Free Radical Generators

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Free radicals are atoms, molecules, or ions with unpaired electrons. In general, the unpaired electrons cause radicals to be highly chemically reactive. Radicals are believed to be involved in the aging process, degenerative diseases, a range of disorders including cancer, arthritis, atherosclerosis, Alzheimer's diseases, and diabetes. The free radical theory of aging implies that antioxidants such as vitamin A, vitamin C, vitamin E, and superoxide dismutase will slow the process of aging by preventing free radicals from oxidizing sensitive biological molecules or reducing the formation of free radicals.

Free radicals are frequently denoted by a dot placed immediately to the right of the atomic symbol or molecular formula as follows:

$Cl_2 \Longrightarrow Cl. + Cl.$

$O_2 \Longrightarrow O. + O.$

Plants naturally have free radicals that are by-products of several chemical processes. However, under normal conditions plants have the ability to mitigate free radical injury by utilizing enzymatic system and antioxidants.

Several herbicide groups can injure plants by generating massive amounts of free radicals. Free radicals are unstable and must obtain an electron from some other chemical to become stable. By taking an electron from another chemical, the other chemical now becomes a free radical and its chemical structure is changed. It must then steal an electron. Thus the chain reaction (of atoms stealing electrons) continues and can be thousands of events long. These events can result in serious damage to cells including lipid periodation, protein damage, and DNA lesions.

In broad terms, there are three groups of herbicides that generate massive amounts of free radicals including those that:

1) Inhibit photosystem II (PSII inhibitors): triazines, triazinone, pyridazinone, phenyl-carbamate, amide, Nitrile, benzothiadiazinone, phenyl ureas, and uracils.

2) Capture electrons in photosystem I (PSI disruptor): bipyridiliums.

3) Inhibit protoporphyrinogen oxidase (Protox inhibitors): diphenyl ethers, phenylpyrazole, N-phenylphthalimide, thiadiazole, oxadiazole, and triazolinone.

The following discussion is about the herbicides under (2) and (3) above, which are often grouped together as free radical generators.

Common properties:

Bipyridiliums (diquat and paraquat, Figure 1), the diphenyl ethers (Figures 2 and 3), and the N-phenyl heterocycles (oxadiazon, carfentrazone, and sulfentrazone, Figures 3) share several properties. Signs of injury to susceptible plants are very similar for all of these herbicides. Symptoms appear a few hours after treatment as dark green areas on foliage, followed by wilting. Necrosis follows, and in a few days the characteristic browning or "burned" appearance is evident. Susceptible species are killed within a few days. Death of tissue is so rapid that none of these herbicides are appreciably translocated. Because of a lack of systemic action, complete coverage is important to prevent weed regrowth. Activity is greater on sunny days, although applications at night that are followed by a bright day may have greatest efficacy.

None of these herbicides are susceptible to leaching from the soil, but for different reasons. Diquat and paraquat are strongly adsorbed by clays and other inorganic soil colloids; thus they are rarely active in the soil (Figure 4). Oxadiazon and the diphenyl ethers are strongly adsorbed by soil organic matter; when applied preemergence, most activity occurs near the soil surface as seedlings emerge. Soil incorporation greatly decreases the activity of oxadiazon and diphenyl ethers.

Principal uses:

Paraquat and diquat are nonselective herbicides. Paraquat is widely used to control vegetation prior to crop emergence, as a dormant season treatment in alfalfa and other perennial crops, and as a directed spray. Diquat is mostly used for aquatic weed control. Application is either postemergent for cattail control, or water-run to control algae and submersed and floating weeds.

Diphenyl ethers and the N-phenyl heterocycles are selective herbicides that must be carefully applied to avoid injury. Sensitivity to these herbicides often varies with crop age, and most crops can outgrow minor, early-season damage. Avoiding contact with crop foliage helps to prevent crop injury, as in directed applications of oxyfluorfen under dormant fruit and nut trees and grapes, or granular applications of oxadiazon in woody ornamentals and turf.

Mode of Action

The mode of action for free radical generators involves membrane degradation. Paraquat and diquat accept electrons from photosystem I (Figure 5) to form free radicals. These free radicals rapidly produce a superoxide radical from molecular oxygen that then undergoes enzymatic dismutation to form hydrogen peroxide (Figures 6 and 7). Hydrogen peroxide and the superoxide radicals interact to produce hydroxyl radicals, which quickly degrade membranes.

The diphenyl ethers and the N-phenyl heterocycles affect the enzyme protoporphyrinogen oxidase (Protox, Figure 8). Protox is found in the chloroplast envelope and in mitochondria (Figure 9). Protox converts protogen IX into protoporphyrin IX (proto IX). Diphenyl ethers and oxadiazon inhibit Protox (Figures 10 and 11). As a result, excess protogen IX moves out of the chloroplast and

into the cytoplasm. Enzymatic oxidation of protogen IX into proto IX results in an accumulation of proto IX. The excess proto IX interacts with oxygen and light to form singlet oxygen ('02), which begins the process of lipid peroxidation (Figures 12 and 13). Both lipids and proteins are oxidized, destroying chlorophyll, carotenoids, and rupturing membranes.

Lipid Peroxidation

All of the free radical generators destroy cell membranes, ultimately leading to the death of plant tissue. A major component of cell membranes are lipids. Lipid peroxidation by free radicals involves three steps: initiation, propagation, and termination (Figure 12). The lipid peroxidation initiation factor varies with the herbicide group and includes: triplet chlorophyll from photosystem II inhibitors, singlet oxygen from Protox inhibitors, and hydroxyl radicals from bipyridylium herbicides. All of these initiating factors remove a methylene group from near the double bond (the unsaturation site) of polyunsaturated fatty acids (Figure 13). This is the initiation reaction. The propagation reaction occurs when the peroxidized lipid radical reduces to lipid peroxides when they extract hydrogen from other polyunsaturated fatty acids in the plant cell membranes. The termination reaction occurs because the lipid peroxides are not stable and undergo degradation to small hydrocarbons such as pentane and ethane.

Bioassays

Diphenyl ethers: *Chlorella* (Kratky and Warren 1971), *Chlamydomonas* (Hess 1980), and sorghum seedlings (Fadayomi and Warren 1977).

Paraquat and diquat: *Lemna* spp. (Funderburk and Lawrence 1963; Damanakis 1970).

Toxicology

Diphenyl ethers and oxadiazon: low avian and mammalian, low to moderate fish toxicity.

Paraquat and diquat: fish toxicity is low for both. Mammalian toxicity is moderate for diquat but HIGH for paraquat. Paraquat is often used in suicide attempts, and can be fatal if enhaled, swallowed, or absorbed through the skin. If ingested, drink fluids and induce vomiting immediately. Flush affected skin areas immediately with water. Respirators are required for many paraquat application situations.

Herbicide Resistance

There are no reports of resistance to diphenyl ethers. At least twenty-seven weed species are resistant to paraquat.

Information on Usage

Paraquat is often used for postemergent control of small weeds, or to destroy foliage of larger weeds. Killing above ground tissue to set back the growth of larger weeds ("burndown") allows crops to form a canopy that shades out weeds. Penetration of paraquat through the plant cuticle is critical, so the use of nonionic surfactants is recommended. Paraquat is rapidly absorbed through plant foliage, and rain occurring 30 minutes or more after application has no effect on activity.

Diquat use is restricted to waters with little outflow to reduce risk of accidental poisoning.

Acifluorfen-sodium is used on more acres than any other diphenyl ether because it is registered in three high acreage crops: soybeans, peanuts, and rice. Soybean and rice use is strictly postemergent, and control is effective for many broadleaf weeds that are missed by other herbicides. Use a nonionic surfactant with postemergent treatments. Peanut use is only as a pre-emergent application. Do not plant root crops (e.g. carrots) for at least 18 months into any soil treated with acifluorfen-sodium.

Oxyfluorfen is used in a number of crops for pre-emergent control, or as a directed postemergent treatment. It will injure most crops if applied over-the-top, but can be safely applied over-the-top in onions. Pre-emergent applications will injure direct-seeded cole crops, but can be safe for transplants. Transplanting breaks through the surface barrier of herbicide-treated soil, allowing the crop to grow without contacting herbicide, but still controlling weeds that emerge through the undisturbed surrounding soil.

Oxadiazon is used on warm season turf grass to control annual grasses and in many ornamentals to control grasses and annual broadleaves.

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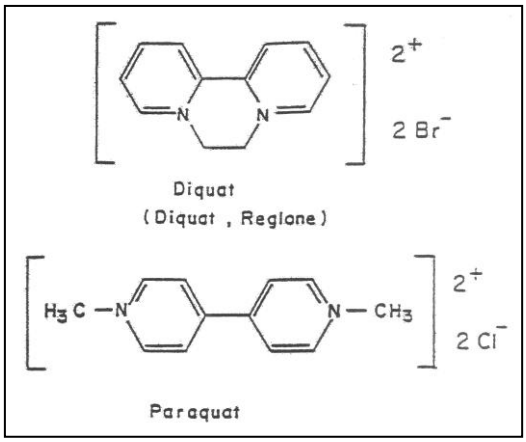


Figure 1. Structure of the two most common bipyridiliums, paraquat and diquat. Note the characteristic herterocyclic rings that contain both carbon and nitrogen atoms. When in solution with water, both paraquat and diquat are bivalent cations, i.e. have two positive charges.

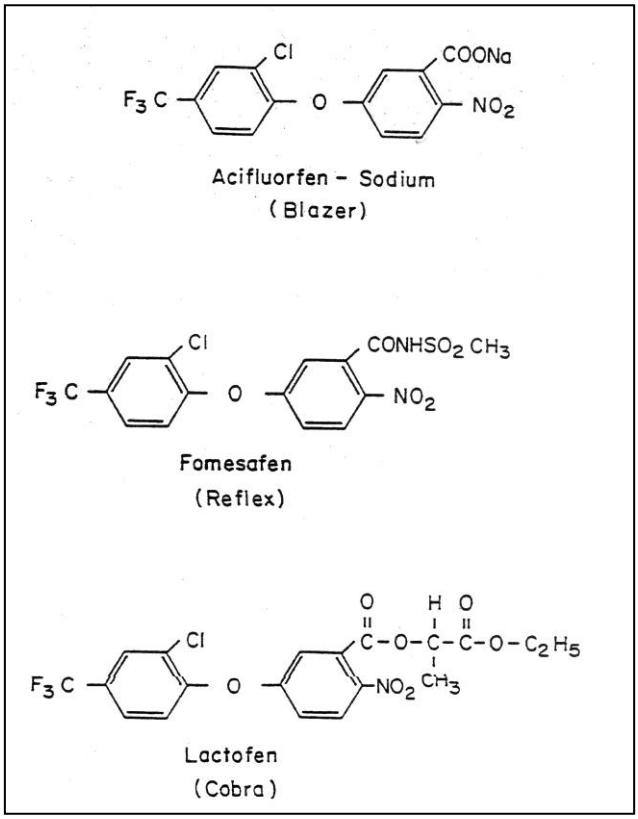
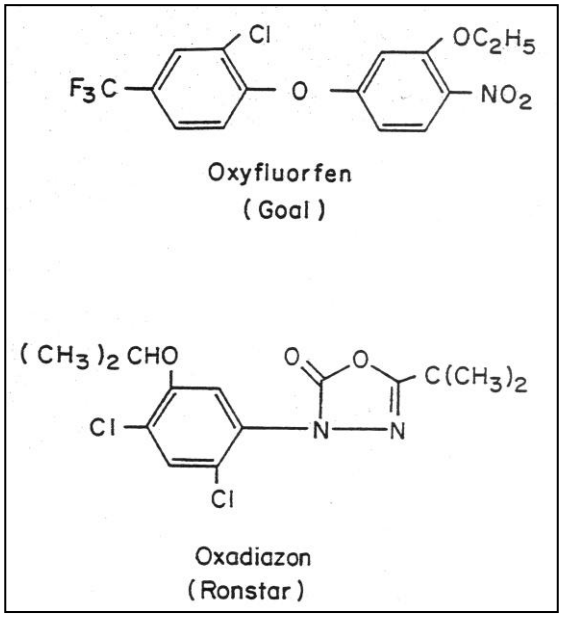


Figure 2. Chemical structure of widely used diphenyl ether herbicides. Diphenyl ethers contain two benzene rings ("phenyl" groups) connected by an ether linkage.

Figure 3. Structure of oxyfluorfen, a diphenyl ether; and oxadiazon, an N-phenyl heterocyclic herbicide. The N-phenyl heterocycles consist of a phenyl group bonded to a nitrogen atom in a heterocyclic ring. Both diphenyl ethers and N-phenyl heterocycles have benzene rings and extensive resonance structures.



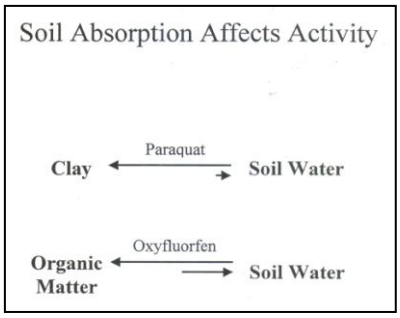


Figure 4. Dynamic equilibrium between soil components and herbicides. Paraquat is so strongly adsorbed onto clay particles that the equilibria is almost completely shifted toward the clay particles, and very little paraquat enters into the soil water solution. As a result, there is usually no injury to plants in soil following paraquat application.

Oxyfluorfen is absorbed onto soil organic matter, which usually prevents significant leaching of oxyfluorfen. However, enough oxyfluorfen enters the soil water solution to injure or kill seedlings that emerge through the surface of oxyfluorfen-treated soil. Thus, oxyfluorfen acts as a barrier to weed emergence. Breaking the barrier, e.g. by digging a hole to plant transplants, prevents injury to plants growing where the soil was disturbed.

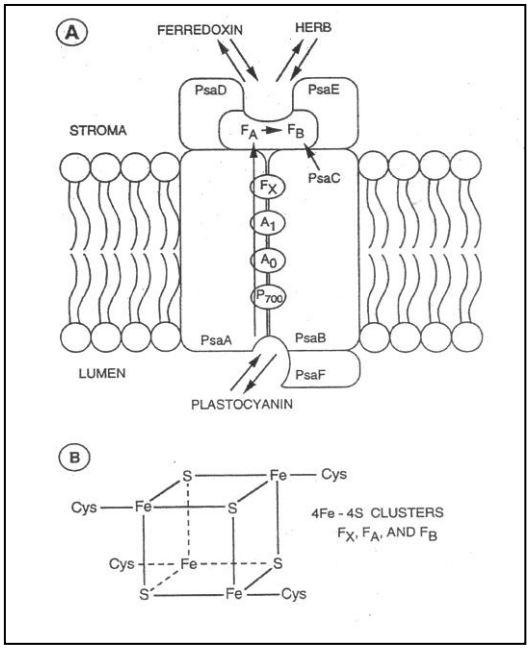


Figure 5. Electron transport in the photosystem I (PS I) complex embedded in thylakoid membranes of chloroplasts. Arrows within the PS I complex represent electron flow. P700, a dimer of chlorophyll a, accepts light energy from the chlorophylls and carotenes associated with PS I. Other electron carriers are: Ao, a chlorophyll a molecule; A1, a phylloquinone; and FX, FA and FBare iron-sulfur clusters (4Fe-4S). Plastocyanin and ferredoxin are soluble electron carrier proteins that dock to PS I. Plastocyanin gives up an electron to PS I (photooxidized) and ferredoxin accepts an electron from PS I (photoreduced). The structural proteins of PS I are termed Psa. PsaA and PsaB make up the core of PS I and are embedded in the thylakoid membrane, whereas PsaC is a peripheral protein housing the iron-sulfur clusters FA and Fe. PsaD and PsaE assist with the docking of ferredoxin, while PsaF assists with the docking of plastocyanin. The bipyridilium herbicides compete with ferredoxin for a binding site at or near PsaC.

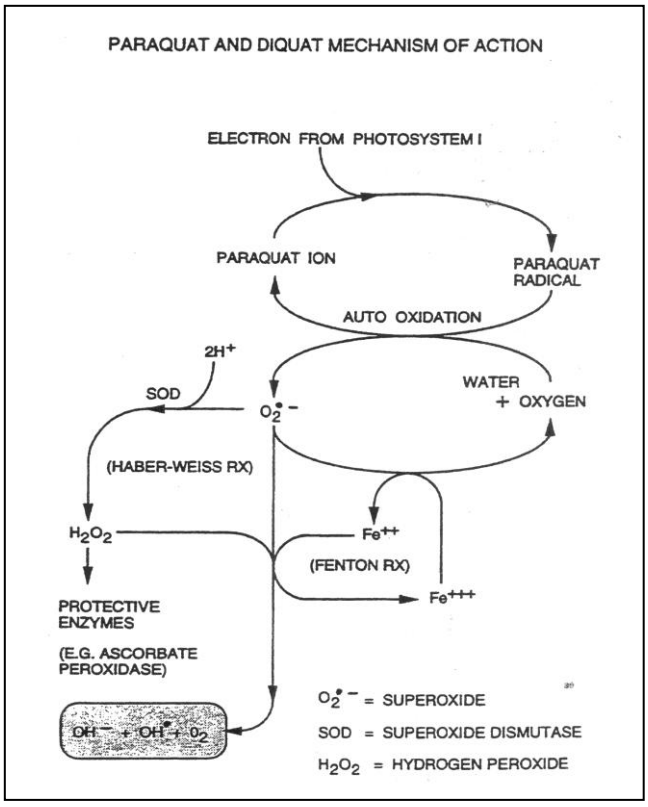


Figure 6. Paraquat, a di-cation bipyridilium herbicide, captures electrons from PS I during electron flow in photosynthesis and becomes a free radical (mono-cation). The paraquat free radical is unstable and rapidly undergoes

auto-oxidation back to the parent ion. During the auto-oxidation process, superoxide radicals (02') are produced from molecular oxygen. Superoxide can undergo enzymatic dismutation (superoxide dismutase - SOD) to form hydrogen peroxide (H202). As hydrogen peroxide and superoxide accumulate in the cell after paraquat treatment, they react to produce hydroxyl radicals (OH') via the Haber-Weiss reaction. The reaction is catalyzed by transition metals, iron or copper, in the Fenton reaction. Hydroxyl radicals efficiently initiate lipid peroxidation in polyunsaturated fatty acids in membranes.

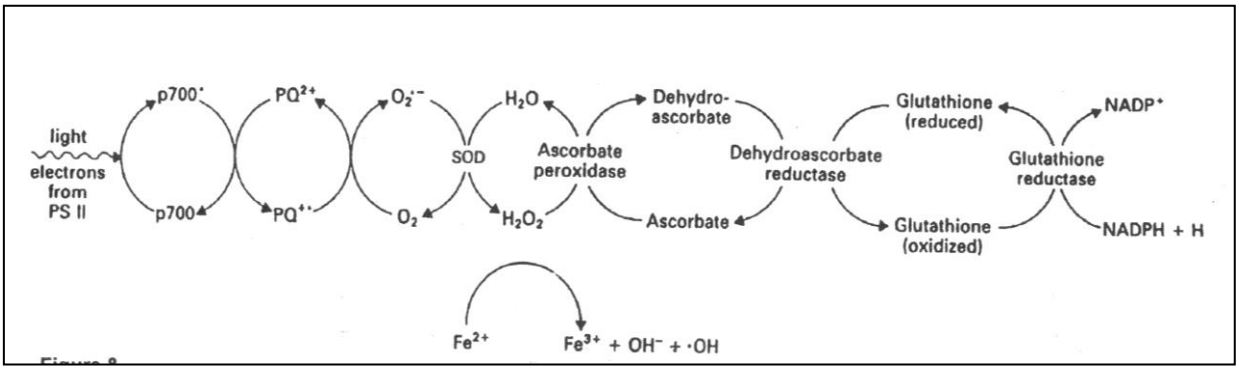


Figure 7. Superoxide generation and detoxification at Photosystem I. PO, paraquat; SOD, superoxide dismutase (after Shaaltiel & Gressel 1986).

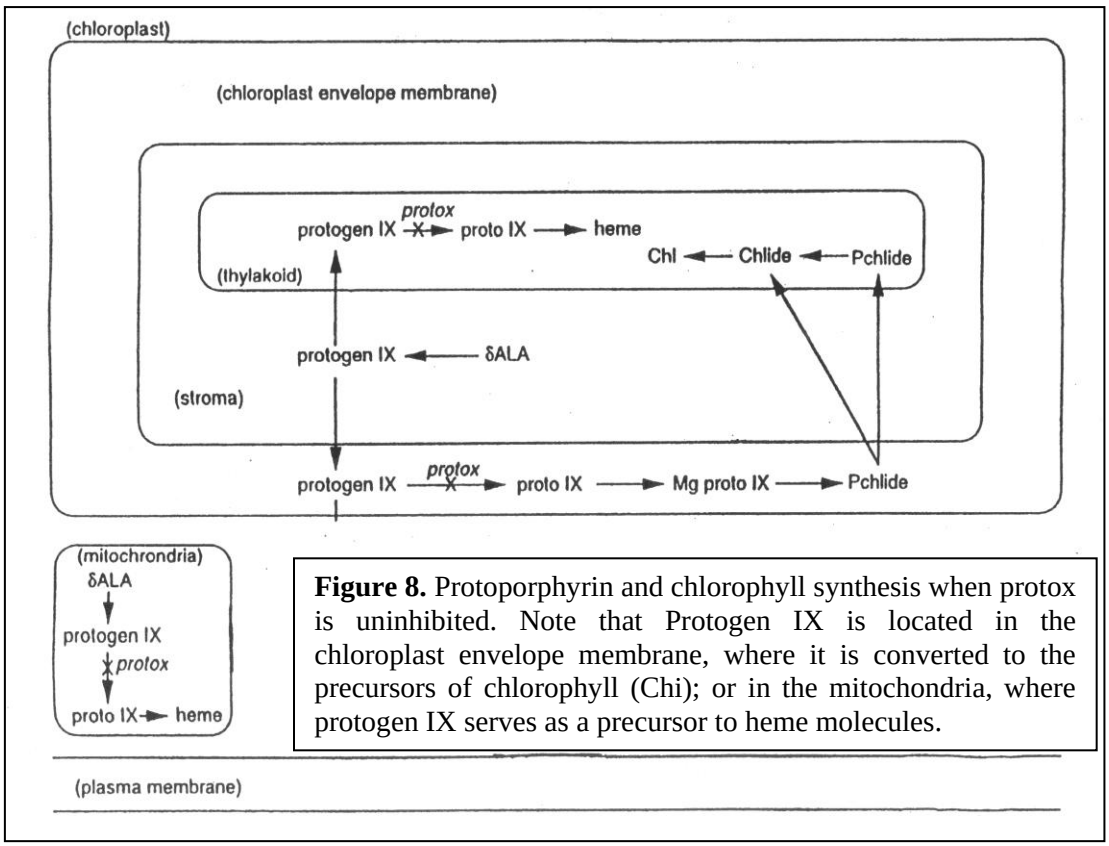
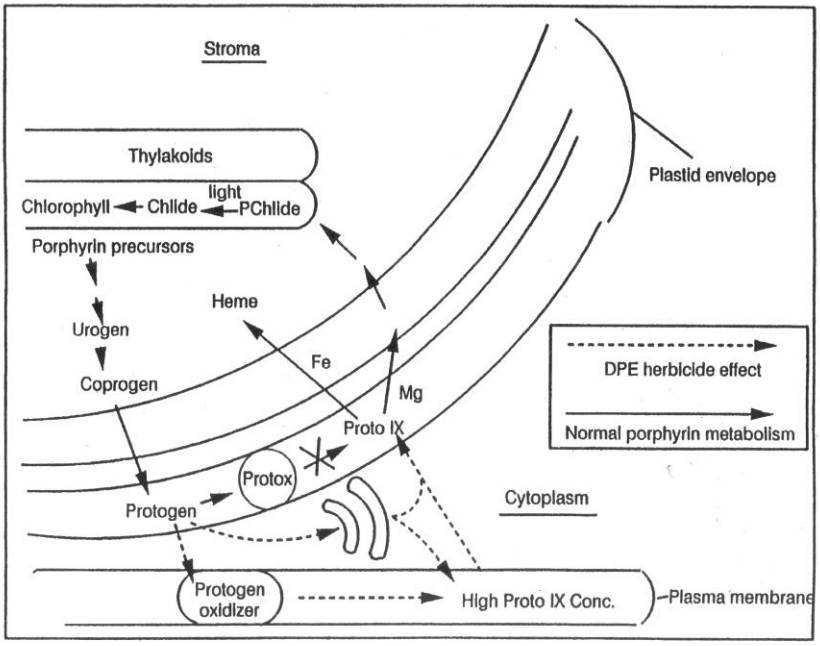


Figure 8. Protoporphyrin and chlorophyll synthesis when protox is uninhibited. Note that Protogen IX is located in the chloroplast envelope membrane, where it is converted to the precursors of chlorophyll (Chi); or in the mitochondria, where protogen IX serves as a precursor to heme molecules.

Figure 9. Diagram of cellular organelles illustrating the locations of precursors and enzymes of chlorophyll synthesis. The solid lines illustrate a normally functioning porphyrin pathway. The dotted line shows how the pathway changes when diphenyl ether herbicides block the activity of the Protox enzyme.



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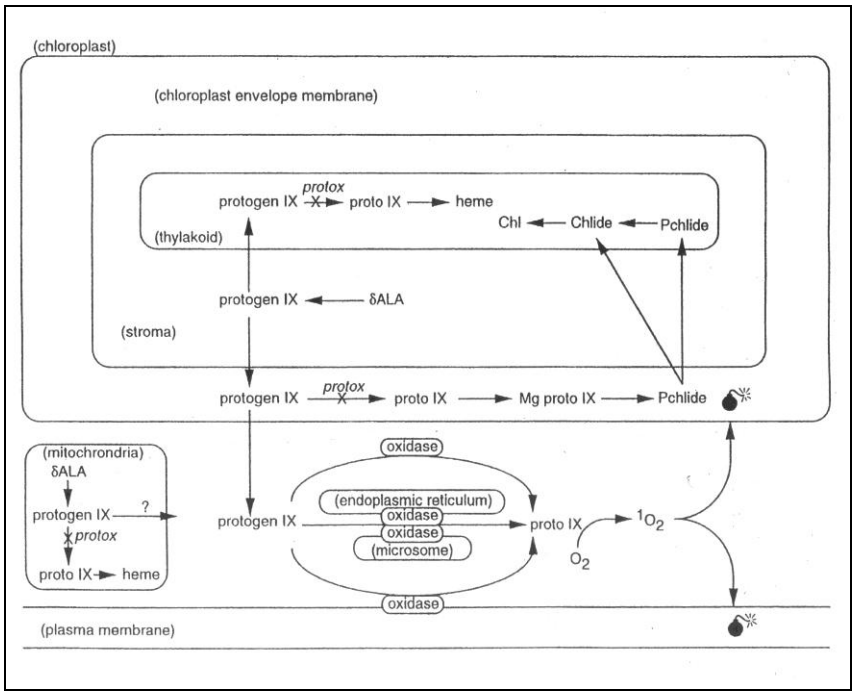

Figure 10. The activity of Protox has been blocked by an herbicide. Protogen IX accumulates and leaks out of the chloroplast envelope membrane, interacting with oxidase and forming singlet oxygen, which ultimately leads to lipid peroxidation and destruction of cellular membranes.

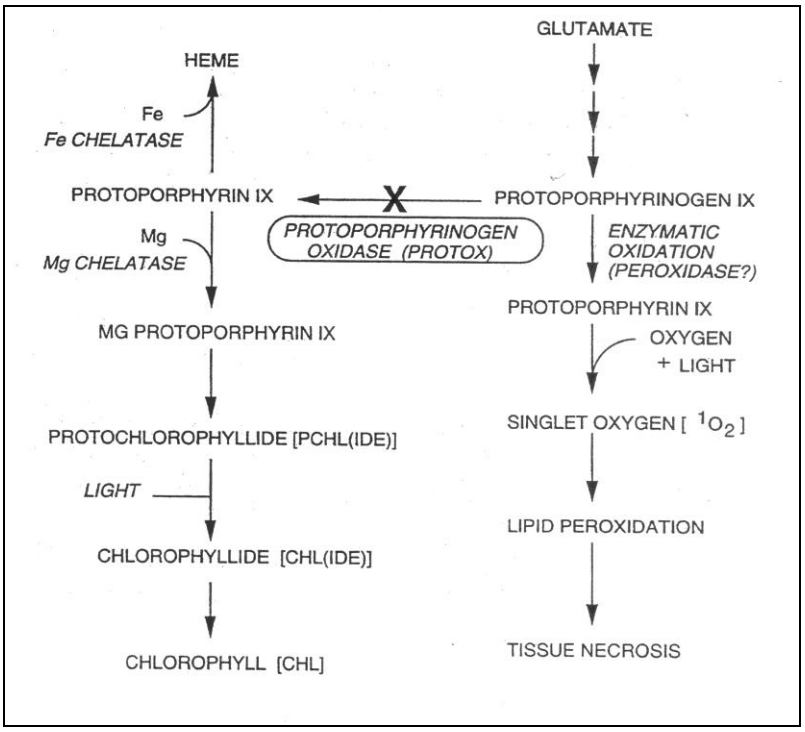

Figure 11. Flow chart of chlorophyll biosynthesis. The left side of the diagram illustrates the pathway when the enzyme protoporphyrinogen oxidase (Protox) functions normally. The right side shows what happens when is inhibited by diphenyl ethers and N-phenyl heterocyclic herbicides.

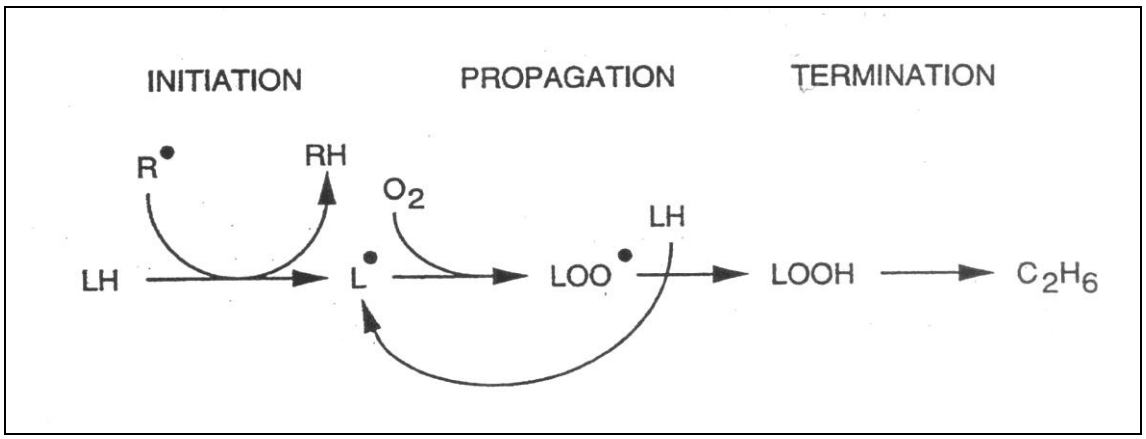

Figure 12. Lipid peroxidation of polyunsaturated fatty acids in plant membranes. An initiating factor (R') such as triplet chlorophyll, singlet oxygen, or a hydroxyl radical removes a hydrogen from a polyunsaturated fatty acid (LH) in the membrane. This hydrogen abstraction process generates a lipid radical (L'). Oxygen reacts with the lipid radical to form a peroxidized lipid radical (LOO'). This peroxidized lipid radical reacts with another polyunsaturated lipid that propagates the reaction within a localized region of the membrane. The lipid peroxides (LOON) formed during propagation are unstable and degrade to short chain hydrocarbon gases such as ethane (CZH6).

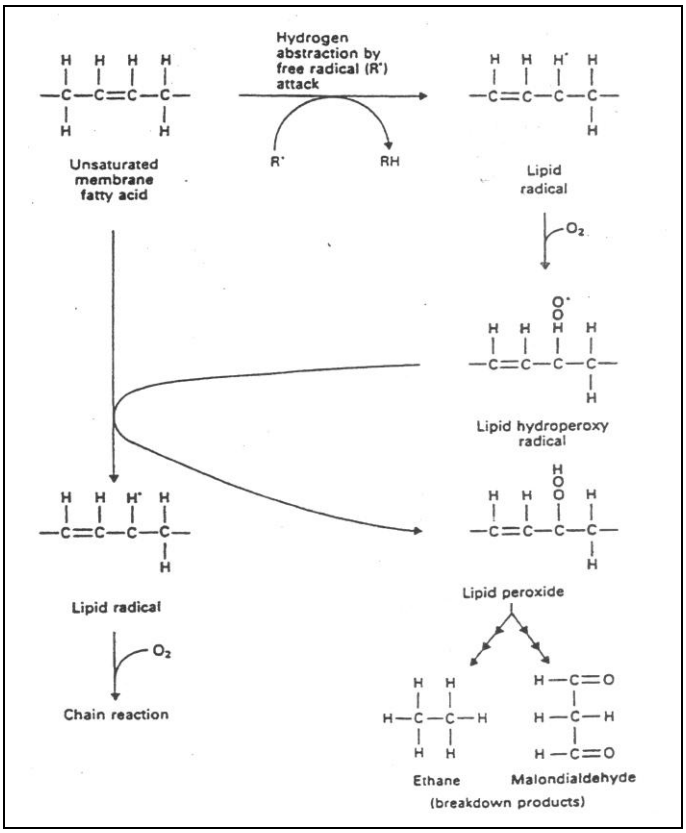

Figure 13. Chemical structure diagrams that illustrate the steps in lipid breakdown that results from free radical generation following herbicide interactions with plant cellular components.