD.

$^b$Total absorbed radioactivity refers to the percentage of total recovered $^3$H following the treated leaf washes.

$^c$Main effect means within a row and factor followed by the same capital letter are not significantly different (p=0.05) according to Fisher's LSD.

Abbreviations used: HI=harvest interval, UV+VL=ultraviolet+visible light, VL=visible light.

† Plants were exposed to UV+VL and VL 24 h after applying the treatments.
Table 4.10. Effects of light regime and harvest interval on the distribution of \(^3\)H within the treated leaf of corn\textsuperscript{a}.

<table>
<thead>
<tr>
<th>Factor</th>
<th>(\text{HI}^\text{f}, \text{h})</th>
<th>(\text{UV}^+\text{VL})</th>
<th>(\text{VL})</th>
<th>(\text{UV}^+\text{VL})</th>
<th>(\text{VL})</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>94\textsuperscript{a}</td>
<td>92\textsuperscript{a}</td>
<td>23\textsuperscript{a}</td>
<td>78\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>76\textsuperscript{a}</td>
<td>92\textsuperscript{a}</td>
<td>21\textsuperscript{a}</td>
<td>20\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>95\textsuperscript{a}</td>
<td>86\textsuperscript{a}</td>
<td>5\textsuperscript{a}</td>
<td>6\textsuperscript{a}</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>95\textsuperscript{a}</td>
<td>87\textsuperscript{a}</td>
<td>0\textsuperscript{a}</td>
<td>3\textsuperscript{a}</td>
<td></td>
</tr>
</tbody>
</table>

LSD (0.05) \(\text{LR} \times \text{HI}\) NS NS

\(\bar{x}^\text{b}\) 90\textsuperscript{A} 89\textsuperscript{A} 12\textsuperscript{A} 26\textsuperscript{A}

\textsuperscript{a}Means within a column and factor followed by the same lower case letter are not significantly different \((P=0.05)\) according to Fisher's LSD.

\(^b\)Main effect means within a row and factor followed by the same capital letter are not significantly different \((P=0.05)\) according to Fisher's LSD.

Abbreviations used: Ribo=riboflavin, \(\text{HI}=\text{harvest interval}, \text{UV}^+\text{VL}=\text{ultraviolet+visible light}, \text{VL}=\text{visible light}, \text{NS}=\text{F values are not significant.}

\textsuperscript{f}Plants were exposed to \(\text{UV}^+\text{VL}\) and \(\text{VL}\) 24 h after applying the treatments.
a photosensitizer is capable of altering the persistence of 2,4-D residues on corn leaves. Our results support the possibility that herbicide residues on plants can be managed by applying a sensitizer which is capable of causing their photodegradation. However, the interactions of sensitizer, herbicide, and cuticular composition, and the overall effect on herbicide efficacy warrant further investigation.
CHAPTER 5

SUMMARY

Photodecomposition is one of the important transformation pathways that determines the fate of herbicides in water, atmosphere, and on soil and plant surfaces. Photochemical processes can be divided into two general categories: direct and indirect photolysis. Substances that undergo direct photolysis absorb UV radiation directly and undergo transformation. Indirect photoreaction occurs through the process of sensitization whereby other compound(s) in the medium absorb light initially and then react with an acceptor molecule inducing its decomposition. Our focus is on indirect photoreaction as a number of naturally produced compounds that occur in the environment are capable of acting as sensitizers and cause indirect photolysis.

Aqueous Photolysis. In the first study, aqueous chlorimuron and imazaquin photolysis were studied in the presence of three phenolic acids, riboflavin, and acetone in a photoreactor. Chlorimuron photolysis was enhanced in presence of riboflavin, acetone, caffeic, and ferulic acid, whereas p-coumaric acid was not an effective sensitizer. Imazaquin photolysis was delayed in the presence of sensitizers and the effect was even more pronounced in the presence of riboflavin.
In the second study, experiments were conducted to determine the effects of riboflavin, pH of the medium, and photoproducts on aqueous photolysis of 2,4-D. The studies were conducted in a controlled environment growth chamber with a UV source that emitted radiation in the wavelength range of 300 to 400 nm. Photolysis of 2,4-D in aqueous solution was dependent on riboflavin concentration. At higher concentrations of riboflavin, the rate of 2,4-D photolysis was increased. Photolysis of 2,4-D in aqueous solution in the presence of riboflavin increased under both UV and visible light as pH of the treatment solution was decreased from 7.5 to 4.5. Lumiflavins, a photoproduction of riboflavin, was also capable of sensitizing 2,4-D in aqueous solution.

Leaf surface photolysis. Riboflavin enhanced the photolysis of 2,4-D and its residues on the corn leaf surface both in the presence of UV and visible light. Riboflavin-sensitized photolysis of 2,4-D resulted in 67% greater degradation of surface 2,4-D residues than in the absence of riboflavin during the first 10 h after treatment. Photolysis of 2,4-D and its residues was also observed in the absence of riboflavin. On glass slides ≥95% of applied 2,4-D was not degraded in the absence of riboflavin and 60% was degraded after 42 h in the presence of riboflavin. Riboflavin did not have any effect on the absorbed 2,4-D distribution within the plant.

Overall conclusions.

1. Some naturally occurring compounds in the environment are capable of enhancing herbicide degradation by acting as photosensitizers in aqueous environments as well as on plant surfaces. The natural sensitizers include riboflavin, acetone, and three phenolic acids.
2. Chlorimuron photolysis rate was increased two to ten fold in aqueous solution in the presence of all sensitizers tested except p-coumaric acid. On the contrary, imazaquin photolysis in aqueous solution was delayed in the presence of sensitizers and this effect was most pronounced with riboflavin. These results illustrate the fact that whether a natural compound acts as a photosensitizer or as a 'protectant' against herbicide photolysis depends on the excited state energy relationships between the specific herbicide and the natural compound.

3. The pH of the reaction medium influences the stability of the sensitizers and the rate of photolysis. 2,4-D underwent rapid photolysis at pH 4.5 compared to pH 6 and 7.5 in aqueous solution. This can be attributed to the stability of the riboflavin molecule.

4. Lumiflavin, a photoprodut of riboflavin, was a stronger sensitizer of 2,4-D photolysis in aqueous solution than riboflavin except when pH of the riboflavin treatment solution was adjusted to 4.5. This might be due to the longer half-life and triplet lifetime of lumiflavin compared to riboflavin under enhanced UV light. Thus lumiflavin may prove to be a more efficient sensitizer than riboflavin for other herbicides as well.

5. Riboflavin enhanced photolysis of 2,4-D residues on the corn leaf surface. The effect was more pronounced under enhanced UV light. 2,4-D photolysis also occurred in the absence of riboflavin. These data suggest that natural compounds may act as in situ sensitizers on the leaf surface. The identification, relative abundance, species specificity, and mechanistic role of these in situ sensitizers merits further investigation.
LIST OF REFERENCES


LIST OF REFERENCES


Fife, D. J. Photochemical Reduction and Degradation of Riboflavin. 1977. Pages 64. MS Thesis in Chemistry submitted to the Utah State University, Logan, Utah.


Gauvrit, C. and P. Gaillardon. 1991. Effect of Low Temperatures on 2,4-D
Behaviour in Maize Plants. Weed Res. 31:135-142.


Slife, F. W., J. L. Key, S. Yamaguchi, and A. S. Crafts. 1962. Penetration, Translocation, and Metabolism of 2,4-D and 2,4-5 T in Wild and Cultivated Cucumber Plants. Weeds. 10:29-35.


