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CHAPTER 6

PHENOXY HERBICIDES AND MICROORGANISMS

A. L. Young

The soil persistence of herbicides is influenced by many environmental and biological factors. Perhaps one of the most important of these is that of the presence or absence of microorganisms. Studies on the interactions of microorganisms and 2,4-D and 2,4,5-T herbicides parallel the release of these chemicals as commercial weed killers. As early as 1945, Smith et al. (31) reported on the effects of 2,4-D to microorganisms. They found that concentrations of 1-100 ppm 2,4-D had no significant effect on total plate counts of actinomycetes, fungi and protozoa in silt loam soil. Similar results were obtained in a sandy soil using 500 ppm 2,4-D. That same year Stevenson and Mitchell (33) were the first to report the effects of 2,4-D on individual microbial species. They found that concentrations of 200 ppm 2,4-D in potato-dextrose agar retarded the growth of Bacillus subtilis, Aerobacter cloacae, Staphylococcus aureus, and Phytomonas tumefaciens. However, a concentration of 800 ppm 2,4-D in potato-dextrose agar did not retard the growth of Fusarium sp. or Penicillium sp.

These two early studies were the first of many studies that have been conducted on microbial interactions with the phenoxy

herbicides. By 1977 over 340 research papers had been published on this subject. This did not count the number of excellent reviews that were available. No other group of herbicides has been as well researched on this subject. Because of this, it will be our intent in this chapter to review only some of the available data. Far more complete reviews on the phenoxy herbicides and microorganism have been recently published by Loos (20), 1975, and Kaufman and Kearney (16), 1976. In addition, some older excellent reviews on phenoxy herbicides and microorganisms are available by Alexander (1), Audus (4), Cullimore (12), Fletcher (14), Kaufman (15), Loeffler and VanOverbeek (19), Pfister (26), and Upchurch (36).

Millions of pounds of phenoxy herbicides have been applied to the environment of the United States. However, in a recent (1969) national survey of pesticide residue in cropland soil for 43 states and noncropland soil for eleven states, Wiersma et al. (38) found that only 3 of 188 soil samples analyzed contained chlorophenoxy herbicides. The herbicide found was 2,4-D, and then only within a concentration range of 0.01 - 0.03 ppm. Moreover, in a survey of soil and crop samples collected from the "Corn Belt" in 1970 (10), no chlorophenoxy herbicides were found although several insecticides were present. The Corn Belt uses about one-fourth of the 2,4-D sprayed annually in the U.S. Although applied extensively

to the environment, it is apparent that the phenoxy herbicides are not persistent.

Brief Review

The interrelationships of soil microorganisms and herbicides are observed in two areas of study: (a) effects of herbicides on microorganisms and (b) effects of microorganisms on herbicides. The first area relates to the effects by direct or indirect action of the herbicide on the growth and physiological processes of the soil microflora. The second area of study deals with the metabolism and breakdown of herbicides into components that are usually less phytotoxic than the original compound.

Soil microorganisms have remarkable adaptive power and several people have shown that microorganisms can, through adaptation or mutation, alter their metabolic pathways for a more efficient utilization of herbicides (8). When microorganisms are exposed to high concentrations of a foreign material, there is usually a lag period before utilization of the material begins. This lag period represents the time required for the microorganism to become adapted. Once breakdown is initiated and completed the soil then retains a capability for rapid breakdown. For example, Audus (4) treated a soil with 100 ppm of 2,4-D and 20 days were required for 80% detoxification but when the soil was treated again only three days were required for 80% detoxification. Colmer (11) found that 5,000 ppm of 2,4-D

was at first inhibitory to a bacterium, but after subculturing three times the organism grew rapidly in the 5,000 ppm concentration. Newman et al. (23) and Rogoff and Reed (29) discovered that 2,4-D disappeared from soil more rapidly with the second application. Walker and Newman (37) found in laboratory tests that three to five days were required for decomposition of 100 ppm, 2,4-D; but when the same soil was treated again with 1,000 ppm then only 10 to 14 days were required for decomposition. Stojanovic et al. (34) added a mixture of 2,4-D and 2,4,5-T (similar to the military formulation of Orange) to soil at a concentration of 5,000 ppm. Seventy-eight percent of the herbicide carbon was given off as CO_2 in 56 days. It also appeared that mixtures of 2,4-D and 2,4,5-T were more rapidly degraded than were the single compounds.

There is considerable evidence available to show that 2,4-D is rapidly decomposed in soils (4). Concentrations of 2,4-D at 100 to 200 times the amounts normally used for weed control usually have no appreciable effect on the soil population of bacteria, fungi, and actinomycetes (25). Reduced bacterial counts have been observed with 2,4-D concentrations as low as 100 ppm, but in several experiments 500 ppm have not altered bacterial counts. More is known about the effects of 2,4-D on soil microflora than about any other herbicide, and some interesting

interactions have been observed (25). The herbicide is more toxic to microorganisms in acid than in alkaline soils and more toxic to aerobes than facultative anaerobes. Spore-forming bacteria appear to be more sensitive than nonspore-formers to 2,4-D. Bacteria are more sensitive than fungi to the herbicide. Even closely related species differ in response to 2,4-D.

If 2,4-D were applied to a moist loam soil under summertime temperature at a rate of 0.56 to 3.36 kg/ha, it would disappear in 7 to 30 days (17). If applied at rates of 4.5 to 61.6 kg/ha, it would probably disappear in one to three months (13). If 2,4-D were applied to the soil at a concentration of 500 ppm and disappeared at a rate proportional to the breakdown of 61.6 kg/ha, the calculated time would be 5.6 years. However, there is evidence that a more realistic time for inactivation of 500 ppm would be less (3).

Persistence of 2,4,5-T in soils is usually two to three times longer than 2,4-D (13), and very few organisms have been identified as having the ability to breakdown the 2,4,5-T molecule (2). Newton (24) has calculated from studies on the kinetics of degradation by microorganisms that 2,4,5-T has a half-life of seven weeks in the forest floor. Blackman et al. (5) have noted that in tropical soils, phytotoxic residues from 28 liters/ha application of the n-butyl esters of 2,4-D and

2,4,5-T at 30 kg active ingredient/ha disappeared within 4 weeks. Leopold et al. (18) found that increasing chlorination of phenoxy-acetic acid decreased its water solubility while increasing its adsorption onto activated carbon and organic matter, thus making it less available for microbial degradation. Moreover, Thiels (35) noted, from reviewing the literature, that 2,4,5-T was less susceptible to attack by microorganisms because the aromatic nucleus of halogenated phenoxyalkyl carboxylic acids and phenols are more biologically inert in compounds containing the halogen (chlorine) in a position meta (the 5 position) to the phenolic hydroxy.

There are some microorganisms that are susceptible to phenoxy herbicides (2,4-D and 2,4,5-T) at concentrations of about 50 ppm (8). However, most microorganisms are resistant to high concentrations. Sherman and Fletcher (30) subjected 38 species of soil bacteria, fungi and actinomycetes to 2,4-D and 2,4,5-T at concentrations of 100 to 10,000 ppm. Twenty-six species were not inhibited by 10,000 ppm 2,4-D. Twenty-four organisms required 10,000 ppm 2,4,5-T for growth restriction to occur. Stojanovic (34) added a mixture of 2,4-D and 2,4,5-T to soil at a concentration of 5,000 ppm and the bacteria and actinomycetes were inhibited but the total number of fungi increased during a 56-day incubation period.

It seems apparent from the literature that over the millennia, microorganisms have developed unbelievable capabilities for handling organic compounds. Moreover, most microorganisms seem to have a latent ability for decomposition of halogenated hydrocarbons. In a recent review, McNew (22) discussed the degradation of just such organic compounds in the soil. He noted that the degradation of such chemicals are dependent upon the enzymatic capabilities of the microorganisms. There are certain types of enzymes that destroy the molecules by hydrolysis at vulnerable spots such as an oxygen group or ester linkage, oxidation over an unsaturated bond or hydroxyl group, reduction, substitute reaction with a carboxyl or halogen substituent, or beta oxidation of an alkyl chain. McNew illustrated this degradation process by discussing the fate of 2,4-D in soil:

In normal loam soils rich in soil microorganisms there is hydrolysis to inactive acetic acid and 2,4-dichlorophenol within 2 to 6 weeks, depending upon the moisture and temperature of the soil. The acetic acid is immediately used as an energy source by entering into the Krebs cycle of almost any microorganism. The 2,4-dichlorophenol is further degraded by those organisms that attack phenols through the hydroxyl group. If instead of 2,4-D, an application is made of the inactive

ester 2,4-dichlorophenoxyethanol sulfate, Bacillus cereus var. mycoides hydrolyzes off the sulfate group, certain species of Pseudomonas or other bacteria oxidize the resultant alcohol to an acid, thereby producing 2,4-D which then undergoes decomposition by the means described above. In substance, the soil microorganisms can be encouraged to generate the herbicide in situ and then decompose it before excessive residues build up. This is an ideal self-regulant device but it has three drawbacks to discourage its general use: more chemical must be applied per acre, it can be ineffective on some soils with low microbial populations, and the system is extremely susceptible to variations in the environmental conditions.

The question can now be asked: "What are the breakdown products from phenoxy herbicides and do they accumulate in the soil?" Loos, et al. (21), and Bollag et al. (6, 7) have extensively studied in cultures the decomposition of 2,4-D by a soil Arthrobacter. They have suggested that the bacterium first enzymatically converts the 2,4-D to 2,4-dichlorophenol and other chlorophenols. These chlorophenols are further metabolized to catechols (e.g., 3,5-dichlorocatechol and 4-chlorocatechol). At low enzyme levels the chlorocatechols are metabolized completely.

At high enzyme levels other compounds are apparently formed. Bollag et al. (6) have identified these as carboxymethylene-butenolides. The butenolides are probably converted to chloromuconic acid and then to chloride ion, acetate and dicarboxylic acid. They concluded by noting that the toxicity of many of these intermediates is unknown and inasmuch as they are found in cultures of a microorganism obtained from soil, they may accumulate during the decomposition of phenoxy herbicides. But do they actually accumulate under field conditions? Investigations by Winston and Ritty (39) and Reigner et al. (27) indicated that both 2,4-D and 2,4,5-T are decomposed to form carbon dioxide, inorganic chlorides, and water; objectionable chlorophenols are not end-products of this decomposition. Further supporting evidence has been provided by Reinhart (28). The upper half of a 24 ha timber watershed in northern West Virginia was logged and treated with a 2,4,5-T ester to kill all vegetation. The volume of herbicide that was applied was 5,015 liters on 12 ha (418 liters/ha). Almost 3,000 liters of this were potential contaminating materials: about 2,800 liters of diesel oil and 200 liters of a commercial formulation of 2,4,5-T (142 kg acid equivalent). Reinhart found no odor contaminants (phenols or catechols) in the numerous water samples taken from the stream draining the treated watershed.

The Effects of Repetitive Applications of Phenoxy Herbicides

In recent years there have been claims that repetitive applications of phenoxy herbicides, especially at rates similar to those applied in South Vietnam, render the soil permanently sterile or at least sterile for a prolonged period. Studies have now been completed that provide results on effects of such repetitive applications.

First, in relation to the effects of herbicides on the soils of South Vietnam, the National Academy of Science published a report by Blackman et al. (5) on persistence and disappearance of herbicides in tropical soils. The 1974 report stated a number of general conclusions, namely:

1. The behavior of herbicides in the soils of Vietnam (and the Phillipines and Thailand) were similar to that reported for soils elsewhere.

2. Only where 2,4-D and 2,4,5-T were applied in very massive doses e.g. at the Pran Buri Calibration Grid in Thailand at rates in the magnitude of 1120 kg/ha, were there still residues in concentrations above the threshold likely to induce phytotoxic symptom in some plant species.

3. When applied to mangrove soils at total doses approaching 112 kg/ha of 2,4-D and of 2,4,5-T, the level of herbicide residues at the end of 30 weeks had no effect on

the establishment of two major mangrove species.

4. In geographical areas subjected to one or two military herbicide missions 1.5 years before sampling, no soil phytotoxic residues could be detected.

5. Soils that received a directed application of Herbicide Orange (a 50:50 mixture of the n-butyl esters of 2,4-D and 2,4,5-T) at the rate of 30 kg/ha safely supported the growth of crops sensitive to 2,4-D or 2,4,5-T four to six months following application.

6. Claims that the herbicides rendered the soil sterile were without any foundation.

Byast and Hance (9) have studied the degradation of 2,4,5-T studied by South Vietnamese soils incubated in the Laboratory. Although care must be exercised in extrapolating laboratory results to field situations, their results suggested that the four Vietnamese soils studied were inherently capable of degrading 2,4,5-T at levels at least as high as 15 ppm which corresponded to roughly twice the rate of military applications in Vietnam.

Young (40) has reported taxonomic and population studies of microorganisms in soils exposed to massive quantities (repetitive aerial applications) of 2,4-D and 2,4,5-T. In tests performed three years after the last application of Herbicide Orange, soils

from Test Area C-52A, Eglin Air Force Base, Florida (a 2.6 km² area that received over 75,650 kg 2,4-D and 75,380 kg 2,4,5-T from 1962 through 1970) exhibited a population of soil microorganisms no different to that found in an adjacent control area of similar soil and vegetative characteristics. Predominant bacteria isolated from either the control or test areas included species of Bacillus and Pseudomonas. The predominant fungi were species of Penicillium, Aspergillus, and Fusarium. The predominant actinomycetes were species of Streptomyces and Nocardia.

A similar study involving soil microorganisms and exposure to heavy rates of phenoxy herbicides has been reported by Stark et al. (32). In support of feasibility tests for the soil disposal of surplus Herbicide Orange, the Air Force established a field study in 1972 on the Air Force Logistics Command Test Range, Hill Air Force Base, Utah. The study consisted of replicated plots subsurface injected with concentrations of either 1,120, 2,240, or 4,480 kg herbicide/ha. Soil samples were taken by Stark et al. three times throughout 1973, and microbial species present (bacteria actinomycetes and fungi) were determined. Bacterial counts were higher for soils with greater concentrations of the herbicide and with greater moisture content, i.e., those samples collected in midwinter

from the 4,480 kg/ha plots. Herbicide Orange, in any concentration, had no significant effect on mycoflora. Arnold et al. (3) monitored the herbicide levels in these plots. They sampled the plots on eight occasions from 1973 through 1975, and determined the concentrations of the n-butyl esters and free acids of both 2,4-D and 2,4,5-T. They suggested that at such massive application rates (soil concentrations greater than 10,000 ppm) and in a alkaline desert environment that the half-life of 2,4-D and 2,4,5-T appeared to be in the range of 150 to 210 days.

The above studies have shown that the application of 2,4-D and 2,4,5-T at massive rates not only did not sterilize the soil, but indeed stimulated the growth of certain microflora. That these bacteria, actinomycetes and fungi proliferated indicated that they probably used the herbicides as a carbon source and, as such, contributed to their degradation.

Literature Conclusions

The literature on the interactions of microorganisms and phenoxy herbicides supported the following generalities:

1. All microorganisms are not affected to the same degree by a particular phenoxy herbicide.
2. Facultative anaerobes are more tolerant of higher concentrations of phenoxy herbicides than aerobic or anaerobic organisms.

3. Spore-forming bacteria are more susceptible to phenoxy herbicides than nonspore-formers.

4. Fungi are more resistant to the phenoxy herbicides than are bacteria.

5. Gram positive bacteria are inhibited by lower concentrations of phenoxy herbicides than are the gram negative bacteria.

6. Populations of microbes specifically induced in soil by addition of a phenoxy herbicide do not immediately disappear even though no energy material of the same composition may be present.

7. Rates of phenoxy herbicides normally used in agriculture do not effect rhizobia or leguminous nodules, and hence nitrogen fixation.

8. The usual order of decreasing toxicity to microorganisms for the phenoxy herbicides is 2,4,5-T, MCPA, and 2,4-D.

9. The phenoxy herbicides are more toxic to soil microorganisms in acid soils than in alkaline soils.

10. Use of the phenoxy herbicides in cereals is likely to reduce the incidence of plant diseases caused by fungi.

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