APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1993, p. 2642–2647
0099-2240/93/082642-06$02.00/0

Vol. 59, No. 8

Degradation of Atrazine and 2,4-Dichlorophenoxyacetic Acid by Mycorrhizal Fungi at Three Nitrogen Concentrations In Vitro†

PAULA K. DONNELLY,[1]‡* JAMES A. ENTRY,[2] AND DON L. CRAWFORD[1]

Department of Bacteriology and Biochemistry, University of Idaho, Moscow, Idaho 83843,[1] and Department of Forest Science, College of Forestry, Oregon State University, Corvallis, Oregon 97331[2]

Received 3 March 1993/Accepted 7 June 1993

Nine mycorrhizal fungi and free-living saprophytic microorganisms were tested for their ability to degrade two chlorinated aromatic herbicides at two herbicide concentrations and three nitrogen concentrations. Radiolabelled 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine) were used as substrates at concentrations of 1 and 4 mM. After 8 weeks, none of the cultures tested grew at 4 mM 2,4-D. However, when the 2,4-D concentration was reduced to 1 mM, *Phanerochaete chrysosporium* 1767 had the highest level of 2,4-D mineralization and degradation under all nitrogen conditions. All cultures tested grew at both atrazine concentrations. In all cases, the ericoid mycorrhizal fungus *Hymenoscyphus ericae* 1318 had the highest level of atrazine carbon incorporated into its tissue. In general, as the nitrogen concentration increased, the total herbicide degradation increased. All of the cultures, except for *Rhizopogon vinicolor* 7534 and *Sclerogaster pacificus* 9011, showed increased degradation at 4 mM compared with 1 mM atrazine. The ability to degrade these two herbicides thus appeared to be dependent on the fungus and the herbicide, with no correlation to fungal ecotype (mycorrhizal versus free living).

Chlorinated aromatic compounds are a major group of chemicals responsible for environmental pollution. They are highly toxic and resistant to degradation, and thus they accumulate in the environment. Many of these compounds are widely used as herbicides (5). Residual amounts of herbicide can persist for a significant period after application. This may have detrimental effects on the ecosystem (4). The degradation of herbicides in the soil environment, like that of any hydrocarbon, is affected by environmental conditions. Two chlorinated aromatic herbicides commonly used in both agriculture and forestry for vegetation control are 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine) and 2,4-dichlorophenoxyacetic acid (2,4-D).

Atrazine is a complex aromatic compound which is commonly used as a pre- and postemergence herbicide for broadleaf and grassy vegetation (4, 18). In Christmas tree plantations and conifer reforestation areas, it is often used for selective weed control. On noncropped land, it is used at higher concentrations for nonselective vegetation control. Application as a preemergence control agent is preferred. The herbicide is absorbed through the plant roots and is translocated through the xylem to the leaves and apical meristem, where chlorosis and death are caused by inhibition of photosynthesis. It can also be absorbed by the leaves, but this amount is usually quite small and is dependent on environmental conditions (4). Atrazine is readily adsorbed to clay soils. Leaching through the soil is limited due to its adsorption to soil particles; however, the adsorption is reversible. Due to its extremely slow degradation, residual amounts of atrazine can be found in the soil for an extended time after application, especially in cold, dry climates (4). Microbial degradation of atrazine has been observed by various researchers (3, 10, 15, 27). Atrazine degradation involves hydroxylation, dealkylation, and ring cleavage (10, 11, 15).

The aromatic compound 2,4-D is widely used as a systemic herbicide for controlling broadleaf vegetation (4, 18). It is usually applied as a postemergence herbicide. The salt forms of 2,4-D are readily absorbed by plant roots and move upward in the transpiration system. The ester forms of 2,4-D are readily absorbed by the leaves and are converted to the acid form by the plant. The acid is then translocated within the phloem. The herbicide accumulates mainly at the meristematic regions of the roots and shoots (4). Because it is an extremely active auxin, 2,4-D causes the plant to grow too fast, changing the normal growth patterns and resulting in death of the plant (12). Microbial degradation of 2,4-D is known to occur in warm, moist soil; however, the rate is very dependent on environmental conditions and soil characteristics (4). Microbial degradation of 2,4-D has been shown with both pure cultures and mixed cultures (16, 21, 25).

Although microbial degradation of herbicides has been observed, it is not known whether ectomycorrhizal fungi can degrade herbicides. The classical thought is that mycorrhizal fungi provide the host plant with minerals as the host plant provides the fungi with carbohydrates (13). Studies show that several mycorrhizal fungi are capable of degrading and utilizing carbohydrates usually found in the litter layer (17, 26). Mycorrhizal fungi have also been shown to degrade lignin, a complex, aromatic plant polymer (7, 14, 26). Several species have also been shown to have the enzymes necessary to degrade other complex, aromatic compounds present in the soil (2, 28). Aust (1) and many others have shown that the ligninolytic system of the nonmycorrhizal fungus *Phanerochaete chrysosporium* is active during the degradation of numerous complex aromatic pollutants. Because several studies have shown that mycorrhizal fungi are capable of degrading lignin, we were interested in learning whether the

* Corresponding author.
† Paper no. 93506 of the Idaho Agricultural Experimental Station.
‡ Present address: Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019.

mycorrhizal fungi would also be able to degrade chlorinated aromatic compounds. The objective of this study was to determine whether selected mycorrhizal fungi would degrade the aromatic herbicides 2,4-D and atrazine in vitro under various physiological conditions.

MATERIALS AND METHODS

Cultures. A total of nine fungi and one actinomycete were tested for their ability to degrade two aromatic herbicides. The fungi were divided into the following five groups: two ericoid mycorrhizal fungi (*Hymenoscyphus ericae* 1318 and *Oidiodendron griseum*), two mat-forming ectomycorrhizal fungi (*Gautieria crispa* 4936 and *Gautieria othii* 6362), one ectomycorrhizal fungus associated with decomposing wood (*Radiigera atrogleba* 9470), one general ectomycorrhizal fungus (*Rhizopogon vinicolor* 7534), and three nonmycorrhizal fungi (*P. chrysosporium* 1767, *Sclerogaster pacificus* 9011, and *Trappea darkeri* 8077). All of the mycorrhizal fungal cultures as well as *Sclerogaster pacificus* 9011 and *Trappea darkeri* 8077 were obtained from the USDA Forest Service, Pacific Northwest Forest Research Station, Corvallis, Ore. The culture of *Phanerochaete chrysosporium* 1767 (ATCC 24725) was supplied by Andrzej Paszczynski, Department of Bacteriology and Biochemistry, University of Idaho, Moscow, Idaho. The actinomycete *Streptomyces viridosporus* T7A (ATCC 39115) was isolated from Idaho soil by D. L. Sinden (24). It conforms to the taxonomic description of *S. viridosporus* by Shirling and Gottlieb (23).

Herbicides. The ^{14}C-ring-labelled 2,4-D was obtained from Sigma Chemical Co. (St. Louis, Mo.). The radiochemical purity was >98%. The ^{14}C-ring-labelled atrazine was supplied by Bruce Thede, Agricultural Division, Ciba-Geigy Corp. (Greensboro, N.C.). The radiochemical purity of the ^{14}C-atrazine was 99.5%.

Culture preparation. The cultures were grown on a solid medium amended with ^{14}C-atrazine or ^{14}C-2,4-D in microcosms as described by Entry et al. (8). Two concentrations of herbicide were tested: 1.0 and 4.0 mM. Three concentrations of ammonium tartrate as the nitrogen source were tested: 0.0, 1.0, and 10.0 mM. The basal medium contained (per liter): glucose, 10 g; KH_2PO_4, 0.4 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $CaCl_2 \cdot 2H_2O$, 100 mg; Fe-citrate, 5 mg; $MnSO_4 \cdot 4H_2O$, 5 mg; $ZnSO_4 \cdot 7H_2O$, 4.4 mg; yeast extract, 50 mg; and agar, 15 g. Uniformly ring-labelled atrazine or 2,4-D was added at final concentrations of 16,430 and 21,131 dpm per sample, respectively. One or 4 mM unlabelled atrazine or 2,4-D per liter was filter sterilized through a 0.2-μm filter and added to each medium.

Ten milliliters of medium with herbicide was pipetted into 60-mm-diameter petri dishes. Each plate was then inoculated with a 3-mm agar plug of the specific culture and placed in a sterile 0.89-liter container with two vials. One vial contained 5.0 ml of 1 N NaOH as a CO_2 trap, and the second vial contained 20 ml of distilled H_2O to maintain the humidity (9). The containers were sealed, and the samples were incubated at 22°C for 8 weeks. There were five replicates of each culture; uninoculated plates were used as controls. Each experiment was repeated; therefore, analysis of data was performed with a total of 10 replicates per treatment.

$^{14}CO_2$ determination. After 8 weeks of incubation, the containers were opened, and the vials containing NaOH were removed. The $^{14}CO_2$ was quantified with standard liquid scintillation methods as follows (8). From each vial, 0.5 ml was removed and transferred to a new vial to which 1.0 ml of distilled H_2O was added and mixed. Then, 17.0 ml of aqueous scintillation cocktail (Bio-Safe II; Research Products International Corp., Mt. Prospect, Ill.) was added, mixed, and allowed to sit overnight at 22°C in the dark for chemiluminescence to subside. The samples were then counted in a Beckman LS 7500 autoscintillation counter.

Fungal dry weight. Fungal dry weight was quantified as follows. The agar and fungal colonies were removed from the petri dish, placed in 200-ml beakers containing distilled H_2O, and steamed until the agar had liquefied (6, 20). The samples were vacuum filtered on 0.2-μm filters and washed with unlabelled herbicide solution at a concentration 10 times that of the herbicide solution used in the incubation medium to replace any labelled herbicide bound to the cell wall surfaces. The fungal tissues were then dried at 70°C and weighed (19).

^{14}C-tissue determination. After weighing, the amount of ^{14}C incorporated into the tissue was quantified by the method of Entry et al. (8). To each tissue sample was added 250 μl of distilled H_2O to rehydrate the tissue. The suspension was allowed to sit overnight at 22°C, and then 2.0 ml of Protosol (NEN Research Products, DuPont, Boston, Mass.) per 200 mg of dry sample was added; samples were incubated at 45°C with shaking at 150 rpm. The samples were removed and allowed to cool to room temperature, and then 0.1 ml of 30% H_2O_2 was added as a decolorization agent. After 30 min at 22°C, 10 ml of scintillation cocktail (Bio-Safe II) was added and mixed. For the reduction of chemiluminescence, the samples were allowed to sit at 22°C in the dark overnight prior to counting by a Beckman LS 7500 autoscintillation counter.

Statistical analysis. All data were transformed with natural logarithmic procedures to achieve a normal distribution and were analyzed with general linear model procedures (22) for a completely random design. Residuals were normally distributed with constant variance. Individual treatment means of log-transformed data were compared with Tukey's Studentized range honestly significant difference (HSD) test at $P \leq 0.05$.

RESULTS

A distinction was made between the terms mineralization and degradation. Mineralization was defined as the complete degradation of the substrate to CO_2 and H_2O. Degradation was defined as the alteration of the original substrate, but not necessarily to CO_2. In this paper, several values are reported: the percentage of $^{14}CO_2$ recovered (the amount of mineralization), the percentage of ^{14}C incorporated into tissue (the amount of incorporation), and the total percentage of ^{14}C recovered (percentage of $^{14}CO_2$ plus percentage of ^{14}C incorporated into tissue; the amount of degradation). Growth was defined as the increase in biomass. The controls were uninoculated plates containing medium.

2,4-D. When the medium was supplemented with 2,4-D at a concentration of 4 mM, none of the cultures grew. All cultures were able to grow at 1 mM. At 0.0 mM added nitrogen, *P. chrysosporium* mineralized significantly more 2,4-D than the other cultures (24.15%). The remaining cultures did not mineralize 2,4-D at significant levels. *H. ericae*, *P. chrysosporium*, and *R. vinicolor* had the highest amount of ^{14}C incorporated into their tissue, although it was not at significant levels. *R. vinicolor* degraded the most 2,4-D (1.91%) among the mycorrhizal fungi.

When the nitrogen concentration was 1 mM, *P. chryso-*

TABLE 1. $^{14}CO_2$ and ^{14}C incorporation into microbial tissue during 2,4-D degradation with 10 mM added nitrogen

Species and description	Incorporation of $^{14}CO_2$ and ^{14}C			
	Wt (mg)[a,b]	CO_2 (%)[a,c]	Tissue (%)[a,d]	Total (%)[a,e]
Ericoid mycorrhizae				
H. ericae 1318	11.1*	0.57†	1.94*	2.51‡
O. griseum	6.5‡	0.99‡	0.48†	1.47†
Mat-forming ectomycorrhizae				
G. crispa 4936	6.5‡	0.28§	1.01‡	1.29†
G. othii 6362	1.4†,§	0.23§	0.08§	0.31§
Ectomycorrhiza associated with decomposing wood; *R. atrogleba* 9470	1.7†,§	0.23§	0.08§	0.31§
General ectomycorrhiza; *R. vinicolor* 7534	0.8†,§	0.25§	0.12§	0.37§
Nonmycorrhizal fungi				
P. chrysosporium 1767	6.7‡	28.91*	2.23*	31.14*
S. pacificus 9011	0.4§	0.26§	0.08§	0.34§
T. darkeri 8077	0.4§	0.29§	0.09§	0.38§
Actinomycete; *S. viridosporus* T7A	4.4†,‡	0.22§	0.15§	0.37§
Control	0.2§	0.20§	0.08§	0.28§

[a] Values are means of 10 samples. In each column, values followed by the same symbol are not significantly different at $P \leq 0.05$ as determined by Tukey's Studentized range (HSD) test.
[b] Values are reported as milligrams of fungal biomass 8 weeks postinoculation.
[c] Values are reported as percentage of $^{14}CO_2$ from ^{14}C recovered.
[d] Values are reported as percentage of ^{14}C incorporated into the fungal tissue.
[e] Values are reported as percentage of $^{14}CO_2$ plus percentage of ^{14}C incorporated into tissue.

sporium mineralized the most 2,4-D (10.17%). There were few other differences in the amount of 2,4-D mineralized. *O. griseum*, *P. chrysosporium*, and *H. ericae* had the most 2,4-D carbon incorporated into their tissue (1.01, 1.08, and 1.37%, respectively). *P. chrysosporium* had the highest total amount of 2,4-D degraded (11.25%). *R. vinicolor* degraded the most 2,4-D among the mycorrhizal fungi (2.14%).

When the medium was amended with 10 mM ammonium tartrate as a nitrogen source, *P. chrysosporium* mineralized the most 2,4-D (Table 1). *P. chrysosporium* and *H. ericae* were not different in the amount of 2,4-D incorporated into their biomass (Table 1). *G. crispa* had more ^{14}C incorporated into its tissue than *O. griseum*. *H. ericae* degraded significantly more 2,4-D than *O. griseum* and *G. crispa*. All of the other cultures degraded lower amounts of 2,4-D than the fungi described above.

Atrazine. When the medium contained 1 mM ammonium tartrate and 4 mM atrazine, all cultures grew, but none mineralized atrazine after 8 weeks of incubation (Table 2). There were significant amounts of carbon from atrazine incorporated into the biomass (Table 2). *H. ericae*, *Rhizopogon vulgaris* S-251, and *T. darkeri* had the highest amounts of atrazine carbon measured in the tissue. The total

TABLE 2. $^{14}CO_2$ and ^{14}C incorporation into microbial tissue during atrazine (4 mM) degradation with 1 mM added nitrogen

Species and description	Incorporation of $^{14}CO_2$ and ^{14}C			
	Wt (mg)[a,b]	CO_2 (%)[a,c]	Tissue (%)[a,d]	Total (%)[a,e]
Ericoid mycorrhizae				
H. ericae 1318	30.5*	0.04†	6.67*	6.71*
O. griseum	11.4‡,§	0.05†	2.67†,‡	2.72†,‡
Mat-forming ectomycorrhizae				
G. crispa 4936	8.6‡,§	0.04†	1.17§,‖	1.21§,‖
G. othii 6362	4.3§	0.05†	1.52‡,§,‖	1.57‡,§,‖
Ectomycorrhiza associated with decomposing wood; *R. atrogleba* 9470	2.9§	0.04†	0.65‖	0.69‖
General ectomycorrhizae				
R. vinicolor 7534	6.0‡,§	0.05†	0.77‖	0.82‖
R. vulgaris S-251	15.6†,‡,§	0.05†	4.22*,†	4.27*,†
Nonmycorrhizal fungi				
P. chrysosporium 1767	24.0*,†	0.04†	2.27†,‡,§	2.31†,‡,§
S. pacificus 9011	4.0§	0.05†	0.65‖	0.70‖
T. darkeri 8077	15.6†,‡	0.04†	3.26*,†	3.30†
Actinomycete; *S. viridosporus* T7A	8.7‡,§	0.17*	2.09†,‡,§,‖	2.26†,‡,§,‖
Control	0.0§	0.03†	0.40‖	0.43‖

[a] Values are means of 10 samples. In each column, values followed by the same symbol are not significantly different at $P \leq 0.05$ as determined by Tukey's Studentized range (HSD) test.
[b] Values are reported as milligrams of fungal biomass 8 weeks postinoculation.
[c] Values are reported as percentage of $^{14}CO_2$ from ^{14}C recovered.
[d] Values are reported as percentage of ^{14}C incorporated into the fungal tissue.
[e] Values are reported as percentage of $^{14}CO_2$ plus percentage of ^{14}C incorporated into tissue.

TABLE 3. $^{14}CO_2$ and ^{14}C incorporation into microbial tissue during atrazine (1 mM) degradation with 1 mM added nitrogen

Species and description	Incorporation of $^{14}CO_2$ and ^{14}C			
	Wt (mg)[a,b]	CO_2 (%)[a,c]	Tissue (%)[a,d]	Total (%)[a,e]
Ericoid mycorrhizae				
H. ericae 1318	23.4*	0.11*	3.61*	3.72*
O. griseum	9.0†,‡,§	0.16*	1.86†	2.02†
Mat-forming ectomycorrhizae				
G. crispa 4936	7.2‡,§	0.11*	0.54‡	0.65§
G. othii 6362	3.9§	0.14*	0.40‡	0.54§
Ectomycorrhiza associated with decomposing wood; *R. atrogleba* 9470	3.8§	0.10*	0.44‡	0.54§
General ectomycorrhiza; *R. vinicolor* 7534	9.3†,‡,§	0.13*	1.39†	1.52†,‡
Nonmycorrhizal fungi				
P. chrysosporium 1767	11.0†,‡	0.23*	0.43‡	0.66§
S. pacificus 9011	14.1†	0.33*	0.50‡	0.83‡,§
T. darkeri 8077	11.0†,‡	0.11*	0.41‡	0.52§
Actinomycete; *S. viridosporus* T7A	6.8‡,§	0.19*	0.53‡	0.72‡,§
Control	0.6§	0.09*	0.35‡	0.43§

[a] Values are means of 10 samples. In each column, values followed by the same symbol are not significantly different at $P \leq 0.05$ as determined by Tukey's Studentized range (HSD) test.
[b] Values are reported as milligrams of fungal biomass 8 weeks postinoculation.
[c] Values are reported as percentage of $^{14}CO_2$ from ^{14}C recovered.
[d] Values are reported as percentage of ^{14}C incorporated into the fungal tissue.
[e] Values are reported as percentage of $^{14}CO_2$ plus percentage of ^{14}C incorporated into tissue.

amount of atrazine degradation did not differ between *H. ericae* and *R. vulgaris* S-251 (Table 2).

When the medium was supplemented with 1 mM atrazine and had no ammonium tartrate added, all of the cultures grew, but there was no mineralization of the herbicide. *H. ericae* had the highest amount of ^{14}C measured in its tissue (1.80%), but was not significantly different from *O. griseum* (1.34%). The total amount of ^{14}C recovered followed the same pattern as the amount of ^{14}C measured in the microbial tissue. *H. ericae* and *O. griseum* had the highest total atrazine degradation levels (1.93 and 1.47%, respectively).

When the atrazine concentration remained at 1 mM and the nitrogen concentration was increased to 1 mM, none of the cultures mineralized the atrazine (Table 3). *H. ericae* had the highest amount of incorporation of radioactivity into its tissue and was significantly higher than all of the other cultures (Table 3). When looking at the total amount of atrazine degraded, *H. ericae* degraded significantly more atrazine than the other cultures and was followed by *O. griseum* and *R. vinicolor* (Table 3).

When the ammonium tartrate concentration was increased to 10 mM and the atrazine concentration remained at 1 mM, no atrazine mineralization occurred (Table 4). The ericoid mycorrhizal fungus *H. ericae* incorporated significantly higher amounts of radioactivity into its tissue than the other cultures (Table 4). *O. griseum* did not incorporate as much ^{14}C-atrazine into its tissue as *H. ericae*, but it did incorporate more ^{14}C-atrazine into its tissue than the remaining cultures.

TABLE 4. $^{14}CO_2$ and ^{14}C incorporation into microbial tissue during atrazine (1 mM) degradation with 10 mM added nitrogen

Species and description	Incorporation of $^{14}CO_2$ and ^{14}C			
	Wt (mg)[a,b]	CO_2 (%)[a,c]	Tissue (%)[a,d]	Total (%)[d,e]
Ericoid mycorrhizae				
H. ericae 1318	51.4*	0.27*	11.11*	11.38*
O. griseum	19.6†,‡	0.34*	6.04§	6.38§
Mat-forming ectomycorrhizae				
G. crispa 4936	22.7†,§	0.29*	2.32†	2.61†
G. othii 6362	12.6†,‡	0.22*	1.55†,‡	1.77†,‡
Ectomycorrhiza associated with decomposing wood; *R. atrogleba* 9470	10.3‡	0.26*	0.64‖,#,**	0.90‖,#
General ectomycorrhiza; *R. vinicolor* 7534	8.7‡	0.27*	0.32**	0.59#
Nonmycorrhizal fungi				
P. chrysosporium 1767	9.2‡	0.28*	1.02‡,‖,#	1.30‡,‖
S. pacificus 9011	12.1†,‡	0.26*	0.77‡,‖,#,**	1.03‡,‖,#
T. darkeri 8077	33.2§	0.28*	1.07‡,‖	1.35‡,‖
Actinomycete; *S. viridosporus* T7A	8.8‡	0.24*	0.46#,**	0.70#
Control	0.1‡	0.20*	0.10**	0.30#

[a] Values are means of 10 samples. In each column, values followed by the same symbol are not significantly different at $P \leq 0.05$ as determined by Tukey's Studentized range (HSD) test.
[b] Values are reported as milligrams of fungal biomass 8 weeks postinoculation.
[c] Values are reported as percentage of $^{14}CO_2$ from ^{14}C recovered.
[d] Values are reported as percentage of ^{14}C incorporated into the fungal tissue.
[e] Values are reported as percentage of $^{14}CO_2$ plus percentage of ^{14}C incorporated into tissue.

H. ericae degraded significantly more total atrazine than the other cultures (Table 4). *O. griseum* degraded less total atrazine than *H. ericae*, but these two fungi degraded more total atrazine than the remaining cultures.

DISCUSSION

The data show that both the mycorrhizal and nonmycorrhizal fungi were capable of degrading the two aromatic herbicides. In all but 2,4-D degradation by *P. chrysosporium*, degradation consisted of incorporation of herbicide carbon into tissue, not mineralization. The assay used did not allow for determination of the chemical form of the incorporated herbicides within the microbial tissues. The amount of ^{14}C recovered either as CO_2 or in the tissue varied considerably among the different fungi. The ability to degrade these aromatic herbicides appears to be dependent on the specific fungus and the herbicide. Our data indicated that *P. chrysosporium* was the best 2,4-D-degrading microorganism, with most of the substrate being mineralized instead of being incorporated into the tissue. However, *P. chrysosporium* was not able to mineralize atrazine. The ericoid mycorrhizal fungi were the best cultures at degrading atrazine. Both the nitrogen and herbicide concentration affected 2,4-D and atrazine incorporation values. As the nitrogen concentration increased, the total herbicide incorporation increased. The values for the percentage of $^{14}CO_2$ recovered, other than for 2,4-D with *P. chrysosporium*, are lower than the level of possible contamination of the ^{14}C-labelled herbicides (1 to 2%). Therefore, we cannot conclusively state that 2,4-D or atrazine was mineralized significantly by any of the microbes other than *P. chrysosporium* (Table 1). Still, the fungi tolerated the herbicides, other than 2,4-D at a 4.0 mM concentration, and incorporated significant amounts into their tissues. The fact that no mineralization of atrazine was detected agrees with the results of Kaufman and Blake (15), who reported no mineralization when using ^{14}C-ring-labelled atrazine. Mineralization was measured only when ^{14}C-chain-labelled atrazine was used (15). Wolf and Martin (27) also reported no mineralization by soil fungi when using ^{14}C-ring-labelled atrazine; however, approximately 25% of the atrazine was associated with the fungal cell material.

Our data are conservative because the substrates were labelled only on the ring structure. Degradation of the side chains of atrazine could have occurred, but would not have been detected. Our methods also only detected the amount of 2,4-D or atrazine mineralized to CO_2 and the amount incorporated into the tissue. We did not measure the amount of intermediate compounds present in the medium or the amount of those which had been exported from the cell. The degradation pathways of 2,4-D and atrazine have been investigated, and the intermediate compounds are known for some systems (5, 11, 15).

When the fungus is growing with the host plant, it has an ample supply of carbohydrates provided by the host plant for growth. It is known that extracellular enzymatic activity of the fungus dramatically increases when the fungus is growing with the host plant versus growing in pure culture. If the herbicide is applied to an established mycorrhizal fungal culture growing with the host plant, there may be an increased degradation rate due to an increase of biomass and enzymatic activity. Future studies are needed to determine how the degradation rate is affected when the mycorrhizal fungi are in the symbiotic relationship with the host plant. The environmental factors which affect the degradation of these herbicides by the mycorrhizal fungi also need to be investigated. These fungi may play an important ecological role in the degradation and/or transport of aromatic compounds in the soil.

ACKNOWLEDGMENTS

We thank Karen Crossley, Jane Smith, and Kermit Cromack, Jr., for technical help.

This project was supported in part by grant DE-FG07-86ER13586 from the U.S. Department of Energy to D.L.C. and by the Idaho Agricultural Experiment Station.

REFERENCES

1. **Aust, S. D.** 1990. Degradation of environmental pollutants by *Phanerochaete chrysosporium*. Microb. Ecol. **20**:197–209.
2. **Bae, K.-S., and L. L. Barton.** 1989. Alkaline phosphatase and other hydrolyases produced by *Cenococcum graniforme*, an ectomycorrhizal fungus. Appl. Environ. Microbiol. **55**:2511–2516.
3. **Behki, R. M., and S. U. Khan.** 1986. Degradation of atrazine by *Pseudomonas*: N-dealkylation and dehalogenation of atrazine and its metabolites. J. Agric. Food Chem. **34**:746–749.
4. **Beste, C. E. (ed.).** 1983. Herbicide handbook of the Weed Science Society of America, 5th ed., p. 515. Weed Science Society of America, Champaign, Ill.
5. **Chaudhry, G. R., and S. Chapalamadugu.** 1991. Biodegradation of halogenated organic compounds. Microbiol. Rev. **55**:59–79.
6. **Cheo, P. C.** 1982. Effects of tannic acid on rhizomorph production by *Armillaria mellea*. Phytopathology **72**:676–679.
7. **Donnelly, P. K.** 1991. Ph.D. thesis. University of Idaho, Moscow.
8. **Entry, J. A., P. K. Donnelly, and K. Cromack, Jr.** 1992. The influence of carbon nutrition on *Armillaria ostoyae* growth and phenolic degradation. Eur. J. For. Pathol. **22**:149–156.
9. **Entry, J. A., N. M. Stark, and H. Loewenstein.** 1987. Timber harvesting: effects on degradation of cellulose and lignin. For. Ecol. Manage. **22**:79–88.
10. **Giardina, M. C., M. T. Giardi, and G. Filacchioni.** 1980. 4-Amino-2-chloro-1,3,5-triazine: a new metabolite of atrazine by a soil bacterium. Agric. Biol. Chem. **44**:2067–2072.
11. **Giardina, M. C., M. T. Giardi, and G. Filacchioni.** 1982. Atrazine metabolism by *Nocardia*: elucidation of initial pathway and synthesis of potential metabolites. Agric. Biol. Chem. **46**:1439–1445.
12. **Harborne, J. B.** 1988. Introduction to ecological biochemistry, 3rd ed., p. 356. Academic Press, Inc., San Diego, Calif.
13. **Harley, J. L.** 1989. The significance of mycorrhiza. Mycol. Res. **92**:129–139.
14. **Haselwandter, K., O. Bobleter, and D. J. Read.** 1990. Degradation of ^{14}C-labelled lignin and dehydropolymer of coniferyl alcohol by ericoid and ectomycorrhizal fungi. Arch. Microbiol. **153**:352–354.
15. **Kaufman, D. D., and J. Blake.** 1970. Degradation of atrazine by soil fungi. Soil Biol. Biochem. **2**:73–80.
16. **Kelly, M. P., K. B. Hallberg, and O. H. Tuovinen.** 1989. Biological degradation of 2,4-dichlorophenoxyacetic acid: chloride mass balance in stirred tank reactors. Appl. Environ. Microbiol. **55**:2717–2719.
17. **Leake, J. R.** 1987. Metabolism of phyto- and fungitoxic phenolic acids by the ericoid mycorrhizal fungus, p. 332. *In* D. M. Sylvia, L. L. Hung, and J. H. Graham (ed.), Mycorrhizae in the next decade: practical applications and research priorities.
18. **Martin, J., and C. R. Worthing (ed.).** 1977. Pesticide manual, 5th ed., p. 593. British Crop Protection Council, Thornton Heath, England.
19. **Molina, R., and J. G. Palmer.** 1982. Isolation, maintenance, and pure culture manipulation of ectomycorrhizal fungi, p. 115–129. *In* N. C. Schenck (ed.), Methods and principles of mycorrhizal research. The American Phytopathological Society, St. Paul, Minn.
20. **Reaves, J. L., C. G. Shaw III, R. E. Martin, and J. E. Mayfield.** 1984. Effects of ash leachates on growth and development of *Armillaria mellea* in culture. USDA Forestry Service Note

PNW-418. U.S. Department of Agriculture, Washington, D.C.
21. **Sahasrabudhe, S. R., and V. V. Modi.** 1987. Microbial degradation of chlorinated aromatic compounds. Microbiol. Sci. **4**:300–303.
22. **SAS Institute, Inc.** 1982. SAS user's guide to statistics. SAS Institute, Inc., Cary, N.C.
23. **Shirling, E. B., and D. Gottlieb.** 1972. Cooperative description of type strains of *Streptomyces*. V. Additional descriptions. Int. J. Syst. Bacteriol. **22**:265–394.
24. **Sinden, D. L.** 1979. M.S. thesis. University of Idaho, Moscow.
25. **Sinton, G. L., L. T. Fan, L. E. Erickson, and S. M. Lee.** 1986. Biodegradation of 2,4-D and related xenobiotic compounds. Enzyme Microb. Technol. **8**:395–403.
26. **Trojanowski, J., K. Haider, and A. Huttermann.** 1984. Decomposition of ^{14}C-labelled lignin, holocellulose and lignocellulose by mycorrhizal fungi. Arch. Microbiol. **139**:202–206.
27. **Wolf, D. C., and J. P. Martin.** 1975. Microbial decomposition of ring-^{14}C atrazine, cyanuric acid, and 2-chloro-4,6-diamino-*s*-triazine. J. Environ. Qual. **4**:134–139.
28. **Zhu, H., D.-C. Guo, and B. P. Dancik.** 1990. Purification and characterization of an extracellular acid proteinase from the ectomycorrhizal fungus *Hebeloma crustuliniforme*. Appl. Environ. Microbiol. **56**:837–843.