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Influence of Microbial Inoculation (*Pseudomonas* Sp. Strain ADP), the Enzyme Atrazine Chlorohydrolase, and Vegetation on the Degradation of Atrazine and Metolachlor in Soil

SHAOHAN ZHAO,† ELLEN L. ARTHUR,‡ AND JOEL R. COATS*,§

ABC Laboratories, 7200 East ABC Lane, Columbia, Missouri, Bayer Corporation, 17745 South Metcalf, ACR II, Stilwell, Kansas 66085, and Department of Entomology, Iowa State University, Ames, Iowa 50011

The concentrations of atrazine in the freshly added soils and the soils that had been incubated for 50 days significantly decreased 1 day after the addition of the enzyme atrazine chlorohydrolase or the soil bacterium *Pseudomonas* sp. strain ADP as compared with those in the uninoculated soils. Atrazine chlorohydrolase or ADP had no effect on the degradation of metolachlor. The half-lives of atrazine in the freshly added soils and in the aged soils after the treatment with atrazine chlorohydrolase or ADP markedly decreased as compared with those in the uninoculated soils. The half-lives of metolachlor in the aged soils were much longer than those of freshly added metolachlor. The percentage atrazine degraded in the freshly treated soils was much higher than that in the aged soils. This indicates that aging significantly decreased the bioavailability of atrazine. Vegetation significantly decreased the concentration of metolachlor. However, vegetation showed no effect on the degradation of atrazine.

KEYWORDS: Atrazine; metolachlor; atrazine chlorohydrolase; *Pseudomonas* sp. strain ADP; vegetation; bioavailability

INTRODUCTION

The frequent detection of atrazine (ATR, 2-chloro-4-(ethylamino)-6-isopropylamino-*s*-triazine), its metabolites, deethylatrazine and deisopropylatrazine, and metolachlor (MET, 2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)-acetamide) in surface and groundwater and high contamination levels of ATR and MET at agrochemical dealership sites have prompted extensive research on the remediation of these compounds. Several bacteria, *Pseudomonas* sp. strain ADP, M91-3, *Agrobacterium radiobacter* strain J14a, and *Pseudomonas* strain YAYA6, which can mineralize ATR completely, have been isolated from ATR-contaminated soils and have been investigated to remediate ATR-contaminated soils in laboratory studies (*1*−*4*). The success of bioaugmentation with the bacteria in soils is affected by several factors. The effectiveness of J14a on the mineralization of ATR was negatively affected by the presence of the indigenous ATR degraders in soils (*4*). An additional carbon source was needed for ADP to mineralize high concentrations of ATR (*2*). Mineralization of ATR by M91-3 was inhibited by exogenous nitrogen (*5*). A successful field scale remediation of ATR-contaminated soil by using ATR chlorohydrolase expressed by recombinant *Escherichia coli* was reported (*6*).

Strain ADP uses ATR as the sole source of nitrogen for growth and metabolizes ATR to CO_2 and ammonia (*2*). The ATR degradation pathway is initiated by three enzymatic steps, which are encoded by three genes. The first gene, *atz*A, encodes ATR chlorohydrolase, which hydrolyzes ATR to hydroxyatrazine (HYA), the first metabolite in the pathway (*7*). HYA is deaminated by HYA ethylaminohydrolase encoded by the second gene, *atz*B, resulting in the formation of *N*-isopropylammelide (*8*). The third gene, *atz*C, encodes a hydrolytic deamination reaction, which results in the formation of cyanuric acid (*9*), a nitrogen source for many bacteria. The *atz*ABC genes are localized on a self-transmissible plasmid (*10*). The sequence identities of the *atz* genes from different ATR-degrading bacteria showed that each *atz* gene in the different genera was derived from a common ancestor (*11*).

Plants have also been used to remediate the soils and water contaminated with organic compounds. Plants may act directly on organic compounds via uptake of organics and transformation of the organics to less toxic metabolites and/or indirectly degrade them via the rhizosphere effect (*12*, *13*). The uptake is influenced by physicochemical properties of the compounds, plant species characteristics, and environmental conditions (*12*, *14*−*17*). Plants can take up moderately hydrophobic organics (octanol−water partition coefficients, Log $K_{ow} = 0.5-3$) quite effectively (*14*). Plant characteristics, such as root surface area, could

* To whom correspondence should be addressed. Tel: 1-515-294-4776. Fax: 1-515-294-4757. E-mail jcoats@iastate.edu.
† ABC Laboratories.
‡ Bayer Corporation.
§ Iowa State University.

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substantially alter adsorption of an organic compound to roots (*16*). Plants not only release exudates for microbial growth or cometabolism but also harbor microbial consortia and mycorrhizal fungi on the root surface. As a result, enhanced degradation of organic compounds occurs in the rhizosphere (*13*, *18*, *19*). However, there are cases in which the rhizosphere did not show enhanced degradation of organic compounds (*17*, *18*).

The bioavailability of organic compounds can influence the success of the various remediation strategies. As organic compounds reside in soil for some time, their bioavailability usually decreases (*20*, *21*). The decline in bioavailability may result from the diffusion of organic compounds through micropores inside of soil particles during aging (*22*, *23*). Hydrophobic compounds can diffuse into soil organic matter slowly, thus becoming increasingly unavailable for biodegradation (*24*).

The objectives of the study were (i) to determine the influence of an enzyme and a soil bacterium on herbicide degradation and to test whether vegetation enhances the capability of the bacterium to degrade the herbicides; (ii) to determine the effectiveness of vegetation on the degradation of herbicides in soil; and (iii) to determine the effect of aging herbicides in soil upon the biodegradability of the herbicides.

MATERIALS AND METHODS

Chemicals. ATR (92.2% pure) and MET (97.3% pure) for treating the soils were obtained from Novartis Crop Protection (Greensboro, NC). ATR (98.0% pure analytical standard) was purchased from Chem Service (West Chester, PA).

Soils. Soil samples were obtained from an agrochemical dealer site in northwest Iowa, denoted as Alpha. Surface soils (top 15 cm) were collected by using hand trowels. Three independent composite samples were taken from vegetated areas. Soils were sieved at 2.4 mm, placed in polyethylene bags, and stored in the dark at 4 °C for less than 6 months. Soils were analyzed by A & L Midwest Laboratories (Omaha, NE) to determine physical and chemical properties. The soil had a sandy loam texture with 68% sand, 21% silt, and 11% clay. The organic matter, total nitrogen, pH, and cation exchange capacity were 2.5%, 0.08%, 7.8, and 10.0 mequiv/100 g, respectively. The residual (background) concentrations of ATR and MET from the Alpha site were less than 0.3 μg g^{-1} soil (*25*). The residual concentration of trifluralin was 0.1 μg g^{-1} soil in the Alpha soil (*25*). The number of indigenous ATR-mineralizing microorganisms was low in the Alpha soil (*25*). The soil was also used in the previous phytoremediation research in this laboratory (*25*). Therefore, this soil was used in the current study.

Microorganism and Enzyme. Strain ADP and the enzyme ATR chlorohydrolase were provided by the Wackett laboratory at the University of Minnesota, St.Paul, MN. The purification of the enzyme from ADP, the characterization, and the kinetics of the enzyme have been described previously (*7*). The culture method for ADP was described previously (*2*). Briefly, ADP was grown in a liquid growth medium containing 0.1% (weight/volume) sodium citrate as the carbon source and ATR (30 ppm) as the only nitrogen source. After the medium was incubated and centrifuged, the cell pellets were concentrated in 50% glycerol solution. The ADP stocks were diluted with sterile phosphate buffer saline (PBS, pH 7.4) for use. ATR chlorohydrolase stocks were diluted with 25 mM morpholinepropanesulfonic acid (MOPS) with 100 mM $CoSO_4$ (pH 6.9).

Experiment 1. An experiment was conducted to examine ATR and MET degradation by ATR chlorohydrolase and strain ADP without the aging of the chemicals in soil. Alpha soils were treated uniformly with a mixture of ATR and MET solutions, using acetone as the solvent. The treatment of the chemicals for each replication was carried out in two 900 mL treating jars, and there were three replications. After the treatment, the soils were mixed well on brown wrapping paper to evaporate acetone and homogenize the treated soils. An aliquot of 25 g of soil (dry weight) was taken from each replication to measure the concentrations of ATR and MET in the soils before the treatment with ADP or ATR chlorohydrolase. The average concentrations of ATR and MET were 97 and 22 μg g^{-1} soil (dry weight), respectively. Eighteen subsamples of 50 g (dry weight) each from each replication were randomly assigned to the following three treatments for six sampling dates: addition of ATR chlorohydrolase (0.01 mg g^{-1} soil), addition of ADP (10^9 cells g^{-1} soil), and control with only PBS and MOPS added. After the treatment of ADP or the enzyme, the soils were placed in Ray Leach "Cone-Tainers" (Stuewe & Sons, Inc., Corvallis, OR) and were incubated in a greenhouse at 27 ± 2 °C. Water was added to the soils on a daily basis to maintain adequate moisture. Concentrations of ATR and MET were determined at day 1, 2, 3, 7, 14, and 28 postenzyme or ADP addition. The reported percentage remaining ATR and MET at day 1, 2, 3, 7, 14, and 28 was calculated by dividing the concentrations of ATR or MET at these six sampling dates by the concentrations before the treatment with ADP or the enzyme and then multiplying by 100.

Experiment 2. A second experiment examined the effects of bioaugmentation of soil with ATR chlorohydrolase and strain ADP on the degradation of the chemicals in the aged soils. Soils were treated uniformly with a mixture of ATR and MET using acetone as the solvent at a concentration of 100 μg ATR g^{-1} soil (dry weight) and 25 μg MET g^{-1} soil (dry weight). After the treatment, the soils were mixed well on brown wrapping paper to evaporate acetone and homogenize the treated soils, and then, they were aged for 50 days in the greenhouse at 27 ± 2 °C. Five milliliters of tap water was added to each 180 g of soil (dry weight) each week through the aging period. After 50 days of aging, aliquots of 25 g (wet weight) of soil were taken for chemical analysis. The measured concentrations of ATR and MET were the concentrations before the treatment with ADP or ATR chlorohydrolase. The remaining soils were treated with the same amount of ATR chlorohydrolase, ADP, or PBS and MOPS per gram of soil as in experiment 1. Then, the procedures exactly followed experiment 1 except that concentrations of ATR and MET were only determined at day 1, 2, 3, 7, and 28 postenzyme or ADP addition.

Experiment 3. The third experiment examined the influence of prairie grasses, ATR chlorohydrolase, and ADP on the degradation of ATR and MET in the aged soils.

Experiment with Vegetation and ATR Chlorohydrolase. Soils were treated uniformly with a mixture of ATR and MET by using acetone as the solvent, providing 100 μg of ATR g^{-1} soil (dry weight) and 25 μg of MET g^{-1} soil (dry weight). After the treatment, the soils were mixed well on brown wrapping paper to evaporate acetone and homogenize the treated soils, and then, they were aged in the greenhouse at 27 ± 2 °C. Approximately 5 mL of water was added to each 100 g of soil (dry weight) each week. After 50 days of aging, 15 g aliquots (dry weight) of soil were taken for chemical analysis. Additionally, 75 g aliquots (dry weight) of soil were randomly assigned to the following four treatments: vegetation, addition of ATR chlorohydrolase (0.01 mg g^{-1} soil), vegetation plus addition of ATR chlorohydrolase (0.01 mg g^{-1} soil), and the control, which was only treated with MOPS. The plants utilized in this study were the mixture of three species of native prairie grasses: big bluestem (*Andropogon gerardii* Vitman), yellow Indian grass (*Sorghastrum nutans* (L.) Nash), and switch grass (*Panicum virgatum* L.). The mixture of grasses was planted in a small tray in the greenhouse until the height range of the grasses was between 10 and 20 cm. The root soils of the grasses were then washed off with tap water, and the grasses were transplanted into the treated soils in the cones. Each cone contained 6−12 grass plants (a mixture of the three species of native prairie grasses). After the treatment with vegetation and the enzyme, the soils were incubated for 28 days at 27 ± 2 °C in the greenhouse. Water was added to the soils on a daily basis to maintain adequate moisture. The concentrations of ATR and MET were determined at 28 days postenzyme addition. The reported percentage remaining ATR and MET at day 28 was calculated by dividing the concentrations of ATR or MET at day 28 by the concentrations before vegetation and addition of ATR chlorohydrolase and then multiplying by 100.

Experiment with Vegetation and ADP. The procedures exactly followed the experiment with vegetation and ATR chlorohydrolase except that the following four treatments were assigned to the aged soils: vegetation, addition of ADP (10^9 cells g^{-1} soil), vegetation plus

Table 1. Percentage Remaining ATR at Each Sampling Date after the Addition of the Enzyme ATR Chlorohydrolase, *Pseudomonas* sp. Strain ADP, or Buffer to Soil Freshly Treated with ATR and MET

day	enzyme[a,b]	ADP[a,b]	buffer[a,b]
1	a 9.1 a	b 23.4 a	c 86.4 ab
2	a 4.6 a	a 11.0 b	c 92.3 ac
3	a 3.0 a	a 6.2 b	c 82.8 ae
7	a 2.7 a	a 5.3 b	c 89.2 ab
14	a 2.6 a	a 3.2 b	c 79.9 be
28	a 1.8 a	a 2.9 b	c 52.8 d

[a] The letters at the right side of the numbers show the comparison among the different sampling dates at each treatment. Values with the same letter in each column are not significantly different ($P = 0.05$). Standard error of the mean = 0.06. [b] The letters at the left side of the numbers show the comparison among the different treatments at each sampling date. Values with the same letter in each row are not significantly different ($P = 0.05$). Standard error of the mean = 0.06.

addition of ADP (10^9 cells g^{-1} soil), and the control, which was treated only with PBS.

Extraction and Gas Chromatographic (GC) Analysis. Soil extraction and analysis exactly followed Anhalt et al. (*26*), except that soils were extracted three times with ethyl acetate instead of twice. Spike recovery tests showed that the extraction efficiency for ATR and MET was 107 ± 9 and $98 \pm 0.1\%$, respectively. The quantitation limit = (the concentration (μg mL^{-1}) of the standards required to give a signal-to-noise ratio of 2:1) × (10 mL of the soil extract)/25 g soil. For ATR and MET, this limit was evaluated as 0.078 and 0.313 μg g^{-1}, respectively.

Statistical Analysis and Half-Life Determination. All data were analyzed by analysis of variance (ANOVA) and least significant difference (LSD). The half-lives of ATR and MET in the soils were first calculated using linear first-order decay kinetics. If the coefficient of determination (r^2) for a linear regression relationship was very low, a nonlinear regression was used to calculate the half-lives. A software program GraphPadTM PRISM (Graph Pad Software, San Diego, CA) was used to calculate the nonlinear decay rate (k). The following equation describes the kinetics, where C_0 and C_t are the reported percentage remaining of ATR at time 0 and time t (days) and C_0 is 100%. The half-life of the decay is $\ln(0.5)/-k$. The decay rate (k) calculated was an "apparent" rate of dissipation. It was used only to estimate the half-lives of ATR after the treatment with ATR chlorohydrolase or ADP. Therefore, care should be taken to use it for predictive calculations.

$$C_t = C_0 \times e^{(-kt)}$$

RESULTS

Experiments 1 and 2. The percentage remaining ATR was significantly less in the soils treated with the enzyme ATR chlorohydrolase than that in the control soils on each sampling date in both experiments 1 and 2 ($P < 0.05$, **Tables 1** and **2**). Therefore, the addition of the enzyme had a statistically significant effect on the degradation of ATR ($P = 0.0001$ for experiment 1 and $P = 0.0034$ for experiment 2). The percentage remaining ATR was significantly less in the ADP-inoculated soils than that in the control soils on each sampling date in experiment 1 and at 1, 3, and 7 days postinoculation in experiment 2 ($P < 0.05$, **Tables 1** and **2**). Overall, the addition of ADP had a significant effect on the degradation of ATR ($P = 0.0001$ for experiment 1 and $P = 0.0263$ for experiment 2). Although on the first day in experiment 1 and on the third day in experiment 2, significantly less ATR was found in the ATR chlorohydrolase-treated soils than in the ADP-treated soils; the degradation of ATR did not differ significantly between ATR chlorohydrolase and ADP-treated soils overall ($P = 0.6022$ for experiment 1 and $P = 0.1326$ for experiment 2). The concentrations of ATR before the treatment with ATR chlorohydrolase, ADP, or buffer in experiment 2 are shown in **Table 2**.

Table 2. Concentrations of ATR at the End of 50 Days of Aging and Percentage Remaining ATR 1, 2, 3, 7, and 28 Days after Treatment with the Enzyme ATR Chlorohydrolase, *Pseudomonas* sp. ADP, or Buffer

day	concentration (μg g^{-1})[a]	enzyme (%)[b,c]	ADP (%)[b,c]	buffer (%)[b,c]
1	75.8 (1.9)	a 34.6 a	a 59.2 a	b 87.4 a
2	72.4 (10.0)	a 30.2 a	ab 52.1 a	b 77.4 a
3	75.3 (7.6)	a 27.4 a	b 59.2 a	c 87.8 a
7	71.4 (8.0)	a 24.3 a	a 33.0 b	b 76.2 a
28	72.9 (10.8)	a 17.6 a	ab 23.9 b	b 46.4 b

[a] Standard deviations are given in parentheses. [b] The letters at the right side of the numbers show the comparison among the different sampling dates at each treatment. Values with the same letter in each column are not significantly different ($P = 0.05$). Standard error of the mean = 0.09. [c] The letters at the left side of the numbers show the comparison among the different treatments at each sampling date. Values with the same letter in each row are not significantly different ($P = 0.05$). Standard error of the mean = 0.13.

The addition of ATR chlorohydrolase or ADP to the Alpha soil resulted in the rapid degradation of ATR both in the soils where ATR was aged for 50 days prior to the treatment with ATR chlorohydrolase or ADP and in the soils where ATR was freshly added prior to the treatment with the enzyme or ADP, and most of the degradation occurred during the first day after the addition of the enzyme or ADP. The percentage remaining ATR did not decrease significantly throughout the 28 day period beyond 1 day after the addition of the enzyme in both experiments 1 and 2. In the ADP-inoculated soils, significant decline of remaining ATR was observed 2 and 7 days after the inoculation in experiments 1 and 2, respectively, and thereafter, no further significant decline occurred in remaining ATR. The percentage remaining ATR in control soils did not significantly decrease until 28 days after the addition of buffer in both experiments 1 and 2. The half-lives of ATR in the ATR chlorohydrolase-treated soils, ADP-treated soils, and the control soils were shown in **Table 3**. For the soils treated with ATR chlorohydrolase or ADP, the half-lives of ATR in the aged soils were much longer (four times or more) than those in the freshly added soils.

Inoculation of ATR chlorohydrolase or ADP into the soils did not significantly increase the rate of degradation of MET in either experiment 1 or 2 ($P = 0.7649$ for experiment 1 and $P = 0.5800$ for experiment 2). The percentage remaining MET in all of the soils did not significantly decrease until 14 and 28 days after the addition of the enzyme, the ADP, or the buffer in experiments 1 and 2, respectively (**Tables 4** and **5**). The concentrationsof MET before the treatment with ATR chlorohydrolase, ADP, or buffer in experiment 2 are shown in **Table 5**. The half-lives of MET in the ATR chlorohydrolase-treated soils, ADP-treated soils, and the control soils were shown in **Table 3**. The half-lives of MET in the aged soils after the treatment with ATR chlorohydrolase, ADP, or buffer were much longer (more than five times) than those in the freshly added soils.

Experiment 3. The average concentrations of ATR and MET in the experiment with vegetation and ATR chlorohydrolase before the treatment with vegetation and ATR chlorohydrolase were 70.3 ± 24.2 and 16.6 ± 2.7 μg g^{-1} soil, respectively. The average concentrations of ATR and MET in the experiment with vegetation and ADP before the treatment with vegetation and ADP were 97.8 ± 9.2 and 18.1 ± 2.5 μg g^{-1} soil, respectively.

Table 3. Half-Lives of ATR and MET Both in the Freshly Added Soils and in the Aged Soils after Treatment with the Enzyme ATR Chlorohydrolase, *Pseudomonas* sp. Strain ADP, or Buffer[a]

	half-life (days)			
treatment	ATR (freshly added)	MET (freshly added)	ATR (aged)	MET (aged)
enzyme	0.30 ($r^2 = 0.99$) a	26.3 ($r^2 = 0.90$) b	1.17 ($r^2 = 0.70$) a	135.9 ($r^2 = 0.78$) b
ADP	0.52 ($r^2 = 0.99$) a	22.3 ($r^2 = 0.90$) b	17.6 ($r^2 = 0.70$) b	123.8 ($r^2 = 0.73$) b
buffer	35.9 ($r^2 = 0.88$) b	23.8 ($r^2 = 0.95$) b	28.5 ($r^2 = 0.93$) b	121.6 ($r^2 = 0.92$) b

[a] r^2, coefficient of determination; a, nonlinear regression was used; b, linear first-order decay kinetics were used.

Table 4. Percentage Remaining MET at Each Sampling Date after the Addition of the Enzyme ATR Chlorohydrolase, *Pseudomonas* sp. Strain ADP, or Buffer to Soil Freshly Treated with ATR and MET

day	enzyme[a]	ADP[a]	buffer[a]
1	87.4 a	77.3 a	83.7 a
2	100.0 a	98.8 b	99.3 b
3	94.8 a	99.1 b	93.1 ab
7	86.1 a	85.2 ab	85.9 ab
14	58.3 b	61.9 c	63.0 c
28	49.8 b	40.7 d	43.9 d

[a] Values with the same letter in each column are not significantly different ($P = 0.05$). Standard error of the mean = 0.07.

Table 5. Concentrations of MET at the End of 50 Days of Aging and Percentage Remaining MET 1, 2, 3, 7, and 28 Days after Treatment with the Enzyme ATR Chlorohydrolase, *Pseudomonas* sp. ADP, or Buffer

day	concentration (μg g^{-1})[a]	enzyme (%)[b]	ADP (%)[b]	buffer (%)[b]
1	17.3 (1.1)	96.5 a	96.5 ac	97.0 a
2	17.3 (0.9)	92.1 ab	92.0 a	97.2 a
3	17.2 (0.8)	98.6 a	100.0 c	100.0 a
7	16.5 (1.0)	94.0 a	90.9 ad	94.9 a
28	16.9 (0.2)	84.8 b	84.3 bd	84.4 b

[a] Standard deviations are given in parentheses. [b] Values with the same letter in each column are not significantly different ($P = 0.05$). Standard error of the mean = 0.04.

As in the previous two experiments, ATR chlorohydrolase or ADP significantly decreased the percentage remaining ATR in the soils ($P = 0.0009$ for ATR chlorohydrolase and $P = 0.0018$ for ADP) (**Table 6**). However, they had no significant effect on the degradation of MET ($P = 0.7975$ for ATR chlorohydrolase and $P = 0.0880$ for ADP) (**Table 6**). The prairie grasses significantly decreased the amount of remaining MET in both the experiment with vegetation and ATR chlorohydrolase and the experiment with vegetation and ADP ($P = 0.0135$ and 0.0009, respectively) (**Table 6**). However, no enhanced ATR degradation was seen with vegetation ($P = 0.4347$ for the experiment with vegetation and ATR chlorohydrolase and $P = 0.1476$ for the experiment with vegetation and ADP) (**Table 6**). There was a statistically significant interaction between the enzyme and the vegetation on the degradation of MET ($P = 0.0045$). However, no significant interaction was seen between ADP and vegetation on the degradation of MET ($P = 0.1209$), between vegetation and ADP on the degradation of ATR ($P = 0.5903$), and between vegetation and ATR chlorohydrolase on the degradation of ATR ($P = 0.7895$).

DISCUSSION

Soil Contaminants. Because the background concentrations of ATR and MET were very low as compared with the spiked concentrations of ATR and MET, these residual concentrations of ATR and MET in the soils were not expected to have an effect on the results in the current study.

Table 6. Degradation of ATR and MET in the Alpha Soil 28 Days after Vegetation and/or Inoculation with the Enzyme ATR Chlorohydrolase, *Pseudomonas* sp. Strain ADP[a]

Treatment with Vegetation and Enzyme			
	enzyme	no enzyme	SEM[b]
ATR	17.6	48.1	0.05
MET	68.6	69.6	0.02
	vegetation	no vegetation	SEM[b]
ATR	35.7	30.1	0.05
MET	64.0	73.6	0.02

Treatment with Vegetation and ADP			
	ADP	no ADP	SEM[b]
ATR	10.9	32.3	0.04
MET	45.9	56.7	0.03
	vegetation	no vegetation	SEM[b]
ATR	17.5	25.7	0.04
MET	39.7	60.8	0.03

[a] ATR and MET were aged for 50 days before vegetation and/or inoculation. [b] SEM, standard error of the mean.

Degradation of ATR by ATR Chlorohydrolase and ADP. ATR chlorohydrolase and ADP enhanced the degradation of ATR both in the freshly added soils and in the aged soils. The degradation of ATR residues in the Alpha soil occurred 1 day after the addition of the enzyme or ADP. Beyond the one day interval, concentrations of ATR remained low and did not significantly differ between 1 and 28 days in the soils treated with the enzyme. Strong et al. reported that 52% of ATR in the soil contaminated with high concentrations of ATR was degraded by killed recombinant *E. coli* cells engineered to overproduce ATR chlorohydrolase (*6*). It took longer for ADP to complete the ATR degradation in the current study as compared with the enzyme. The degradation of freshly added ATR was complete (11% of the applied ATR remaining) 2 days postinoculation. However, the degradation of ATR in the aged soil by ADP was not complete until 1 week after the inoculation. Shapir et al. reported that the *atz*A gene copy number in ADP declined at least 2 orders of magnitude 1 day after the inoculation of ADP to sands, but the *atz*A gene persisted in soils and continually mineralized ATR for at least 18 days in the presence of the competition from the indigenous ATR degraders (*27*). The addition of the enzyme or ADP markedly decreased the half-life of ATR in the current study.

In the current study, only the remaining ATR after the addition of ATR chlorohydrolase and ADP was measured. No effort was made to confirm whether the amount of ATR degraded by the enzyme or ADP was totally mineralized or only partially transformed. The dechlorination of ATR to HYA by ATR chlorohydrolase is the first step in the catabolic breakdown of ATR by ADP (*7*). Therefore, in the enzyme-treated soils,

ATR was presumably transformed to HYA. HYA does not have the phytotoxic activity (*28*, *29*) and is more susceptible to microbial or chemical degradation than ATR (*17*, *30*, *31*). However, it has been shown to be persistent in some studies (*32*, *33*). It also tends to adsorb to soil strongly (*34*, *35*). Therefore, HYA may be less of an environmental concern.

ATR can be completely mineralized by ADP (*2*). Addition of ADP and citrate to the ^{14}C-ATR-treated soil, which was aged for 1 day, resulted in 90−100% mineralization of the ^{14}C applied 15 days postinoculation (*36*). Bichat et al. noted that 80% of freshly added ATR in soil was mineralized by ADP during the first week (*5*). However, Newcombe and Crowley reported that only 33% of the applied ATR was mineralized by ADP in 12 days (*37*). Therefore, the efficiency of ADP from the literature is not completely consistent.

ADP metabolizes ATR as its sole N-source (*2*). Mandelbaum et al. reported that 70% of the aged ATR was degraded by ADP with the addition of sodium citrate; however, without the addition of citrate, only 17% of the aged ATR was degraded by ADP (*2*). They and others all showed that citrate can stimulate the degradation of high concentrations of ATR by ADP (*2*, *36*, *38*). Citrate amendment was not necessary in the degradation of low concentrations of ATR by ADP (*38*). No additional C-source was added to soil in this study; however, 97.1% of the freshly added ATR was degraded 28 days after the inoculation of ADP. Our results with freshly added ATR are consistent with those of Bichat et al. (*5*). The rapid degradation of ATR suggests that the nutrient levels in the Alpha soil, especially C-source, could support the activity of ADP in this study.

Influence of Aging on the Bioavailability of ATR. To evaluate the influence of aging on the bioavailability of ATR in both experiments 1 and 2, all of the data in both experiments 1 and 2 were recalculated by dividing the concentrations of ATR degraded at each sampling date by the initial concentrations applied (data not shown) and then were analyzed by ANOVA and LSD. For the soils treated with ATR chlorohydrolase or ADP, the percentage ATR degraded in the aged soils was significantly smaller than that in the freshly added soils on each corresponding sampling point ($P < 0.05$). Therefore, more ATR was degraded in the soils with freshly added ATR. This indicates that the bioavailability of ATR residue in the aged soils is significantly decreased as compared with that in the freshly added soils. Our results were consistent with the finding of Radosevich et al. and Yanze-Kontchou and Gschwind. Radosevich et al. reported that the mineralization of ATR by the bacterium M91-3 significantly decreased as the aging of the ATR-treated soils increased from 1 day to 3 months (*20*). Yanze-Kontchou and Gschwind also noted the reduced bioavailability of ATR after aging. The half-life of ATR, which was aged for 3 weeks prior to the inoculation of YAYA6, doubled as compared with that of freshly added ATR when both were degraded by YAYA6 (*1*).

Our results indicate that ATR and MET in the aged soils are more persistent than freshly added ATR and MET. This is probably related with the decreased bioavailability after aging. The bioavailability is related to the partition of a chemical into soil organic matter and penetration of the chemical into soil matrix (nanopores) (*22*, *24*, *39*). Sorption of ATR to soil aggregates was initially rapid (within 24 h), followed by a period of slow sorption (*20*, *40*). During the period of slow sorption, the aqueous phase concentration of ATR decreased slowly (*20*).

Influence of Vegetation on the Degradation of ATR and MET. The addition of the prairie grasses significantly reduced the concentration of MET but not ATR. Previous research has also showed that MET dissipation was more rapid in soil planted with corn (*Zea mays* L.) (*19*) as compared to the unvegetated soil, as well as in water containing live aquatic plants coontail (*Ceratophyllum demersum*), American elodea (*Elodea canadensis*), and common duckweed (*Lemna minor*) (*41*) as compared to unvegetated water. In another study conducted in this laboratory, the mixture of the three native prairie grasses significantly decreased ATR residues after 57 days with vegetation (*42*). Therefore, the plants need more time to decrease the concentrations of ATR significantly in the current study. The degradative ability of ATR chlorohydrolase or ADP was not influenced by the presence of the plants in the current study since there was no statistically significant interaction between vegetation and ADP or between the vegetation and the enzyme on the degradation of ATR.

In conclusion, results from this study clearly indicate that addition of ATR chlorohydrolase and ADP enhanced the degradation of ATR both in the freshly added soils and in the aged soils, and the prairie grasses enhanced the degradation of MET in the Alpha soil. Aging the ATR-treated soils significantly decreased the bioavailability of ATR as indicated by the lower amount of ATR degraded by ATR chlorohydrolase or ADP in the aged soils than in the freshly added soils. Bioaugmentation with *Pseudomonas* sp. strain ADP may be an effective method for remediating soils contaminated with high concentrations of ATR. In addition, the potential for using ATR chlorohydrolase to remediate ATR-contaminated soils and prairie grasses to remediate MET-contaminated soils is also promising.

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