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Physiological Aspects of Atrazine Degradation by Higher Marine Fungi

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Abstract. The influence of glucose and NH_4NO_3 on the degradation of the herbicide atrazine was studied with the marine fungus *Periconia prolifica* Anastasiou. The bioaccumulation of ^{14}C-atrazine by fungal cultures was substantially increased at increased concentrations of glucose. Overall, 34.1% of the initial atrazine concentration was removed from the culture filtrate of the cultures grown in 0.5% (w/v) glucose and 0.007% (w/v) NH_4NO_3, and 40.4% of the initial atrazine concentration was removed when the same media contained 0.08% (w/v) NH_4NO_3. The majority of internalized radioactivity from both sets of cultures could be extracted from the mycelia as undegraded atrazine. However, examination of both the culture filtrates and mycelia of cultures grown under 0.5% (w/v) glucose and 0.08% (w/v) NH_4NO_3 revealed the presence of both dealkylated and dechlorinated hydrolysis products of atrazine. The fungal cultures, compared with uninoculated controls, showed a 5-fold increase in 2-chloro-4-ethylamino-6-amino-*s*-triazine (deisopropylatrazine), a 1.9-fold increase in 2-hydroxy - 4 - ethylamino - 6 - isopropylamino - *s* - triazine (hydroxyatrazine), and a 1.5-fold increase in other metabolites not extracted into ethyl acetate, suggesting two separate degradation pathways caused by a combination of metabolic and physicochemical interactions. Although mineralization of [ring-^{14}C] atrazine did not occur under the conditions employed, considerable radioactivity was found in an unextractable form associated with cell fragments of *Periconia* cultures indicating further metabolism of the initial degradation products.

The higher marine fungi inhabit such lignocellulosic substrates as wood, stems and leaves (Hughes 1975) and may aid in the biological transformation of plant material into detritus (Kohlmeyer and Kohlmeyer 1979; Gessner 1980). The demonstration of cellulolytic and lignolytic activities in these fungi provides additional evidence for their role in the estuarine food web (Meyers and Reynolds 1959; MacDonald and Speedie 1982; Sutherland *et al.* 1982). The marine fungi are well-positioned to interact with pollutants, including herbicides, as they enter estuaries from bordering agricultural lands. The interaction may involve adsorption, uptake or degradation, all of which would modify the fate of pollutants.

Although the herbicide atrazine, 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine, has been extensively studied in terrestrial environments, little is known concerning its fate in estuaries. Previous studies in this laboratory have indicated that selected marine fungi can bioaccumulate atrazine through the processes of adsorption and uptake (Schocken and Speedie 1982a, 1982b). Our studies with eight species of higher marine fungi have additionally indicated that two species can utilize atrazine as a source of nitrogen (*Periconia prolifica* Anastasiou) or as a source of carbon and nitrogen (*Leptosphaeria oraemaris* Linder) (Schocken *et al.* 1982). While the utilization of atrazine as a carbon source could be achieved by dealkylation, utilization as a nitrogen source requires more extensive

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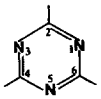
Table 1. *s*-Triazine nomenclature and chemical structure



Chemical name	Trivial name
2-Chloro-4-ethylamino-6-isopropylamino-*s*-triazine	Atrazine
2-Hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine	Hydroxyatrazine
2-Chloro-4-amino-6-isopropylamino-*s*-triazine	Deethylatrazine
2-Chloro-4-ethylamino-6-amino-*s*-triazine	Deisopropylatrazine
2-Chloro-4,6-diamino-*s*-triazine	—
2,4-Diamino-6-hydroxy-*s*-triazine	Ammeline
2,4-Dihydroxy-6-amino-*s*-triazine	Ammelide
2,4,6-Trihydroxy-*s*-triazine	Cyanuric Acid

degradation, at least to the stage of deamination and possibly involving ring cleavage.

In this investigation, attention was focused on the influence of differing concentrations of glucose and NH_4NO_3 on the uptake and/or degradation of atrazine. *P. prolifica* was selected because it utilized atrazine as a sole nitrogen source and mediated substantial atrazine loss under cometabolic conditions (Schocken *et al.* 1982). Also, *P. prolifica* is among the most abundant marine fungi in both the upper (Shearer 1972) and lower (Kirk and Brandt 1980) Chesapeake Bay.

Methods

Maintenance and Growth of P. prolifica

The marine fungus was maintained on slants of a complex medium and grown from filtered spore/mycelia suspensions in an artificial seawater medium (ASWM) supplemented with glucose and NH_4NO_3. Both media, as well as preparation of inocula, have been previously described (Schocken *et al.* 1982).

Chemicals

Analytical standards of atrazine (99.24%) were provided by the United States Environmental Protection Agency, Beltsville, MD [Ring-^{14}C]atrazine (40.0 $\mu Ci \cdot mg^{-1}$), [ethyl-^{14}C]atrazine (7.3 $\mu Ci \cdot mg^{-1}$) and the unlabeled dealkylated and dechlorinated *s*-triazine derivatives (Table 1) were gifts of the Ciba-Geigy Corporation (Greensboro, NC). Radiochemical purity of [ring-^{14}C]atrazine (96.7%) and [ethyl-^{14}C]atrazine (96.5%) were determined with a TLC linear analyzer (Berthold). Solvents used for extraction and thin-layer chromatography (TLC) were reagent grade or better. All other chemicals used were of the highest purity available from the suppliers.

Analytical Methods

TLC was performed on silica gel precoated aluminum-backed strips with fluorescent indicator (EM Laboratories, Elmsford, NY). The solvent systems used to separate atrazine and its metabolites are given in Table 2.

High-pressure liquid chromatography was performed with a component system consisting of a Waters Associates Model 6000A solvent delivery system, a Model U-6K septumless injector, and a Varian Techtron Model 635 UV-visible spectrophotometer operated at 234 nm. Chromatographic conditions employed to separate and quantify atrazine from its potential ethyl acetate-soluble derivatives have been previously described (Schocken *et al.* 1982).

^{14}C-Radioactivity was determined with a Mark III Liquid Scintillation Counter (Searle Analytic, Inc., Des Plaines, IL). Samples were counted to a 2% statistical error; counts were corrected for background and quench by the channels-ratio method.

Growth, Sampling, and Harvesting of Cultures

The fungus was inoculated (in triplicate) into ASWM (2.7% salinity) containing various concentrations of glucose [0.1% (w/v) or 0.5% (w/v)] and NH_4NO_3 [0.007% (w/v) or 0.08% (w/v)]. All media additionally contained 0.31 μCi [ring-^{14}C]atrazine and 30 mg/L unlabeled atrazine. Uninoculated media served as controls. Media were sterilized by filtration and atrazine was sterilized by dissolution in anhydrous ethyl ether. Cultures were grown under conditions previously described (Schocken *et al.* 1982). Every 2 to 3 days, 1.0 ml aliquots were removed from the cultures and control flasks, and radioactivity in 0.1 ml determined by liquid scintillation counting (LSC). The remainder of each sample was frozen at −20°C.

Cultures and control flasks were harvested after a 23-day incubation period and examined microscopically for the presence of contamination (none was detected). Mycelia were collected by suction filtration over pre-weighed Whatman no. 4 filter paper, washed 3 times with 5.0 ml ASWM (to remove atrazine loosely adsorbed to the cell surface), dried at 40°C for 48 hr, cooled in a desiccator for 30 min and weighed. Mycelial replicates were pooled, sonified in methanol, and solubilized as previously described (Schocken and Speedie 1982a). Radioactivity removed by cell washings and released upon sonification and solubilization of mycelia was determined by LSC.

Detection and Quantitation of Atrazine Metabolites

The ethyl acetate extracts of pooled culture filtrates of two of the experimental media described above [i.e., (1) ASWM with 0.5% (w/v) glucose and 0.08% (w/v) NH_4NO_3, designated high glucose-high NH_4NO_3, and (2) ASWM with 0.5% (w/v) glucose and 0.007% (w/v) NH_4NO_3, designated high glucose-low NH_4NO_3 as well as the ethyl acetate extract of the uninoculated control media, were evaporated under reduced pressure and redissolved in several ml of methanol. One drop of 1N HCl was added to insure that atrazine, N-dealkylated and dechlorinated metabolites were solubilized. The concentrated extracts of the cultures and controls were stored at −20°C.

The extracted aqueous phases were applied to an XAD-2 resin column according to the method of Ramsteiner and Horman

Table 2. TLC separation of potential atrazine metabolites[a]

	R_f values					
	A	B	C	D	E	F
Atrazine	0.93	0.80	0.77	0.86	1.00	1.00
2-Chloro-4-amino-6-isopropylamino-*s*-triazine	0.87	0.73		0.68	0.95	0.42
2-Chloro-4-ethylamino-6-amino-*s*-triazine	0.84	0.62		0.55	0.94	0.23
2-Chloro-4,6-diamino-*s*-triazine	0.64	0.43		0.26	0.89	0.05
2-Hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine	0.15	0.04	0.21	0.04	0.66	0.00
2,4-Dihydroxy-6-amino-*s*-triazine	0.00		0.21			
2,4-Diamino-6-hydroxy-*s*-triazine	0.00		0.07			
2,4,6-Trihydroxy-*s*-triazine	0.04	0.24	0.24			

[a] TLC solvent system:—A: chloroform:ethanol:acetic acid (90:5:5). B: hexane:*n*-butanol:acetic acid (40:50:1). C: benzene:acetic acid:water (50:50:3). D: hexane:*n*-butanol:acetic acid (60:40:1). E: chloroform:methanol:acetic acid (15:4:1). F: ethyl acetate:hexane (3:7); multiple development (R_f values relative to atrazine)

(1979). Silanized glass wool plugs were used to prevent adsorption of the *s*-triazines to the plugs. Bound hydroxyatrazine and some polar metabolites were eluted with methanol and concentrated under reduced pressure and were subsequently separated by TLC. Other polar metabolites remained bound to the column and were calculated by difference between applied and eluted radioactivity. Aliquots of the concentrated ethyl acetate extracts, the methanol extract of the mycelia, and the eluate from the XAD-2 column were subsequently spotted along with unlabeled reference standards on silica gel TLC plates. The plates were developed in various solvent systems (Table 2) to separate atrazine from its dealkylated and dechlorinated hydrolysis products. Spots corresponding to the unlabeled standards were located by the quenching of the fluorescent indicator. The location of radioactivity on the developed plates was determined by scraping 1 cm sections of the developed chromatograms into counting vials, adding scintillation fluid and determining radioactivity by LSC. The relative amounts of ^{14}C-atrazine and metabolites were determined and expressed as a percentage of the total ^{14}C-activity on the chromatogram. The overall amounts of ^{14}C-atrazine and metabolites were calculated from these data and the total radioactivity contained in each fraction. (Analysis of the ^{14}C-atrazine at day 0 indicated that small amounts of impurities were present whose radioactivity corresponded to authentic standards of *s*-triazine derivatives (Table 5). Moreover, after 23 days of incubation, the uninoculated controls broke down even further. Therefore, for accurate quantification, the radioactive spots from the fungal cultures had to be directly compared to the radioactivity corresponding to the authentic standards in the 23-day uninoculated controls.

Time Course of Atrazine Loss in High Glucose-High NH_4NO_3 Cultures

Loss of atrazine from cultures grown in the high glucose-high NH_4NO_3 medium was determined by HPLC throughout the 23-day incubation period. To avoid confounding due to abiotic-induced changes in atrazine concentration, atrazine loss mediated by the fungi was expressed in the following manner:

$$\text{loss } (\%) = 100\,[1 - A_f/A_u)]$$

where A_f = atrazine in the fungal culture, and A_u = atrazine in an uninoculated control culture.

$^{14}CO_2$ Evolution from Ring ^{14}C and Ethyl ^{14}C Atrazine in High Glucose-High NH_4NO_3 Cultures

An apparatus similar to the biometer flask described by Pramer and Bartha (1965) was used to assess the ability of *P. prolifica* to evolve $^{14}CO_2$ from [ring-^{14}C] and [ethyl-^{14}C]atrazine. Fifty ml of ASWM containing glucose, 0.5% (w/v); NH_4NO_3, 0.08% (w/v); unlabeled atrazine 10mg/L; and either [ring-^{14}C]atrazine, 0.40 μCi, or [ethyl-^{14}C]atrazine, 0.97 μCi, were placed in the 250-ml compartment and 10.0 ml of freshly prepared 2N NaOH were added to the side-arm compartment. Cultures were grown at 27 ± 1°C on a rotary shaker set at 100 rpm. The CO_2-trapping solution was regularly sampled, neutralized with 2N HCl, and the absorbed radioactivity determined by LSC.

Results

The study was designed to examine the effects of varying concentrations of glucose and nitrogen on the overall disposition and compartmentalization of radioactivity from ^{14}C-atrazine following 23 days of incubation. Subsequently, the two culture conditions showing the greatest loss of atrazine from the medium were further examined to identify atrazine degradation products.

The results of the study determining the effects of glucose and NH_4NO_3 on atrazine disposition are given in Table 3. Radioactivity (^{14}C-atrazine and/or metabolites) loosely associated with fungal cell surfaces was fairly uniform, ranging from 1.3% to 1.7%. Larger amounts of radioactivity were removed from the medium and internalized within mycelia as either atrazine or methanol-extractable metabolites (ranging from 1.8% to 16.4%). Internalization of ^{14}C-atrazine and/or metabolites may have been underestimated due to lysis of fungal cells, since the cultures had reached a stationary growth phase.

The bioaccumulation of radioactivity by cultures of *P. prolifica* was substantially increased when glucose was supplied to the medium (Table 3). When

Table 3. Distribution of radioactivity in *P. prolifica* cultures after 23 days of growth under various physiological conditions

Conditions[a]						
Glucose (%, w/v)	NH_4NO_3 (%, w/v)	Desorbable by ASWM washings (%)	Internalized within mycelium[b] (%)	Non-extractable[c] (%)	Culture filtrate (%)	Recovery of radioactivity[d] (%)
0.1	0.007	1.3 ± 0.0	1.8[e]	ND[f]	96.9 ± 1.3	100
0.1	0.08	1.3 ± 0.2	4.3	ND	92.7 ± 1.1	98.3
0.5	0.007	1.7 ± 0.1	16.4	2.5	76.0 ± 2.5	97.5
0.5	0.08	1.5 ± 0.2	11.3	5.2	76.4 ± 5.2	94.4

[a] All cultures contained [ring-^{14}C] atrazine plus 30 mg/liter unlabeled atrazine
[b] Radioactivity extracted into methanol after sonification of mycelia
[c] Determined by solubilization of mycelia
[d] Relative to controls
[e] Data not given as mean ± SEM represent determinations based on pooled replicate cultures (see **Methods** section)
[f] Not determined

removal of radioactivity from the medium was standardized for biomass (Table 4), the loss per mg mycelium in the high glucose-low nitrogen cultures was almost twice that observed in cultures grown under any other condition. The addition of 0.08% (w/v) nitrogen to the high glucose condition yielded an increased biomass but decreased bioaccumulation per unit of biomass. The pH of the high glucose-high nitrogen cultures dropped considerably, indicating that buffer capacity had been exceeded.

Experiments were also conducted at a lower salinity (0.8%). Distribution of radioactivity (data not given), however, was nearly identical to results observed at the higher salinity. Therefore, salinity apparently has no effect on atrazine disposition.

These results led us to further identify the radioactive compounds in the various compartments of the high glucose-low nitrogen cultures and of the high glucose-high nitrogen cultures to determine if atrazine degradation had occurred. As in Table 5, in both cases the majority of the radioactivity extracted from the mycelium into methanol was identified as undegraded atrazine. Undegraded atrazine in the mycelia accounted for 14.7% and 9.2% of initial atrazine in the low nitrogen cultures and in the high nitrogen cultures, respectively (Table 5).

Examination by TLC of the culture filtrates and mycelia of cultures grown under high nitrogen conditions revealed the presence of atrazine degradation products. Cultures grown under low nitrogen conditions had no detectable degradation products. Specifically, the methanol extract of the mycelium of the high glucose-high nitrogen culture contained hydroxyatrazine (0.6% of initial radioactivity) and other polar metabolites (0.7%) which arise from further metabolism of dealkylated, dechlorinated atrazine. Examination by TLC of the ethyl acetate extract and the extracted aqueous phase of these cultures also revealed the presence of atrazine metabolites. Overall, the fungal cultures grown under high glucose-high nitrogen conditions compared with uninoculated control flasks, showed a 5-fold increase in radioactivity corresponding to 2-chloro-4-ethylamino-6-amino-*s*-triazine (deisopropylatrazine), a 1.9-fold increase in radioactivity corresponding to 2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine (hydroxyatrazine) and a 1.5-fold increase in other metabolites not extracted into ethyl acetate.

Furthermore, 5.2% of initial radioactivity remained associated with the mycelium even after exhaustive extraction with methanol. This amount, which represents 32% of the internalized radioactivity, is indicative of atrazine degradation, since it was shown that free atrazine is fully extractable. On the other hand, metabolites such as ammeline (2,4-diamino-6-hydroxy-*s*-triazine) and ammelide (2,4-dihydroxy-6-amino-*s*-triazine) remain tightly bound to many polymeric materials (e.g. XAD-2 resin) and presumably, could also bind tightly to cellular constituents (Speedie and Schocken, unpublished results). Conceivably, the unextractable radioactivity could also represent covalently bound or polymerized atrazine or atrazine metabolites, or incorporation of carbon from fully-degraded atrazine into cellular constituents. These possibilities will be assessed more fully later in this paper.

In contrast, for cultures grown under the high glucose-low nitrogen conditions the presence of breakdown products in the mycelial extract and in the medium was not significantly different from the controls. The only evidence of degradation under these conditions was the 2.5% of initial radioactivity not extractable from the sonified mycelium.

Table 4. Determination of biomass, pH and radioactivity bioaccumulated in *Periconia prolifica* cultures after 23 days of growth

Conditions[a]				
Glucose (%, w/v)	NH_4NO_3 (%, w/v)	Mycelial dry weight (mg)	Final pH	Radioactivity bioaccumulated[b] (dpm/mg of mycelium)
0.1	0.007	21.8 ± 1.0	6.7 ± 0.0	715
0.1	0.08	25.6 ± 1.4	6.2 ± 0.0	1625
0.5	0.007	54.1 ± 0.0	6.5 ± 0.0	2670
0.5	0.08	92.3 ± 12.4	3.9 ± 0.1	1535
0.1	0.007	Controls	6.6 ± 0.0	—

[a] All cultures and controls contained $6.8 \cdot 10^5$ dpm [ring-^{14}C] atrazine + 30 mg/L unlabeled atrazine
[b] Radioactivity bioaccumulated (dpm) = $6.8 \cdot 10^5$ dpm [1 − (% remaining in the culture filtrate 10^{-2})]/mg mycelium

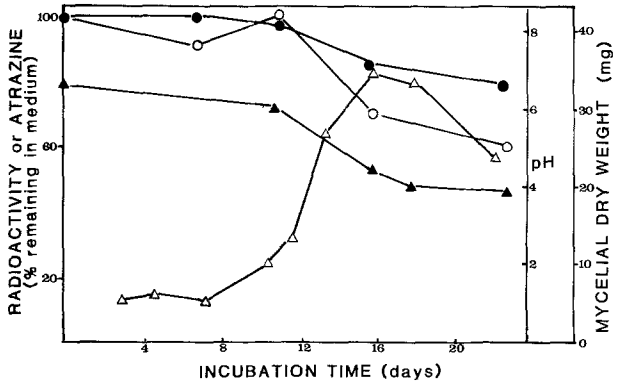


Fig. 1. Time course of loss of radioactivity (—●—) and atrazine (—○—) from the medium of cultures of *Periconia prolifica* grown in ASWM containing 0.5% (w/v) glucose and 0.08% (w/v) NH_4NO_3. Corresponding changes in pH (—▲—) and mycelial dry weight (—△—) are shown. Data points represent means of triplicate cultures. The growth curve was taken from a previous experiment (unpublished data) in which identical cultures were harvested after the various lengths of incubation time, in order to determine the effects of incubation conditions on the growth and sporulation pattern

The loss of atrazine from the culture filtrate over the time course of growth was determined for the high glucose-high nitrogen cultures by HPLC analysis of the aliquots withdrawn during the incubation period. The results (Figure 1) supported our previous finding that 40% of the initial atrazine had been removed from the culture filtrate through uptake and/or degradation by the end of the 23-day growth period. Atrazine loss commenced after 11 days of growth, concurrent with the fall in pH and the middle of the exponential growth phase. Loss continued through the stationary growth phase.

The ability of *P. prolifica* to mineralize ^{14}C-labeled portions of the atrazine molecule when grown in high glucose-high NH_4NO_3 conditions was determined. After 33 days of incubation in the [ring-^{14}C]atrazine-containing cultures, 0.7% of the initial radioactivity was evolved as $^{14}CO_2$ (compared to 0.5% evolved in uninoculated controls; not statistically significant). After 27 days of incubation in the [ethyl-^{14}C]atrazine-containing cultures, 1.4% of the initial radioactivity was evolved as $^{14}CO_2$ (compared to 1.0% evolved in the uninoculated controls; significant at $p < 0.05$ level).

Discussion

The fate of the herbicide atrazine in *P. prolifica* cultures is controlled, in part, by nutritional conditions. The concentrations of both NH_4NO_3 and glucose in the medium affect the rate and nature of atrazine degradation (Tables 3 and 5) whereas increased glucose alone substantially increased atrazine bioaccumulation (Table 4). The decreased bioaccumulation per unit of biomass observed in the high glucose-high nitrogen culture may not be a regulatory effect of nitrogen but rather a result of the growth pattern of *Periconia* (pellets) which results in a less than proportional increase in surface area for increased biomass. This is supported by the fact that a similar effect was not observed when nitrogen was increased in the glucose-limited cultures.

Based upon the presence of both hydroxyatrazine and deisopropylatrazine in extracts of the broth and mycelium, we hypothesize that two degradation pathways exist in this fungus. First, atrazine is hydrolyzed to hydroxyatrazine by a combination of biological and physicochemical processes. A second pathway, operating simultaneously, involves N-dealkylation without prior hydrolysis. The relative importance of these pathways are apparently affected by nutritional conditions.

The results indicate that both pathways are operating in the high glucose-high NH_4NO_3 medium. The greater amounts of hydroxyatrazine and other non-ethyl acetate-extractable metabolites in the culture filtrate concomitant with the lowered pH of the medium (Figure 1) suggests a biologically facilitated chemical reaction resulting in the hydrolysis of atra-

Table 5. Distribution of metabolites from [ring-^{14}C]atrazine formed in cultures of *P. prolifica*[a]

Condition	Compartment	% of initial radioactivity						
		ATR[b]	DEA	DIPA	DAA	HA[c]	Polar metabolites[d]	Total
[^{14}C]-ATR purity at Day 0		96.7	1.3	0.7	0.4	0.3	0.1	99.5
Uninoculated controls at Day 23	Ethyl acetate extract of medium	88.4	2.0	1.1	1.6	2.2	0.3	95.6
	Aqueous phase of extracted medium					0.8	2.0	2.8
P. prolifica cultures grown in high glucose-high nitrogen	Methanol extract of mycelium	9.2	1.0	0.2	0.2	0.6	0.7	11.0
	Ethyl acetate extract of culture filtrate	59.6	2.3	5.4	1.3	0.6	0.2	69.4
	Adsorbed and desorbed from mycelium							1.5
	Non-extractable radioactivity							5.2
	Aqueous phase of extracted culture filtrate					4.5	2.5	7.0
P. prolifica cultures grown in high glucose-low nitrogen	Methanol extract of mycelium	14.7	0.2	0.1	0.3	0.7	0.2	16.2
	Ethyl acetate extract of culture filtrate	65.9	1.4	1.0	1.0	0.8	0.1	70.2
	Adsorbed and desorbed from mycelium							1.7
	Non-extractable radioactivity							2.5
	Aqueous phase of extracted culture filtrate					1.6	0.8	2.4

[a] Distribution of metabolites and atrazine breakdown products were calculated from pooled replicate cultures and controls
[b] Abbreviations: ATR, atrazine; DEA, 2-chloro-4-amino-6-isopropylamino-*s*-triazine; DIPA, 2-chloro-4-ethylamino-6-amino-*s*-triazine; DAA, 2-chloro-4,6-diamino-*s*-triazine; HA, 2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine
[c] HA in aqueous phase of extracted culture filtrates determined by XAD-2 procedure followed by TLC
[d] Polar metabolites refers to radioactivity remaining near the origin of the TLC plate in solvent system A plus radioactivity remaining irreversibly bound to the XAD-2 column

zine to its 2-hydroxy analog. Such an interaction is supported by the work of Armstrong and Chesters (1968) and of Russell *et al.* (1968), which demonstrated that adsorption to and protonation by clay or carboxyl resin facilitated hydrolysis of atrazine. Very possibly, the fungal surface and excreted organic acids are acting in an analogous manner. In a recent review, Bollag (1982) addressed the importance of microbial participation in non-enzymatic transformation reactions.

The presence of deisopropylatrazine in the high glucose-high nitrogen culture filtrates indicates that an alternative pathway, involving N-dealkylation as the first step, is also occurring. This pathway is most likely enzymatically mediated. N-dealkylation reactions are known to occur in biological systems through monooxygenase enzymes (Rosazza and Smith 1979). The predominance of 2-chloro-4-ethylamino-6-amino-*s*-triazine (instead of 2-chloro-4-amino-6-isopropylamino-*s*-triazine) in the extracts, as well as the small amount of $^{14}CO_2$ evolved from ^{14}C-ethyl atrazine, suggest preferential cleavage of the isopropyl moiety of atrazine. This is in contrast to the findings of Kaufman and Blake (1970), who found that soil fungi preferentially removed the ethyl moiety. Although N-dealkylation can also occur nonbiologically through free-radical mechanisms (Plimmer *et al.* 1971), it would be unlikely that such dealkylation would involve the isopropyl moiety predominantly over the ethyl.

The fact that small amounts of dealkylated and dechlorinated hydrolysis products were generated nonbiologically in the uninoculated controls confirmed the report by Geller (1980) and necessitated the quantitative comparisons of metabolites produced from *Periconia* cultures to those formed in the uninoculated controls.

Since the products of both postulated initial steps

are extractable from mycelium, the substantial non-extractable radioactivity (5.2%) associated with the sonified and extracted mycelia suggests that, regardless of the mechanism of the initial steps, further metabolism is occurring. While the unextractable radioactivity may represent irreversible binding of further degradation products, it could also represent covalent coupling of atrazine metabolites to cellular constituents, and/or triazine polymers formed as a result of oxidative coupling reactions. The ability of atrazine or, more likely, its hydrolysis products, to undergo oxidative coupling will be the subject of future studies. In any case, the data suggest that a substantial portion of the metabolites created in the initial steps are further metabolized, though not to the extent of ring cleavage. It is unlikely that the unextractable radioactivity results from incorporation of carbon atoms from fully degraded atrazine into cellular constituents since significant amounts of $^{14}CO_2$ did not evolve from the cultures, as would be expected if the ring were cleaved to intermediates useful for the biosynthesis of cellular components.

The extracted mycelia from the high glucose-low NH_4NO_3 cultures also contained 2.5% of the initial radioactivity, again suggesting that some degradation of atrazine to metabolites considerably more polar than hydroxyatrazine had occurred. However, levels of hydroxyatrazine and other non-ethylacetate-extractable metabolites were not elevated over control levels. These results can be explained if the initial metabolites are formed at a rate less than subsequent metabolism resulting in a low steady-state level of the intermediates. Such an explanation is consistent with the observation that the pH remained above 6.0 in all high glucose-low NH_4NO_3 cultures, thus reducing the rate of a cell-surface mediated hydrolysis, and also with reports demonstrating that hydroxyatrazine is a better substrate for further metabolism than atrazine (Goswami and Green 1971; Wolf and Martin 1975). There is no evidence that deisopropylatrazine is formed under these nutritional conditions.

Recently, Jones *et al.* (1982) studied two estuarine water/sediment microcosm systems for atrazine degradation and found a rapid conversion of atrazine to hydroxyatrazine. Although they interpreted their data as indicating a minor role for microbes, our data indicate that the estuarine fungi may be playing a role both in generating hydroxyatrazine and in its further degradation once it is formed.

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