



Enhanced herbicide degradation and dissipation of clomazone in two Montana soils
by Eric Robert Gallandt

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Agronomy
Montana State University
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Abstract:

Clomazone is a preplant herbicide that provides excellent control of many weeds which are common in fallow land however little is known about the residual period or potential for injury to subsequent wheat crops. Clomazone dissipation was examined at two locations in Montana. Clomazone at 2.2 kg/ha applied to a loam soil dissipated to below 0.1 ppmw in 3 months and applied to a silty clay loam soil dissipated to 0.2 ppmw 6 months after application. Half-lives, determined from first order rate plots, were 33 and 37 days in the Willow Creek loam and Bozeman silty clay loam, respectively. Thus, clomazone residue from labeled-use rates should not inhibit wheat in a wheat-fallow-wheat cropping system in Montana.

Enhanced herbicide degradation is a phenomenon of accelerated herbicide decomposition in soil which is induced by prior treatment of that soil with the herbicide.

Enhanced degradation of EPTC was first reported in 1979 and is caused by an adaptation of the constitutive microbial population due to prior EPTC exposure. A soil inoculation technique was used to examine the geographic extent and degree of EPTC enhancement in 166 United States soils. Enhanced degradation of EPTC is a wide-spread phenomenon and EPTC half-lives ranged from less than 1 day to greater than 20 days in the 166 soils examined.

A soil inoculation technique was also used to determine if soils exist which contain microorganisms with the ability to enhance the rate of degradation of persistent soil herbicides such as chlorsulfuron, picloram, atrazine, or clomazone. None of the 166 soils tested contained microorganisms able to enhance the degradation of atrazine or clomazone however, several soils significantly altered the rate of chlorsulfuron or picloram degradation.

A minimal medium plating screen was conducted to isolate microorganisms capable of metabolizing persistent herbicides. Soil extracts from 166 soils were plated on minimal medium which contained chlorsulfuron, picloram, atrazine, clomazone, or EPTC as a sole carbon source.

Twenty-five isolates rapidly grew in liquid medium containing chlorsulfuron, picloram, or atrazine as a sole carbon source. HPLC was used to measure herbicide concentration in liquid cultures. Although attempts to show disappearance of the parent herbicide molecule failed repeatedly, several isolates appeared to produce unidentified metabolite peaks.

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Eric Robert Gallandt

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Clomazone is a preplant herbicide that provides excellent control of many weeds which are common in fallow land however little is known about the residual period or potential for injury to subsequent wheat crops. Clomazone dissipation was examined at two locations in Montana. Clomazone at 2.2 kg/ha applied to a loam soil dissipated to below 0.1 ppmw in 3 months and applied to a silty clay loam soil dissipated to 0.2 ppmw 6 months after application. Half-lives, determined from first order rate plots, were 33 and 37 days in the Willow Creek loam and Bozeman silty clay loam, respectively. Thus, clomazone residue from labeled-use rates should not inhibit wheat in a wheat-fallow-wheat cropping system in Montana.

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CHAPTER 1**LITERATURE REVIEW**General Herbicide Degradation

The ultimate fate of a herbicide molecule in soil is regulated by properties of the herbicide molecule, and environmental and edaphic conditions. Some herbicide molecules are volatile and are lost to the atmosphere. Others bind tightly to clay surfaces or soil organic matter and become unavailable for plant uptake. Molecules which do not interact strongly with soil constituents may leach. While volatilization, binding, and leaching reduce herbicide activity, the herbicide molecule remains intact.

Degradation of the herbicide molecule in soil can be caused by both biological and nonbiological mechanisms. Some herbicide molecules absorb ultraviolet light and undergo photochemical degradation. Absorbed ultraviolet energy is released by spontaneous bond cleavage with the formation of free radicals (45). Free radicals are often unstable and react immediately with the solvent system or other reactants. The amount of light energy which penetrates soil is very low; therefore, photodegradation of most soil-active herbicides is insignificant. Alternatively,

photodegradation is very significant for picloram [4-amino-3,5,6-trichloro-2-pyridine carboxylic acid], and napropamide [N,N-diethyl-2-(1-naphthalenyloxy)propanamide] if they are not leached into soil by precipitation (26). Pesticide photodegradation has been reviewed by Plimmer (45).

Chemical hydrolysis represents a major abiotic mechanism of degradation. Hydrolytic reactions involving pesticides are often facilitated by binding to soil reactive sites which favor catalysis (5). Armstrong and Chesters (4) examined the effect of model sorbents and soil on atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)1,3,5-triazine-2,4-diamine] hydrolysis. Cellulose acetate, montmorillonite clay, carboxylic resin, and a phenolic resin had high adsorption capacities for atrazine however only the carboxylic resin was more effective in catalyzing atrazine hydrolysis than soil. The authors proposed that the triazine molecule is weakly bound to carboxylic acid functional groups of soil organic matter. The adsorbed chloro-s-triazine is then susceptible to hydrolysis by nucleophilic substitution of -Cl by -OH forming hydroxy-s-triazine (4).

Studies designed to elucidate the degradative pathway of a herbicide in soil often fail to adequately separate biotic (microbial) and abiotic (photolysis/hydrolysis) factors. Separation of these factors is difficult because of the interaction of microbial populations and soil

chemistry. Microbial activity in soil is dependent upon soil pH, organic matter content, temperature, moisture content, and texture.

Soil microorganisms have the ability to partially degrade and/or mineralize an impressive array of natural and man-made organic compounds including herbicides. While microorganisms occupy approximately 0.1% of the soil volume, bacterial cell density can reach levels as high as 10^9 cells per g of soil (55).

Soil microorganisms may directly metabolize a herbicide, or indirectly affect degradation by inducing changes in the chemical environment (55). Direct catabolism occurs when a molecule is utilized as both a carbon and an energy source. This is typically a growth-linked process and often results in complete reduction of the molecule to carbon dioxide and water (1).

Early research on microbial degradation of synthetic organic molecules focused on catabolic metabolism. Considerable research was conducted with microorganisms growing on minimal media containing the chemical of interest as the sole source of energy for the organism. Lanzelotta and Pramer (36) used an enriched soil to select a strain of Fusarium solani capable of growth in a minimal salts medium containing the herbicide propanil [3',4'-dichloro-propionanilide] as the sole carbon source. The selected

fungus degraded propanil more rapidly when additional nutrients were added to the culture. The major metabolite, 3,4-dichloroaniline, eventually accumulated to levels which inhibited additional catabolism.

Hartman et al. (25) used a chemostat and stepwise selection to isolate a Pseudomonas spp. capable of utilizing 3,5-dichlorobenzoate as a sole carbon source. A chemostat system permits a culture to remain in the logarithmic phase of growth indefinitely. Fresh media is metered into the culture vessel as used media containing dead cells is removed. The growth rate of the bacterial culture increased significantly during the stepwise selection process as the sole carbon source was changed from 3-chloro- to 4-chloro-, and finally to 3,5-dichlorobenzoate. Unfortunately, the selected bacteria was not tested for its ability to degrade the compound in soil or water.

Daughton and Hsieh (13) used a chemostat to select microorganisms capable of using the insecticide parathion [O,O-diethyl O-P-nitrophenyl phosphorothionate] as a sole growth substrate. They selected two bacterial species which symbiotically metabolized parathion. P. stutzeri cometabolically hydrolyzed parathion to diethylthiophosphate and p-nitrophenol. P. aeruginosa utilized p-nitrophenol as a sole energy source. This culture was tested for its ability to degrade parathion in soil. Barles et al. (8) found that the culture obtained by Daughton and

Hsieh was capable of complete degradation of parathion with initial concentrations as high as 5000 ppmw in soil. The degradation rate of the commercial emulsifiable concentrate formulation was much slower than for technical grade parathion, or for rates of technical parathion above 5000 ppmw.

Rache and Lichtenstein (46) found that soil microorganisms could degrade unextractable, bound residues of ^{14}C parathion in soil. The addition of glucose to soil reduced the amount of nonextractable ^{14}C residues and increased $^{14}\text{CO}_2$ evolution. While the concept of cometabolism and pesticide degradation is receiving increased attention, systems utilizing cometabolism are exceptionally difficult to study (34).

The occurrence of microorganisms capable of normal growth on a single substrate such as a pesticide molecule is rare. Cometabolic degradation, in which an organism derives its energy from one substrate and incidentally metabolizes a pesticide molecule, is far more common both in pure cultures and in naturally occurring microbial populations (34). Wang et al. (59) examined the microbial degradation of monuron [N'-(4-chlorophenyl)-N,N-dimethylurea], linuron [N'-(3,4-dichlorophenyl)-N-methoxy-N-methylurea], and diuron [N'-(3,4-dichlorophenyl)-N,N-dimethylurea] at several concentrations in several environments. All three compounds

were mineralized (converted to CO₂ and H₂O) when added to sewage at 10 ug/L. However, when the concentration of monuron was increased to 10 mg/L the compound was cometabolically altered, not mineralized.

Lappin et al. (37) washed soil adhering to wheat (Triticum aestivum L.) roots into a minimal salts media containing the herbicide mecoprop [2-(2-methyl-4-chlorophenoxy) propionic acid] as a sole carbon source. They isolated a microbial community containing five species capable of growth on mecoprop. While none of the organisms could grow alone, combinations of two or more species were able to degrade the compound. Maximum culture growth and mecoprop degradation occurred when all five species were present.

Clomazone

Clomazone [2-(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxazolidinone] (Figure 1) is a broad spectrum herbicide discovered in 1979. It is being developed by the FMC Corporation under the trade name Command^R. Clomazone is currently labeled for use as a preemergence or preplant incorporated herbicide in soybeans (Glycine max L. Merr.) and as a fallow herbicide. The herbicide controls many problem weeds of soybeans including barnyardgrass (Echinochloa crus-galli L.), fall panicum (Panicum dichotomiflorum Michx.), foxtails (Setaria spp.), velvetleaf

(Abutilon theophrasti Medico.), common lambsquarters
 (Chenopodium album L.), and black nightshade (Solanum nigrum
 L.).

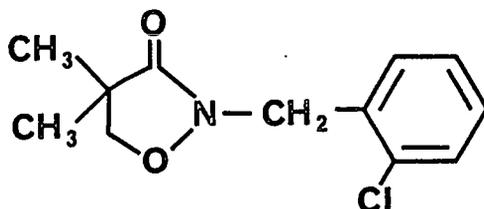


Figure 1. Structure of clomazone.

Bellman et al. (10) found that clomazone provided better residual control of giant foxtail (Setaria fabre Herrm.) than several other grass herbicides commonly used in soybean production including trifluralin [2,6-dinitro-N,N-dipropyl-4-(trifluoromethyl)benzenamine], metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide], and alachlor [2-chloro-N-(2,6-diethylphenyl)N(methoxymethyl) acetimide]. Velvetleaf was most susceptible to clomazone followed in order by giant foxtail, common lambsquarters, and redroot pigweed (Amaranthus retroflexus L.) (10). Clomazone alone or in combination with metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one] controlled

velvetleaf and common lambsquarters for a longer period of time than metribuzin alone or metribuzin in combination with the grass herbicides mentioned above.

Clomazone has been tested for use in chemical fallow in Montana for several years. Results from field trials established in 1984 through 1986 indicated good to excellent control of several common weed species associated with fallow (18) (Table 1).

Soil factors influence the activity of clomazone. In leaching studies, the herbicide demonstrated low mobility in sandy loam, silt loam, and clay loam soils, and intermediate mobility in fine sand (2). In field studies, the half-life was 15 to 45 days depending upon soil type (2).

Tymonko and Guscar (39) applied clomazone to 21 soil types and examined its effect on the growth of ten plant species in the greenhouse. High levels of soil organic matter reduced activity. Activity was also reduced in soils with high clay content or high cation exchange capacity. Activity was highest in sandy soils. There was no correlation between soil pH and herbicide activity.

Loux and Slife (41) compared the activity of clomazone in sandy clay loam (2.7% organic matter) and sandy loam (1.3% organic matter) soils using sorghum (Sorghum bicolor L.) as a bioassay indicator species. Initial sorghum injury was greater in low organic matter soil. Herbicide incorporation did not reduce activity.

Table 1. Weed control results obtained with clomazone in chemical fallow field trials conducted at 3 locations in Montana from 1984 through 1986 (18).

Clomazone Rate	No. of Trials	Bare Ground	Vol. Wheat	Downy-Brome ²	Russian thistle ³
(kg ai/ha)		-%-		Percent Control ¹	
0.6	4	85	70	63	73
0.8	3	89	98	65	88
1.1	2	94	100	100	88
1.4	1	90	90	100	85

¹Control was rated visually with 0 = no control and 100 = complete control.

²Bromus tectorum L.

³Salsola iberica L.

They found that mobility in soil columns varied with soil texture and organic matter content. Significantly greater movement occurred in a soil with 1.3% organic matter compared to a soil with 5.7% organic matter. These findings are supported by the results of other studies which used soil thin-layer chromatography and soil leaching columns to examine clomazone mobility (20).

Keifer and ElNagger (33) also examined the role of soil organic matter on the activity of clomazone. The herbicide was applied to seven field soils and three clays and allowed to dry overnight. The herbicide was extracted from soil using 5mM CaSO₄ to determine the partition coefficient between soil and water. Soil/water partition coefficients ranged from 8 for a kaolin clay to 60 for a muck soil with 76% organic matter indicating that the herbicide is

partitioned into organic matter. The average of soil organic matter/water partition coefficients was 960 over a range of field soils which indicates that on a weight basis clomazone has more affinity for organic matter than clay.

Aerobic degradation of ^{14}C -methyl-labeled clomazone led to the evolution of $^{14}\text{CO}_2$ and unidentified soil-bound residues (20). Under anaerobic soil conditions the herbicide was rapidly degraded to an experimental compound, FMC 65317 (N-[(2'chlorophenyl)methyl]-3-hydroxy-2,2-dimethylpropanamide) (20).

Enhanced Thiocarbamate Degradation

Enhanced herbicide degradation is the phenomenon where accelerated herbicide decomposition in soil is induced by prior treatment of that soil with the herbicide (51). Enhanced degradation was first reported by Audus (6) in 1949. Soil pretreated with 2,4-D [2,4-dichloro-phenoxy acetic acid] showed an enhanced degradation rate of subsequent 2,4-D applications. Enhanced degradation in soil has also been shown for several other herbicides that have foliar but not soil activity (35).

Enhanced herbicide degradation was first reported for the soil applied herbicide EPTC [s-ethyl dipropyl-carbamothioate], in 1979 (49). EPTC is a preemergence herbicide introduced in 1959 to control broadleaf and grass weeds in corn (Zea mays L.), potatoes (Solanum tuberosum

L.), alfalfa (Medicago sativa L.), and many vegetable crops. Rhaman et al. (49) found that EPTC applied to plots which had previously received three annual EPTC applications failed to control bristly foxtail (Setaria verticillata L. Beauv.). The herbicide provided excellent control in adjacent plots which had no prior EPTC history. The authors proposed that the herbicide failure was caused by rapid degradation from enhanced microbial activity, a theory which has been supported by many studies in the United States (23,38,41,43,54,60).

Obrigawitch et al. (43) measured accelerated $^{14}\text{CO}_2$ evolution when ^{14}C -carbonyl-labeled EPTC was applied to a soil with a history of EPTC use. In other studies a single EPTC application was sufficient to enhance the degradation of subsequent applications in some soils (42,43). The half-life of EPTC was reduced from 13 to 3 days in a soil in which EPTC had been applied for eight consecutive years (42).

Applications of EPTC may induce enhanced degradation of other thiocarbamate herbicides, a phenomenon termed cross-enhancement (54). While EPTC degradation was greatly accelerated in a soil which received 9 annual EPTC applications, the rate of degradation of butylate [s-ethyl bis(2-methylpropyl)carbamothioate], a thiocarbamate analog of EPTC, was accelerated far less than EPTC (42).

