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Microbial Degradation of Fluridone[1]

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ABSTRACT

Microbial consortia collected from two lakes degraded fluridone [1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4-(lH)-pyridinone] when placed in liquid culture. Microorganisms from Lake McLeod (no previous fluridone application) degraded 40% of the fluridone in solution while microbes from Lake Pierce (prior fluridone application) degraded 26% of the compound over a 150 day period. When heterogeneous cultures from Lake McLeod were transferred to new liquid cultures which contained fluridone, 99% of the herbicide was degraded over a 370 day period. These enriched cultures were grown using media that contained alternate carbon sources plus fluridone or minerals plus fluridone. Ninety-nine percent of the fluridone in the minerals plus fluridone medium was degraded. Cultures in the other media grew rapidly, but little fluridone was degraded. This indicates that there are carbon sources preferentially assimilated over fluridone. These degradation rates do not indicate that past fluridone use influences fluridone degradation. However, continued exposure to high rates of the compound can increase the rate of microbial breakdown under experimental conditions.

Key words: microorganisms, environmental fate, persistence, enrichment, herbicide degradation.

INTRODUCTION

Fluridone is used in Florida to control the exotic submersed plant hydrilla (*Hydrilla verticillata* (L.f.) Royle). Although fluridone concentration as low as 8 ppb can be phytotoxic to hydrilla (6), control has been variable. A possible cause of variable results with fluridone may be differences in microbial degradation of the herbicide. If microorganisms are involved in the degradation of fluridone, variability in their activity could result in changes in concentration after application. Although most herbicides are degraded by microorganisms, the rate is usually too slow to have an important effect on weed control. However, in certain cases, (e.g. thiocarbamates) the ability of a microorganism population to degrade a herbicide increases after repeated application, to a point where significant loss of weed control occurs due to a rapid decrease in the herbicide concentration in the soil. This phenomenon is known as microbial enrichment.

Moorman (9) demonstrated that in two field soils, EPTC (S-ethyl dipropylthiocarbamate) and butylate (S-ethyl diisobutylthiocarbamate) were degraded 66% and 92% more rapidly, respectively, when the soils were treated with EPTC for three consecutive years. This accelerated rate of herbicide degradation was due to an increase in the rate of microbial metabolism rather than an increase in population. In addition to the thiocarbamates, dicamba (3,6-dichloro-2-methoxy-benzoic acid), propanil [N-(3,4-dichlorophenyl)propanamide] and glyphosate [N-(phosphonomethyl)glycine] have been shown to be degraded by certain species of bacteria and fungi (4, 8, 12). However, there is a difference between enrichment with a subsequent loss in weed control and simple degradation of herbicides by microorganisms.

Results of studies conducted to assess the rate of dissipation of fluridone in terrestrial and aquatic environments indicate a possible interaction between microorganisms and fluridone. Banks et al. (2) demonstrated that fluridone degradation was reduced in sterilized soils and Muir and Grift (11) were able to isolate ^{14}C-fluridone metabolites from a sediment-water culture incubated for 26 months. The fluridone metabolite, 1,4-dihydro-1-methyl-4-oxo-5-[3-trifluoromethylphenyl]-3-pyridine carboxylic acid, accounted for 48 to 54% of the applied radioactivity. Other metabolites present were two unidentified phenolic compounds and desphenylfluridone. Muir et al. (10) demonstrated that re-treatment of a pond with 100 $ug \cdot l^{-1}$ of fluridone resulted in a decrease of fluridone half-life in sediment from 1 year to 20 weeks. Freund and Rubin (5) found that "history soils" (soils previously treated with fluridone) showed a more rapid decline in phytotoxicity than "non-history soils".

The objective of this study was to determine if microbial metabolism was responsible for variability in fluridone efficacy. Microbial response to repetitive fluridone exposure and the possible role of preferential carbon assimilation was also investigated.

MATERIALS AND METHODS

Seven inocula, consisting of hydrosoil and water, were collected by Eckman dredge from lakes in Florida and South Georgia. Samples were placed in plastic bags and cooled for transport. Lakes Orange, Istokpoga, Pierce, Ray's and Biven's Arm had previously been treated with fluridone. Lakes McLeod and Mize had never been treated with fluridone. Two ml of homogenized sample suspension were placed in 50 ml of sterile mineral medium plus fluridone[3] (approx. 12 ppm). The medium contained the following (in g/1): KNO_3, 0.2; N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES) buffer, 4.77; K_2HPO_4, 0.2; NaCl, 0.02; KC1, 0.08; $CaCl_2 \cdot 2H_2O$, 0.06; $MgSO_4 \cdot 7H_2O$, 0.1; H_3BO_3, 0.014; $FeSO_4 \cdot 7H_2O$, 1.9×10^{-3}; $MnCl_2 \cdot 4H_2O$, 1.7×10^{-3}; $ZnSO_4 \cdot 7H_2O$, 2.2×10^{-5}; $CuSO_4 \cdot 5H_2O$,

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$1.8x10^{-6}$; $CoCl_2 \cdot 6H_2O$, $6.0x10^{-7}$; Thiamine, $5.0x10^{-6}$; cyanocobalamine (B_{12}), $5.0x10^{-8}$; Biotin, $5.0x10^{-8}$. The pH of the liquid mineral medium was adjusted to 6.5. Both sterile and inoculated medium were placed in either a 12 hr photoperiod (100 $\mu E \cdot m^{-2} \cdot sec^{-1}$) or continuous darkness at 20 C. Two ml samples were collected every 50 days for 150 days.

The growth of microorganisms was determined by monitoring the absorbance of each sample at 600 nm. Each 2 ml sample was filtered (0.2 μm pore size) and rinsed with 2 ml of methanol to remove residual fluridone, which has been shown to bind to plastic when in aqueous solution (data not shown). Fluridone concentration was determined by high performance liquid chromatography (HPLC).[4] A 25 μl injection of the culture solution was analyzed using a Supelco[5] C8-DB column (nominal particle size 5.0 μm) with a 70:30 methanol/water mobile phase and a flow rate of 0.75 $ml \cdot min^{-1}$. Retention time for fluridone was 5.6 to 6.1 min. Detection of fluridone was accomplished using a UV-visible detector[4] set at 300 nm (0.01 AUFS).

Cultures testing positive for fluridone degradation were transferred to new mineral medium plus fluridone (ca. 12 ppm). However, no sediment (and therefore external carbon sources) was present. Fluridone and HEPES buffer served as the sole carbon sources. Cultures were kept at 29 C and agitated at 100 rpm with no illumination. Samples were removed periodically and analyzed for fluridone as stated before.

These same heterogeneous cultures were added to either soy-peptone[6] plus fluridone, thioglycollate[6] plus fluridone or mineral medium plus fluridone solutions. Fluridone concentration was monitored over a 75 day period.

From heterogeneous cultures that degraded fluridone, 0.1 ml of inoculum was spread onto silica gel or purified agar plates which contained approximately 10 ppm fluridone. Growth occurring on these plates was transferred to standard bacterial and fungal media. Isolates from these plates were added to mineral medium plus fluridone solutions which were monitored for fluridone loss.

All studies were conducted twice and replicated four times. The effect of the various lake inocula on the degradation of fluridone are presented as percent loss (PL). Percent loss of fluridone was calculated by the following equation:

$$PL = \left[\frac{\text{(sterile concentration - treatment concentration)}}{\text{(sterile concentration)}}\right] X100$$

Analysis of variance (7) was used to test for main factor effects (i.e., different lake inocula, illumination and time) and interactions. When fluridone degradation over time was evaluated, mean values were subjected to regression analysis to develop predictive models. These models were used to determine the predicted percent loss of fluridone at the termination of each experiment as influenced by different inocula.

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[5]Supelco, Inc. Bellefonte, PA 16823.
[6]Difco Laboratories. Detroit, MI 48232.

RESULTS AND DISCUSSION

Inocula from two of the seven lakes degraded fluridone (Table 1). Inocula from the other five lakes grew in the medium, but did not degrade fluridone (data not shown). The growth of these five inocula was probably due to the utilization of carbon-containing compounds that were added in the initial hydrosoil plus water suspension.

Cultures from Lakes McLeod and Pierce that were maintained in total darkness for 150 days decreased the concentration of fluridone in solution by 40 and 26%, respectively (Figure 1). The predicted percent loss of fluridone after 150 days was 38 and 20% for cultures inoculated from Lake McLeod and Lake Pierce, respectively (Table 1). Percent loss of fluridone in cultures exposed to the 12 hr photoperiod (Figure 2) was less than that of the cultures in continuous darkness. There was a 29% loss of fluridone in the Lake McLeod cultures at the end of the sampling period. The predicted percent loss of fluridone was 27% (Table 1). Fluridone loss in cultures from Lake Pierce was 9% after 150 days. Data from this treatment were quite variable and a regression model was not constructed. An interpretation of these results is that repeated fluridone use in a lake may not influence the rate of fluridone degradation by microorganisms. However, our sample size was small and microbial enrichment cannot be discounted. Possibly more important than the phenomenon of enrichment is the ability of microorganisms to actually degrade fluridone in the aquatic environment, which this study has demonstrated.

Microbial growth tended to plateau in the dark treatments (Figure 1), demonstrating that there was a shift in microbial metabolism, or that microorganisms capable of degrading fluridone grew at a constant rate. Both of these phenomena can lead to enrichment situations.

Fluridone was degraded more rapidly in the continuous darkness than the light/dark treatments. This may have been due to competition for space and nutrients between fluridone degrading and light requiring microorganisms. Absorbance data (Figures 1 & 2) indicate that microbial biomass was greater in the 12 hr light/dark treatment due to the presence of photosynthetic microorganisms. Visual observation indicated that the dark treatments did not contain these types of chlorophyllous microorganisms.

Three replicate heterogeneous cultures (A,B,C) that demonstrated the highest percentage of fluridone degra-

TABLE 1. REGRESSION EQUATIONS AND PREDICTED PERCENT LOSS (PPL) OF FLURIDONE EXPOSED TO VARIOUS LAKE INOCULA AND ILLUMINATION REGIMES AFTER 150 DAYS OF INCUBATION.

Lake	Regime	Equation	R^2	PPL
McLeod	light	%loss = 0.31 + 0.18(time)	0.93	27
McLeod	dark	%loss = 0.07 + 0.25(time)	0.96	38
Pierce	light	NC[a]		
Pierce	dark	%loss = -2.46 + 0.15(time)	0.79	20

[a]NC = regression model not constructed from data.

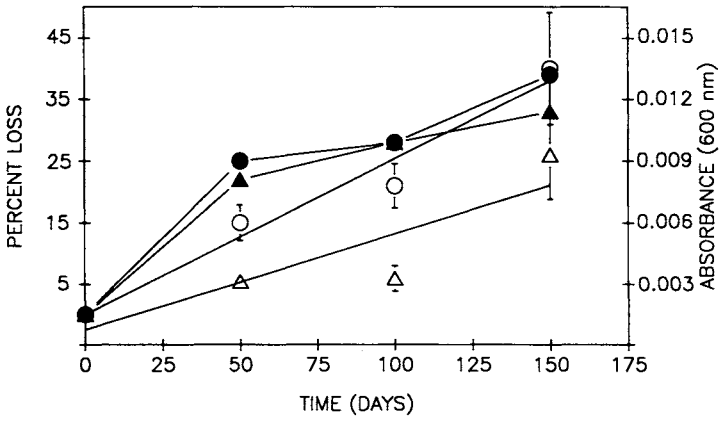

Figure 1. Percent loss of fluridone (open symbols) and absorbance (closed symbols) in cultures exposed to inocula from two lakes in continuous darkness:

○,● -Lake McLeod

Δ,▲ -Lake Pierce.

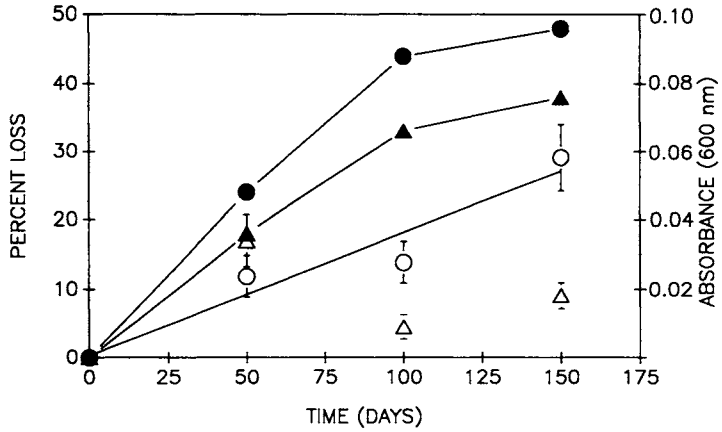

Figure 2. Percent loss of fluridone (open symbols) and absorbance (closed symbols) in cultures exposed to inocula from two lakes in a 12 h photoperiod:

○,● -Lake McLeod

Δ,▲ -Lake Pierce.

dation in the Lake McLeod dark treatments were transferred to a liquid medium that contained fluridone and HEPES buffer as the sole carbon sources. Culture A degraded 10% of the fluridone over the 370 day time period while cultures B and C degraded 99% of the fluridone. The predicted percent loss of the three cultures was 12, 100 and 110%, respectively (Table 2).

In the second enrichment, culture A demonstrated a 15% loss over the 210 day period. However, variable results or an insignificant amount of fluridone degradation precluded a regression model from being fit to the data. Cultures B and C again degraded 99% of the fluridone present by the end of the sampling period. The predicted percent loss of fluridone for cultures B and C were 117 and 108%, respectively (Table 2).

In both enrichment series, culture A degraded fluridone more slowly than cultures B and C, which were

TABLE 2. REGRESSION EQUATIONS AND PREDICTED PERCENT LOSS (PPL) OF FLURIDONE EXPOSED TO INOCULA FROM LAKE MCLEOD THROUGH TWO ENRICHMENTS.

Enrichment	Culture	Equation	R^2	PPL[a]
one[b]	A	%loss = 1.39 + 0.03(time)	0.83	12
one	B	%loss = -3.64 + 0.28(time)	0.97	100
one	C	%loss = -1.06 + 0.30(time)	0.97	110
two[c]	A	NC[d]		
two	B	%loss = 18.54 + 0.47(time)	0.80	117
two	C	%loss = 5.55 + 0.49(time)	0.95	108

[a]PPL was computed at the time the incubation was terminated.
[b]Enrichment one was terminated after 370 days of incubation.
[c]Enrichment two was terminated after 210 days of incubation.
[d]NC = regression model not constructed from data.

similar. Since all inocula came from the same hydrosoil and water samples, the differences in loss of fluridone indicates that microbial metabolism increased in cultures B and C (i.e., increased fluridone degradation). This phenomenon may have been a result of enzyme induction, which has been shown to occur with the thiocarbamate herbicides (3,9). The increase in rate of fluridone degradation (i.e., 370 and 210 days required to degrade 99% of the fluridone after 1 and 2 enrichments, respectively) indicates that it is possible to cause an increase in fluridone degradation by continuously exposing the microbial populations to high rates of fluridone.

These same heterogeneous cultures added to the soy peptone plus fluridone and thioglycollate plus fluridone solutions degraded 4% and 8% of the herbicide over a 75 day period, respectively. In contrast, the same cultures added to the mineral medium plus fluridone solution degraded 99% of the herbicide over the same time period. This difference in percent loss of fluridone suggests that the rate of fluridone degradation by microbial cultures is influenced by other carbon sources. When a broad spectrum of carbon compounds was available, only small amounts of fluridone were degraded. However, when the cultures were exposed to a single carbon source (i.e., only fluridone), degradation proceeded rapidly. These findings imply that in a lake system, fluridone would not be rapidly degraded if other carbon sources were available. However, naturally occurring carboniferous detrital material such as cellulose and lignin, which contain the majority of the carbon in the environment, are relatively long-lived (1). Therefore, it is difficult to predict whether these compounds would be degraded preferentially to fluridone.

When heterogeneous cultures capable of degrading fluridone were transferred from liquid to silica and agar solid media, both bacterial and fungal growth was observed. However, when individual isolates were added to the mineral medium plus fluridone solution, no degradation of the herbicide occurred over the 60 day period. This observation indicates that a mixed culture may be required to degrade fluridone. This is similar to findings obtained with metolachlor, in which individual isolates could not degrade the compound (13).

This is the first reported research which demonstrates *in vitro* microbial degradation of fluridone. Although the initial rate of fluridone degradation is slower than those reported for other herbicides (4,8,9,12), this rate can be

increased by constant exposure to high rates of the compound. It is also apparent that the presence of other carbon-containing compounds can decrease the rate of fluridone degradation. Although mixed cultures are capable of degrading fluridone, attempts to isolate individual microorganisms of this culture that degrade fluridone were unsuccessful.

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