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Ian J. Murphy
Iowa State University

Joel R. Coats
Iowa State University, jcoats@iastate.edu

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Abstract
Atrazine is a widely used herbicide in agriculture. Non-point source contamination of groundwater and drinking water may pose a significant threat to humans, wildlife, and the environment. Phytoremediation may provide a cost-effective strategy for reducing non-point source contamination of atrazine from agricultural runoff. Previous studies have shown that the rhizosphere of the native prairie grass, switchgrass (Panicum virgatum) is capable of enhancing the degradation of atrazine in soils. Biodegradation also may occur within the plant biomass; however, the extent to which this occurs has not been studied. We hypothesize that switchgrass has the capacity to degrade atrazine in vivo, in addition to the microbial biotransformation that occurs in its rhizosphere. The goals of this study were to characterize the ability of switchgrass to take up atrazine from soils, quantify the amount of biodegradation occurring in the plant, and quantify the amount of degradation occurring in the rhizosphere. Switchgrass seedlings were transplanted into autoclaved and non-autoclaved sand containing 10 μg/g atrazine in sand. Treatments were sacrificed on days 0, 3, and 7. Sand and plant tissue extracts were analyzed by gas chromatography to determine the concentration of atrazine and metabolites in sand and plant tissues. Results demonstrated that leaf biomass is capable of detoxifying atrazine, because metabolites were present in leaf material and not in the sand or root.

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Joel R. Coats
Iowa State University
Department of Entomology
116 Insectary
Ames, IA 50011-3140
Phone: 515-294-4776
Fax: 515-294-4757
E-mail: jcoats@iastate.edu
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The capacity of switchgrass (*Panicum virgatum*) to degrade atrazine in a phytoremediation setting

Ian J. Murphy† and Joel R. Coats†

†Department of Entomology, Iowa State University, Ames, Iowa 50011

Joel R. Coats
E-mail: jcoats@iastate.edu

Abstract

Atrazine is a widely used herbicide in agriculture. Non-point source (NPS) contamination of groundwater and drinking water may pose a significant threat to humans, wildlife, and the environment. Phytoremediation may provide a cost-effective strategy for reducing NPS contamination from agricultural runoff. Previous studies have shown that switchgrass (*Panicum virgatum*) can enhance atrazine degradation in contaminated soils. Phytoremediation is partially a result of microbial processes in the rhizosphere. Degradation may also occur within the plant biomass; however the extent to which this occurs remains unknown. We hypothesize that switchgrass has the capacity to degrade atrazine. The goal of this study was to: 1) characterize the ability of switchgrass to accumulate atrazine from soils; 2) quantify the amount of degradation occurring in the plant; and 3) quantify the amount of degradation occurring in the rhizosphere. Switchgrass seedlings were transplanted into autoclaved and non-autoclaved sand containing 10 µg of atrazine per gram of sand. Treatments were sacrificed on Days 0, 15, and 30. Sand and plant tissue extracts were analyzed on gas chromatograph equipped with a thermionic specific detector to determine the concentration of atrazine and metabolites in sand and plant tissues. Autoclaved treatments remained relatively sterile for the duration of experiment, ensuring atrazine degradation as the sole result of plant enzymes in vegetated and autoclaved treatments. Concentrations of atrazine and metabolites in leaf tissue were not affected by the presence or absence of microbes in the sand, as concentrations were statistically the same in both autoclaved and non-autoclaved treatments. This supports leaf biomass being capable of detoxifying atrazine as metabolites were present in leaf material and not in the sand or root.

Keywords: Atrazine, Phytoremediation, Degradation, Metabolism, Herbicide

Introduction

Atrazine (6-chloro-N-ethyl-N-isopropyl-1,3,5-triazine-2,4-diamine) is an herbicide that is registered in the United States for control against broadleaf and grassy weeds within row crop agricultural settings [1]. Atrazine's mode of action relies on inhibiting D-1 quinone binding involved in photosystem II [2]. It is possible for atrazine to be detoxified in soil, but it's half-life in soil can vary from 13 to 261 days [3]. Market estimates from 2000 and 2001 report that approximately 74 million pounds of atrazine are applied annually in the United States [4]. Globally, atrazine is one of the most commonly used herbicides [5].

Even though atrazine is a popular herbicide in the United States and globally, there is concern about its effects on surrounding ecosystems as well as on human health. Atrazine has been shown to have a number of negative effects on aquatic organisms including photosynthesis disruption in phytoplankton and algae, unsuccessful pupation in chironomids, and low survival rates of first generation *Daphnia magna* offspring [6]. Mammals including humans have been shown to be affected by atrazine as well. Hamster ovaries [7], mouse bone marrow, and farm worker lymphocytes [8] have been shown to have chromosomal damage when exposed to atrazine. Atrazine has also been linked to breast cancer, by interfering with the metabolism of estradiol [9]. Due to these potential detrimental effects, the Environmental Protection Agency (EPA) has set the Maximum Containment Level (MCL) for atrazine in drinking water at 3 μg/L [10].

Because of its widespread use, atrazine can be found in drinking water, mostly in the corn-belt region of the United States. This is typically due to non-point source runoff [11, 12]. There have been numerous instances in which the MCL for atrazine has been exceeded [13, 14, 15]. It is therefore essential that atrazine be remediated in the environment if possible. Many

full-scale remediation projects are time consuming, intrusive, and very costly [12, 16]. Instead of conventional technologies, it has been suggested to use plants to remediate atrazine contaminated sites. This is known as phytoremediation and has been shown to be more cost effective, less invasive, and a more aesthetically acceptable way to remediate contaminants in the environment [17]. It is also important to note that transformation of atrazine into its metabolites can be referred to as detoxification since these products are thought to less toxic than the parent compound.

In recent years, switchgrass (*Panicum virgatum*) has emerged as a promising candidate concerning the remediation of atrazine in the environment. Switchgrass can be found growing naturally in most of the United States, and it is able to grow in various soil types and pH levels, as well as withstand extreme weather conditions [18]. Switchgrass has been used previously in phytoremediation studies concerning atrazine contamination. A study using a mixture of prairie grasses including switchgrass, found that the parent compound atrazine and its metabolites were present in both the root and above-ground biomass of the plant [19]. In a similar study, switchgrass was shown to contain detoxification products of atrazine, 94.3% of what was applied was found as metabolites [20]. Metabolites of atrazine have been found in soil, the root zone, and in the leaves of above-ground biomass. Despite this, it is still unclear if above-ground biomass is capable of detoxifying atrazine.

The goal of this study is to determine the capacity of switchgrass' above-ground biomass to degrade atrazine by (1) characterizing the ability of above-ground leaf material of switchgrass to accumulate atrazine from sand, (2) quantifying the amount of detoxification occurring in the plant, (3) and quantifying the amount of detoxification occurring in the rhizosphere. To accomplish these objectives, atrazine was applied to sand and its fate was monitored. At the end

of 7 days, soil and grass tissues were evaluated for identification and quantification of atrazine and its chlorinated metabolites (Figure 1).

Materials and Methods

Experimental setup

Approximately 14.4 kg of commercial sand (Lowe's) was obtained, washed with water, and sifted in an effort to eliminate dust and other particulate matter. Sand was completely dried using heat lamps for five days. Thirty-six polypropylene pots (8.5 cm x 8.5 cm x 10 cm) were each filled with 400 g of sand. Each pot also had a hollow propylene tube (9.5 cm x 2.7 cm) placed vertically in the center of the pot with the sand occupying the space around the tube. Eighteen of these pots were autoclaved at 121°C for one hour once a day for three days. After autoclaving, all 36 pots had a piece of plastic cling wrap (10 cm x 10 cm) attached to the top of the pot, covering the sand below. Each piece of the plastic cling wrap was cut from the center of the square to one edge to allow for watering and the potential presence of switchgrass.

Switchgrass groups

Three months prior to the beginning of the experiment, switchgrass seeds were grown in sand washed and sifted according to the procedure mentioned above. These plants were watered with Hoagland's solution [21] over the preceding three months. A total of 54 switchgrass plants (with roots) were extracted from the sand, and then transplanted at 3 plants per pot into 18 pots; each group of three plants weighing approximately 3.2 g.

Transplantation

To transplant groups of switchgrass, each group's root system was dipped into a 10% hydrogen peroxide solution for 10 seconds and then rinsed for 10 seconds in autoclaved water. The transplant group was then placed, roots first, into the hollow polypropylene tube of a pot.

The tube was then lifted vertically out of the pot allowing the surrounding sand to cover the roots under the surface. When all transplants were made, four treatment groups were designated: nine pots with autoclaved sand and three plants (AP), nine pots with autoclaved sand and no plants (AN), nine pots with non-autoclaved sand and 3 plants (NAP), and nine pots with non-autoclaved sand and no plants (NAN).

Conditions and timeframe

All four treatment groups were kept in an environmental chamber for a total of 10 days on a 16 h light: 8 h dark schedule at 27°C/day and 22°C/night. On Day -3, plant groups were transplanted and all pots were watered with an autoclaved Hoagland's solution to the field capacity of the sand. Field capacity was determined prior to the experiment using percolation tubes [22]. On Day 0, 10 µg of atrazine per gram of sand was applied to all pots in all treatments. The concentration of atrazine was added to each pot based on the weight of the sand. The spiking solution was made by mixing 24.5 mL of water with 0.5 mL of a 8000 µg/mL atrazine solution (in acetone) which made the final concentration of atrazine in each pot 10 µg of chemical per gram of sand. Three pots from each treatment were sacrificed on Day 0 (after atrazine application), Day 3, and Day 7. Plants were watered with autoclaved water on Days 0, 2, 4, and 6. Field capacity was maintained by weighing each pot as it was watered.

Colony-forming unit analysis

On each day of extraction, the entire amount of sand from each of three pots from each treatment was placed in an autoclaved mason jar and mechanically shaken horizontally at 300 rpm's for 20 minutes. From each of the 12 mason jars, three one-gram samples were taken and placed in autoclaved plastic vials with caps. Each one-gram sample was then placed in a French square bottle containing 99 mL of an autoclaved phosphate buffer solution (0.5 M KH_2PO_4, 0.5

M K_2HPO_4, pH 7.1), making a 10^{-2} dilution of the sand. This mixture was then horizontally mechanically shaken at 300 rpm's for 20 minutes. Next, 0.1 µL of each solution was placed in a petri dish containing tryptic soy agar and spread with an L-shaped spreader. Petri dishes were sealed using paraffin film and kept in an incubator with no light at 28°C for 72 hours. Colony-forming units (CFU) were then counted from each plate.

Extraction of switchgrass material

On each extraction day, six plant groups were cut off at the level of the sand surface for their respective vegetated treatment group. Above-ground switchgrass biomass from each group was collected into a one-gram sample. Roots that had been teased out of the sand were collected in 0.1 g samples. Each sample of switchgrass biomass was rinsed with water and cut into pieces that were approximately one-half inch in length. The sample was then placed in a glass homogenizer with approximately 10 mL of ethyl acetate. Each extract was then homogenized with a Teflon®-tipped homogenizer, using a drill press, for three minutes. The solvent was decanted off through a filter containing 15 g of anhydrous granular sodium sulfate to absorb any water contained in the sample. The above procedure was repeated for a total of three times for all switchgrass biomass samples.

Extraction of sand

After three one-gram samples were taken from each of three pots from each treatment group, 20 g of sand was collected and placed in a French square bottle. Each French square bottle had 60 mL of ethyl acetate added to it and mechanically shaken horizontally at 300 rpm's for 20 minutes. The excess ethyl acetate was decanted off into a paper filter with 15 g of anhydrous granular sodium sulfate. This procedure was repeated for a total of three times.

Concentration of switchgrass and sand samples

All solvent extractions were then placed in a N-evaporator. Samples were dried down with nitrogen to a total of 1 mL. The extract was then pipetted into a syringe with a 0.45-µm micropore filter attached. The tube originally containing the solvent extract was subsequently rinsed with ethyl acetate, and the rinse was also placed into the syringe. The extract was passed through the filter into a volumetric to a total of 5 mL. A 2-mL volume of this was then pipetted into a gas chromatograph (GC) vial, and kept at -20°C until analysis.

Analysis for atrazine and metabolites

Extracts for all samples were analyzed on a Varian 3400 GC equipped with a Varian 8100 auto sampler and a thermionic specific detector (TSD). The column was a DB5 (5% phenyl-methylpolysiloxane nonpolar stationary phase), 0.25 µm film thickness, 30 m in length, 0.25 mm ID (J&W Scientific). GC operating conditions were as follows: oven parameters, 80°C held for 2 min, increased 8°C/min to 190°C held for 4 min, increased 6.5°C/min to 230°C held for 0.25 min; inlet, splitless mode, 220°C; carrier gas, ultrahigh purity helium, flow 30 mL/min; detector, 300°C, hydrogen flow 4.25 mL/min, air flow 175 mL/min. The retention times for the ethyl acetate solvent, didealkylatrazine (DDA), deisopropylatrazine (DIA), deethylatrazine (DEA), and atrazine (ATR) were 2.92, 18.78, 20.23, 20.73, and 22.43 minutes, respectively. Limits of detection for ATR and all chlorinated metabolites was 0.1 µg per g of extracted material. Peak areas were integrated using Peak Simple® [23].

Statistical analysis

All reported statistical differences were assessed using paired t-tests (two-sided) and compared concentrations of various treatments within each day. Statistical differences were also made between the number of CFU's between autoclaved and non-autoclaved treatments. This comparison of CFU numbers was made within Days. Means are expressed with standard error.

Each test performed utilized 3 degrees of freedom (9 degrees of freedom for CFU's) and tested the null hypothesis that the difference between treatments was equal to zero. Statistical calculations were performed using SAS [24].

Results

Colony-forming units

Colony-forming units (CFU's) were plated out from each sample to ensure that sterility had been achieved in autoclaved treatments. Each day that samples were extracted, the sand of nine pots from each treatment group was assessed for sterility based on the number of CFU's that were produced. Over the course of the 7-day study, sterility of autoclaved treatments was achieved. On each extraction day, treatments containing autoclaved sand were shown to have significantly fewer CFU's when compared to their respective non-autoclaved counterparts. Even after 7 days, autoclaved sand treatments remained virtually sterile as indicated by the amount of CFU's: Autoclaved treatments with no plants (AN) and 3 plants (AP) had 0.89±0.56 and 3.56±2.25 CFU's respectively, whereas non-autoclaved treatments with no plants (NAN) and 3 plants (NAP) had 449±47 and 561±59, respectively. It is also important to note that each pot containing switchgrass had more CFU's present on each extraction day when compared to its non-vegetated counterpart. Table 1 details the amount of CFU's for each treatment on each extraction day.

Sand residues

Levels of atrazine in the sand over the 7-day period followed a predictable pattern. On Day 0, levels of atrazine in all four treatments were found to be at or just under 10 µg/g of sand and not statistically different from one another. Based on an average of all four treatments, the percentage recovery of atrazine from sand using the described method above was 99.0%.

On Day 3, the levels of atrazine in AN did not change significantly. Levels of atrazine were reduced to 7.02±0.26 µg/g of sand in NAN. AP levels of atrazine were reduced to 6.04±0.14 µg/g of sand. Finally, levels of atrazine in NAP were recorded to be 4.25±0.16 µg/g of sand.

Day 7 of the experiment showed that levels of atrazine in NAP had been reduced below detection. AN atrazine levels were 6.37±0.10 µg/g of sand; NAN atrazine levels were 2.59±0.05 µg/g of sand; and AP atrazine levels were 1.04±0.05 µg/g of sand.

The levels of atrazine observed in the sand of all four treatments followed a predictable pattern of decline due to metabolism. Over the 7-day experiment, AN showed the least amount of atrazine detoxification. This makes sense as shown by the CFU analysis, as there were few microbes available in the sand to degrade atrazine. Also, since there were no plants present in these treatments, no plant enzymes could contribute to the detoxification of atrazine either. NAN showed the next least amount of atrazine detoxification overall as only sand microbes were available to degrade atrazine. Plant enzymes present in AP were solely responsible for atrazine in this treatment to be detoxified, which were the second highest. Lastly, NAP, with both sand microbes and plant enzymes present, showed the highest amount of atrazine detoxification, with no atrazine present at the end of 7 days (Figure 2).

Atrazine and metabolite concentrations in all four treatments were compared against one another to obtain information on the possible influence of soil and rhizosphere microbes. When comparing AP and NAP, a few differences stand out. On Day 7 in NAP, no ATR or DEA is detectable, while both are present in AP. While the concentration of atrazine in AP is not statistically different from 0, the concentration of DEA however, is significant.

Investigation of the role of soil microbes in atrazine detoxification was made by comparing AN and NAN. On Day 3 in NAN, the concentration of DDA reached 2.98±1.52 µg/g. No DDA was found in AN, and the concentration of atrazine was significantly less in NAN. By Day 7, concentrations of all metabolites were not shown to be statistically different from one another. Atrazine concentrations however, were significantly lower in NAN than in AN.

To investigate the influence of primarily rhizosphere enzymes on atrazine detoxification in the sand, comparisons were made between parent and metabolite concentrations of AP and AN. On Day 3, the concentrations of atrazine in AP and AN were 6.04±0.14 µg/g and 10.0±0.23 µg/g, respectively. These concentrations were found to be statistically different. On Day 7, only DIA was found to be statistically similar. Concentrations of both ATR and DDA were significantly less in AP than in AN. The concentration of DEA however was found to be significantly more in AP than AN.

The influence of innate soil microorganisms and enzymes in the rhizosphere was investigated by comparing NAP and NAN. No significant influence of rhizosphere enzymes and microbes was seen on Days 0 and 3. By Day 7, no significant difference could be seen in the concentrations of the metabolites. The concentration of atrazine in NAN, 2.59±0.05 µg/g, however, was significantly higher than in NAP, where no atrazine could be detected.

Plant biomass residues

Concerning plant biomass, only two of the four treatment groups contained switchgrass. Comparing autoclaved vs. non-autoclaved vegetated treatments, no significant differences were seen between treatments concerning the concentration of atrazine or metabolites found in both root and leaf tissue (Figures 3 and 4).

Discussion

Colony-forming units

The first underlying reason for using sand in this experiment was that sand has an inherently low amount of microbes present. This is evidenced from the fact that sand has a higher bulk density than silt or clay. This results in sand having less total pore space, a lower amount of surface area, and a much lower cation exchange capacity, resulting in microbial communities being less attracted and more hindered by their physical size to be present in sand [25]. The second reason is that since sand has an inherently low microbial population, it would therefore be easier to decimate any microbes that were present by utilizing an autoclave. Won et al. [26] reports that autoclaving sand for 1 h at 110°C results in no detectable numbers of heterotrophic microbes.

Over the course of our study, autoclaved treatments of sand were shown to have a very limited microbial population when compared to non-autoclaved treatments. This was essential to the main objective of the experiment concerning whether switchgrass in a phytoremediation system has the capacity to degrade atrazine on its own without the aid of microbes in the soil. Essentially, detoxification of atrazine could be attributed solely to plant enzymatic activity in treatments where no microbial populations of sand were present. It must be noted that even though the tryptic soy agar (TSA) used in this experiment is a general growth medium on which most microorganisms will grow, it may not grow every microbe present in a soil sample [27]. Despite this, microbes that are known to degrade atrazine have been shown to be maintained on TSA [28, 29].

Residues in sand

The amount of atrazine found in the sand in each of the 4 treatment groups proved to be distinct and gave insight into different microbial communities' influence on degrading atrazine. NAP showed to be most proficient in degrading atrazine with both the plant and the sand microbes present. AP, with only the influence of plants, showed to be the second most capable of degrading atrazine. With only the presence of microbes in the sand, NAN was less capable of degrading atrazine. Not surprisingly, the least amount of atrazine detoxification occurred in AN where there was no influence of any biotic system (plants and/or microbes). The detoxification of atrazine has been observed to be not only a biological process of microbial activity but also can be attributed to chemical processes in soils [30]. This is the most plausible explanation for detoxification of atrazine that occurred in AN where there were no microbes present.

DEA was found in sand of AP on Day 7 of the experiment. Looking at NAP, however, it is noted that there is neither DEA nor ATR found in sand by the end of the experiment. This seems to indicate that with the presence of both plant enzymes and sand microbes, ATR is broken down more quickly and results in metabolites that require further processing than the single dealkylation reaction required to form DEA. It has been well established in literature that the presence of vegetation is able to significantly increase the detoxification of contaminants. This is evidenced in a study looking on the mass balance of metolachlor in a phytoremediation setting [31]. That study showed evidence of plants and soil microbes/enzymes contributing to more detoxification of metolachlor, where 16.2% of the originally applied metolachlor was found in vegetated systems compared to 25.3% remaining in unvegetated systems. Concentrations of DDA and DIA in the sand however remain similar in both treatments suggesting that ATR was detoxified beyond these metabolites into a compound that did not contain chlorine, such as deethylhydroxyatrazine, and thus was not of concern in this study.

Comparing AN against NAN, the non-autoclaved treatment was shown to have significantly less ATR, a significant concentration of DDA, and no other metabolites on Day 3. On the same day, AN showed the formation of no metabolites and the same concentration of ATR that was originally applied. This may be evidence that the microbes that were in the sand preferred two sequential dealkylation reactions as opposed to just one. Quick conversion of atrazine to DDA in soil has been hypothesized to occur in previous literature. According to study by Mills et al. 1994 [32], there is a preferential removal of ATR's ethyl side chains in soils as opposed to removal of the isopropyl group. They went on to hypothesize that these deethylation reactions proceeded at two to three times the speed of deisopropylation reactions. Subsequently, they predicted that production of DIA may be slow, but its conversion to DDA could be much quicker. This rapid removal of both side chains has also been shown to occur when atrazine is exposed for a soil bacterium, *Agrobacterium radiobacter* J14a [28]. By Day 7 however, concentrations of the three metabolites had reached similar levels in both AN and NAN. Even though there was significantly more ATR present in AN, the formation of statistically similar levels of metabolites suggests that chemical detoxification may be as effective as microbial detoxification. Evidence on the influence of chemical and microbial detoxification of atrazine is highly prevalent in literature. Instances where chemical detoxification dominated [33, 34], as well as instances where microbial detoxification dominated [35, 36] have both been reported. Thus, it is fully plausible that atrazine was efficiently degraded under the influence of chemical detoxification in our study.

To assess the influence of plant enzymes on the detoxification of atrazine in sand, AP and AN were compared. By the end of the study, both ATR and DDA concentrations were significantly more in AN than AP. Concentrations of DEA however were significantly more in

AP than AN. Concentrations of DIA were similar in both treatments. While ATR was less detoxified in AN, the fact that the majority of the detoxification product found was DDA suggests that chemical detoxification can result in two sequential dealkylation reactions. Plant enzymes present in the sand however seem to prefer removing the ethyl group of atrazine, as the majority of metabolites found were DEA which would be the result of only one dealkylation. Similar findings have been reported in the past where it was observed that freshly cut foliar tissue from poplar trees degrading atrazine resulted in forming nine times more DEA than DIA [37]. Another possible explanation for the presence of DEA in the sand of AP could be that the plant is taking up ATR, degrading it, and subsequently exuding or passively releasing DEA back into sand. To our knowledge, there is no documented evidence of this phenomena, and further studies would be required to address whether this is occurring or not.

To see if the addition of plant enzymes and rhizosphere microbes further enhanced atrazine detoxification in the sand, NAP and NAN were compared. Over the course of the experiment, levels of ATR and all metabolites remained relatively the same. The only instance of a difference is seen on Day 7 where the concentration of atrazine is significantly greater in NAN than that of ATR in NAP. Because of the significant decrease of ATR in NAP, it seems that vegetation has the ability to significantly increase the detoxification of atrazine. As mentioned previously, this was also observed in a study concerning the detoxification of metolachlor [31]. Even though the concentration of ATR by Day 7 is higher in NAN, concentrations of metabolites are not significantly different between the treatments. This may be another indication of an instance where ATR was detoxified beyond the chlorinated metabolites, e.g., hydroxyatrazine, and a distinctive pattern cannot be elucidated as these metabolites were not of concern in this study.

Residues in root material

A few noticeable things stand out when assessing the concentration of atrazine in root material. On Day 0, immediately after application, small amounts of atrazine were observed in root material; there were only a few hours between application of atrazine and the extraction of the roots, and it appears that atrazine may have simply adsorbed to the surface of the roots and wasn't necessarily taken up by the root at this time. However, there is still the possibility that roots had taken up this amount of atrazine even in a short period of time. Previous experiments have shown that corn, soybean, and cotton plants were able to take up atrazine into their foliar tissue within seven hours of application [38]. Since surface-applied atrazine is typically taken up into plants via an aqueous phase through the roots, it is feasible that atrazine was absorbed into root material only after a short period of time. By the end of the experiment, ATR, DEA, and DDA were all found to be present in root material, but none was significantly more or less when AP and NAP were compared. This indicates that roots are not influenced by the presence or absence of microbes in the sand concerning their ability to degrade atrazine. Also of interest is that in all days of extraction, there is no DIA seen in either of the treatments. This could suggest that detoxification of atrazine in the root occurs via removal of the ethyl group from atrazine first before removal of the isopropyl group. Roots of mature and immature pea plants were shown to be able to degrade atrazine, with the primary metabolite being DEA [39, 40]. This incidence gives further evidence supporting that plant roots may preferentially form DEA over DIA.

Residues in leaf material

On Day 0, both vegetated treatments showed no parent or metabolite in the leaf material. By Day 3, concentrations of ATR, DEA, and DDA could be seen. Again, this is evidence that

plant enzymes prefer to remove the ethyl group from atrazine prior to the subsequent dealkylation required to form DDA. (See above). It is important to note that the concentrations in both treatments were statistically similar. On Day 7, concentrations of ATR and all metabolites including DIA could be seen. Again, concentrations for both treatments were not statistically different.

The fact that the concentrations of ATR and all metabolites were found to be statistically similar throughout the experiment suggests that plants' ability to degrade atrazine is not influenced by the presence or absence of sand microbes. The fact that metabolites are seen in leaf tissue in treatments that had no influence of microbes in the sand suggests that switchgrass plants are fully capable of taking up atrazine and degrading it on their own. There is a wide array of literature that reports the detoxification of atrazine as solely the result of plant enzymatic activity. Plants capable of degrading atrazine include pea plants, corn, soybeans, cotton, and poplar trees [37, 38, 39, 40]. In this experiment the switchgrass plants are not simply taking up metabolites from the sand that are the result of chemical or biological reactions in the sand. In fact, in treatments where sand microbes had been eradicated there was only atrazine found in the sand on Day 3. Therefore, metabolites found in the leaf on Day 3 cannot be attributed to chemical reactions or rhizosphere microbes but due to plant enzymes degrading atrazine. It is unclear if plant enzymes are capable of removing the isopropyl group from atrazine as levels of DIA in the sand of vegetated treatments were statistically similar and could have simply been taken up by the plant.

It must be noted that detoxification of ATR to another metabolite, hydroxyatrazine (HYA), also has the potential to occur. This metabolite forms when the chlorine of atrazine is replaced by a hydroxyl group and has been observed in many other experiments where the

detoxification of atrazine has been studied [19, 20, 37]. This experiment however, was concerned with chlorinated metabolites of atrazine, and thus the presence of HYA was not determined.

Conclusions

It is clear from the data presented above that switchgrass is fully capable of taking up and detoxifying atrazine. Evidence has also been presented to suggest that atrazine can be detoxified not only by microbial reactions but by chemical reactions as well. These results also suggest sand microbes as well as the chemical reactions taking place therein prefer to degrade atrazine by way of two dealkylation reactions followed quickly after one another. The concentrations in both the root and leaf suggest that the enzymes present there prefer to break atrazine down by way of removing the ethyl group first.

To our knowledge, this is the first laboratory study examining specifically the atrazine-detoxification capacity of switchgrass itself. We feel that the results here further support the use of switchgrass to remediate soils the United States where atrazine contamination is of concern. Further studies are required to determine the preferred method of detoxification of leaf, root, and soil microbes. Even though this study has shown that switchgrass is capable of removing and metabolizing atrazine, full-scale field studies are required to determine if the same results will be exhibited in the actual environment. The use of switchgrass in buffer strips, grass waterways, and on terraces in fields of corn, sorghum, or sugarcane may be feasible for in-field phytoremediation. In addition, further studies examining switchgrass' potential ability to exude or diffuse detoxified contaminants through its roots back into its growing medium are required to see if this actually occurs. Currently, our laboratory is performing experiments in an effort to address this interesting question.

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Table

Table 1. Number of colony-forming units from all four treatments from each day of extraction.

	Number of colony-forming units ± se[a]		
	Day 0	**Day 3**	**Day 7**
AN	0.67 ± 0.29	2.78 ± 0.91	0.889 ± 0.564
AP	2.67 ± 1.16	11.1 ± 3.64	3.56 ± 2.26
NAN	339 ± 42.4	637 ± 91.1	449 ± 47.4
NAP	424 ± 53.0	797 ± 114	562 ± 59.2

[a]Abbreviation: se = standard error

[AN]Autoclaved treatments with no plants

[AP]Autoclaved treatments with 3 plants

[NAN]Non-autoclaved treatments with no plants

[NAP]Non-autoclaved treatments with 3 plants

Figure Legends

Figure 1. Atrazine pathway of degradation. Chlorinated metabolites are highlighted. Note that the degradation of atrazine is not shown in its entirety.

Figure 2. Concentration of atrazine (in µg/g of sand) based on the dry weight of sand from all four treatments over the course of the experiment. Different letters signify a significant difference between concentrations within each Day.

Day 0 p-value: 0.8380

Day 3 p-value: <0.0001

Day 7 p-value: <0.0001

^{AP}Autoclaved treatments with 3 plants

^{AN}Autoclaved treatments with no plants

^{NAP}Non-autoclaved treatments with 3 plants

^{NAN}Non-autoclaved treatments with no plants

Figure 3. Concentration of atrazine and metabolites (in µg/g of root material) based on the dry weight of root material in vegetated treatments. Different letters signify a significant difference between concentrations within each Day.

Day 0 p-value: <0.0001

Day 3 p-value: 0.0203

Day 7 p-value: <0.0001

^{AP}Autoclaved treatments with 3 plants

^{NAP}Non-autoclaved treatments with 3 plants

Figure 4. Concentration of atrazine and metabolites (in µg/g of leaf material) based on the dry weight of above-ground leaf biomass in vegetated treatments. Different letters signify a significant difference between concentrations within each Day.

Day 3 p-value: 0.4039

Day 7 p-value: 0.5484

[AP]Autoclaved treatments with 3 plants

[NAP]Non-autoclaved treatments with 3 plants

Figures

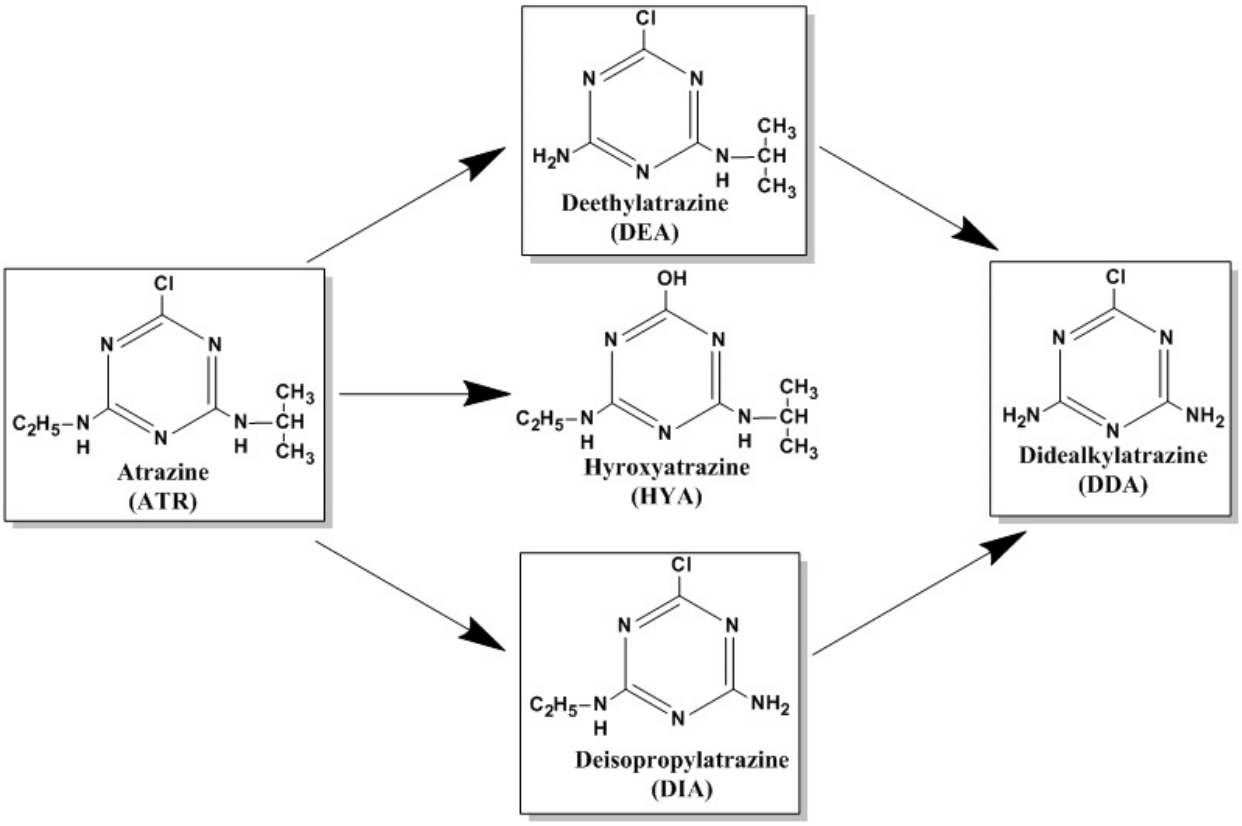

Figure 1

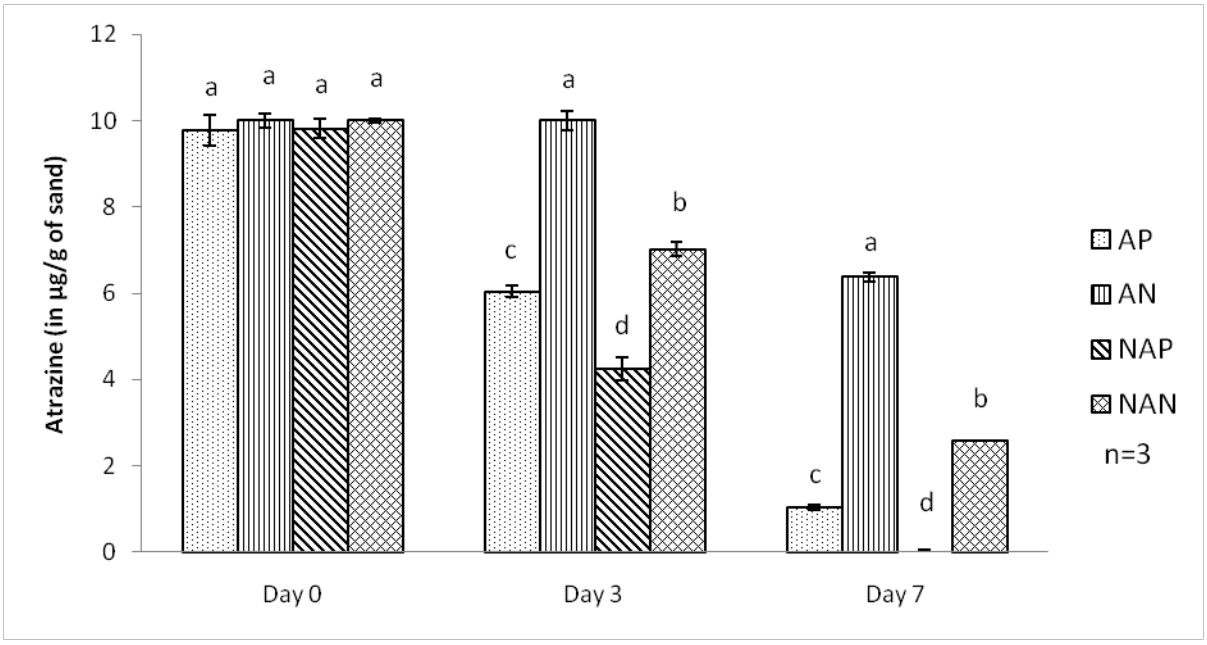

Figure 2

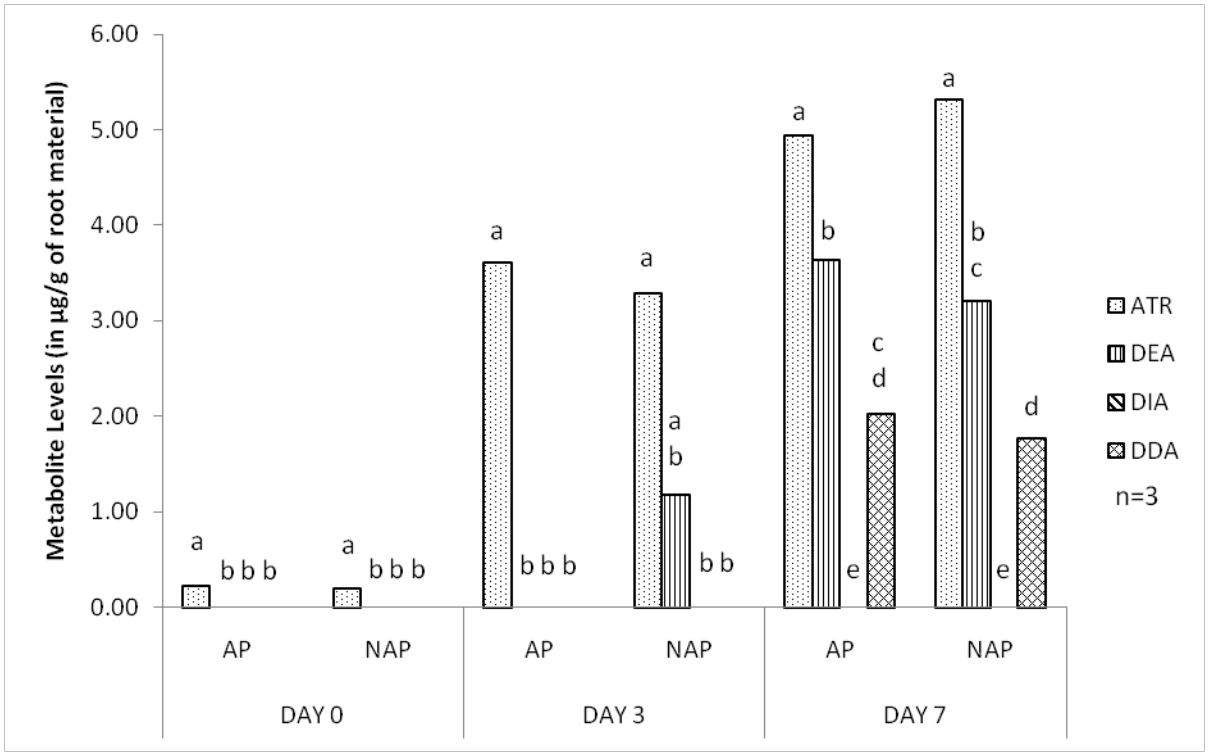

Figure 3

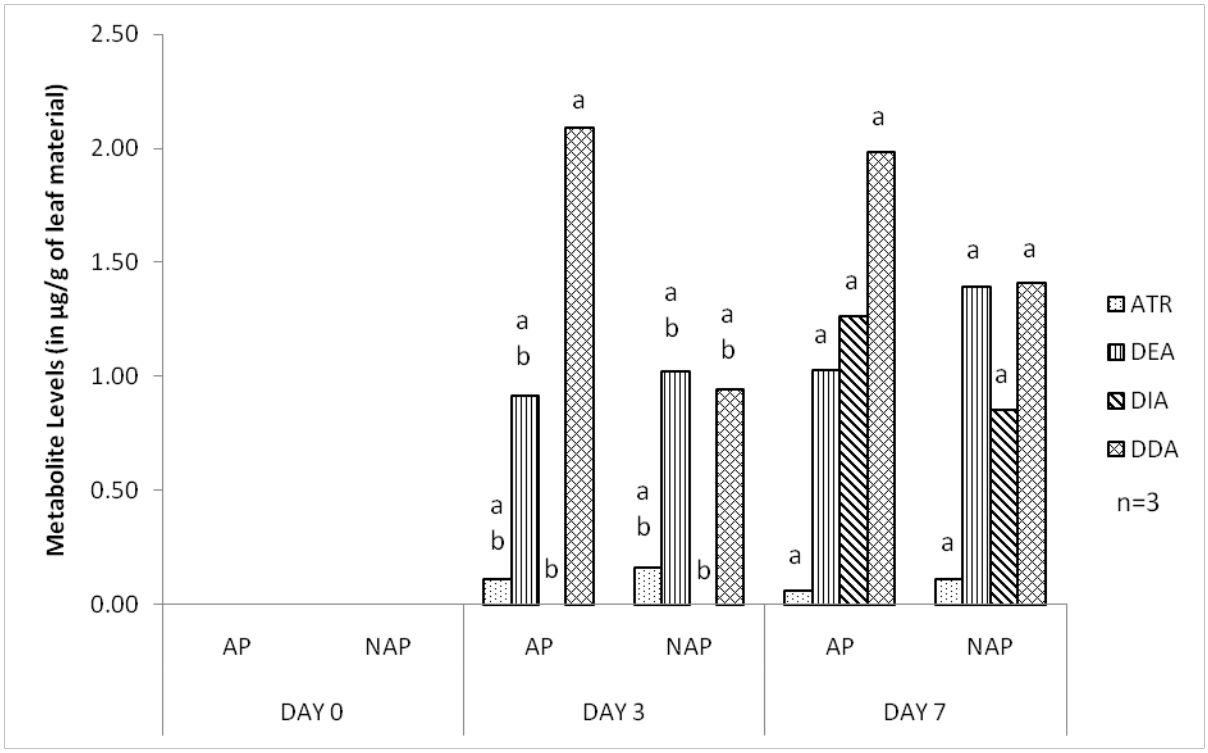

Figure 4