2011

Investigations into the capabilities of switchgrass to phytoremediate atrazine contamination in surface water runoff

Vurtice Albright  
Iowa State University

Follow this and additional works at: http://lib.dr.iastate.edu/etd
Part of the Entomology Commons

Recommended Citation
Albright, Vurtice, "Investigations into the capabilities of switchgrass to phytoremediate atrazine contamination in surface water runoff" (2011). Graduate Theses and Dissertations. 10436.  
http://lib.dr.iastate.edu/etd/10436

This Thesis is brought to you for free and open access by the Graduate College at Iowa State University Digital Repository. It has been accepted for inclusion in Graduate Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Investigations into the capabilities of switchgrass to phytoremediate atrazine contamination in surface water runoff

By

Vurtice Carroll Albright III

A thesis submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Toxicology

Program of Study Committee:
Joel R. Coats, Major Professor
Matthew J. Helmers
Thomas B. Moorman

Iowa State University
Ames, Iowa
2011

Copyright © Vurtice Carroll Albright III, 2011. All rights reserved.
# TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION ................................................................. 1  
   INTRODUCTION/LITERATURE REVIEW ...................................................... 1  
   THESIS ORGANIZATION ........................................................................ 10  
   REFERENCES ............................................................................................ 11  

CHAPTER 2. EXUDATION OF ATRAZINE METABOLITES FOLLOWING UPTAKE AND DEGRADATION OF ATRAZINE BY SWITCHGRASS .......... 15  
   ABSTRACT .................................................................................................. 15  
   INTRODUCTION ........................................................................................ 15  
   METHODS AND MATERIALS .................................................................... 17  
   RESULTS .................................................................................................. 21  
   DISCUSSION ............................................................................................. 22  
   ACKNOWLEDGEMENT .......................................................................... 25  
   TABLES ................................................................................................... 26  
   REFERENCES ........................................................................................... 29  

CHAPTER 3. STUDY ON THE PHYTOREMEDIATION CAPABILITIES OF SWITCHGRASS USING $^{14}$C ATRAZINE .......................................................... 31  
   ABSTRACT ............................................................................................... 31  
   INTRODUCTION ....................................................................................... 31  
   METHODS AND MATERIALS .................................................................. 34  
   RESULTS .................................................................................................. 36  
   DISCUSSION ............................................................................................. 37  
   ACKNOWLEDGEMENT .......................................................................... 41  
   FIGURES ................................................................................................... 42  
   TABLES ................................................................................................... 49  
   REFERENCES ........................................................................................... 51  

CHAPTER 4. GENERAL CONCLUSIONS .................................................................. 53  
   ACKNOWLEDGEMENT .......................................................................... 55
INTRODUCTION/LITERATURE REVIEW

Atrazine Introduction

Atrazine is one of the most widely used herbicides in the agricultural industry today. It is a triazine herbicide that is used for control of broad leaf weeds, mainly in corn, sorghum, and sugarcane crops by a reversible inhibition of photosynthesis [1-3]. Atrazine was first introduced for commercial use in the United States in 1959 [4]. In 2010, 51 million pounds of atrazine were applied to 61% of corn planted across 18 program states surveyed by the National Agricultural Statistics Service; only glyphosate isopropylamine sale, more commonly known as Roundup® was used in greater amounts, 57 million pounds [http://quickstats.nass.usda.gov/]. In Iowa, 6.8 million pounds of atrazine were applied, making it the second most used herbicide in the state, and that makes Iowa the state with the second highest usage of atrazine behind only Illinois [http://quickstats.nass.usda.gov/]. Like many other pesticides, atrazine can also pose a risk in the environment. It is moderately persistent in soil, demonstrated by a half-life of 41-231 days, and can leach into ground water where it is moderately soluble and has a half-life of >200 days. [1, 5]. Contamination of water sources due to atrazine and its metabolites, deethylatrazine (DEA), deisopropylatrazine (DIA), didealkylatrazine (DDA) and hydroxyatrazine (HA), has been widely reported. The United States Environmental Protection Agency (EPA) estimated that atrazine is present in 1,570 community water source wells and 70,800 private wells, but these numbers may be as high as 2,700 and 214,000 wells respectively [6]. Battaglin et al. [7] found atrazine was
present in 100% of 129 samples from 75 Midwestern rivers and streams in 1998. Another study reported that in Iowa alone, atrazine was present in 41% of 106 municipal wells [8].

One major source of atrazine contamination is non-point source runoff from agricultural fields. In fact, 2-5% of the atrazine applied to a field may be lost to water runoff; this number may be even higher if a storm event occurs immediately after application [9]. As a result, surface water bodies have been shown to be more susceptible to atrazine contamination than ground water supplies [10]. Atrazine can also enter the ground water through soil infiltration [10]. Contaminated soils at agrochemical dealerships and manufacturing facilities are another source of atrazine pollution. The potential for spills is higher at these sites, and large amounts of spilled atrazine may persist at these sites for several years, thus increasing the chances of atrazine leaching into the ground water or being carried by runoff to surface water bodies [11].

*Atrazine Toxicity*

The presence of atrazine and its metabolites, DEA and DIA, in waterways are of concern as they can have many detrimental effects on the ecosystem. Atrazine has been shown to have negative effects in many aquatic organisms. The metabolites DEA and DIA have also been shown to be phytotoxic but less so than the parent compound [12]. Atrazine can inhibit photosynthesis in algae, phytoplankton, and macrophytes [13]. In a study by DeNoyelles et al. [14], atrazine was shown to significantly decrease phytoplankton growth across a wide range of concentrations (1-500 ug/L); additionally, some species of phytoplankton were shown to be resistant to atrazine. This reduction of phytoplankton and macrophytes can negatively affect the survival and development of aquatic insects and
crustaceans. The abundance of the chronomid *Labrundinis pilosella* and other nonpredatory insects has been shown to be greatly reduced at concentrations of atrazine as low as 20 ug/L [15]. No effects were found on predatory insects, leading the author to conclude that decreases in insect populations were most likely due to the decrease in phytoplankton and macrophytes, a food source for many herbivorous insects, which was also observed in the study [15]. Earlier than normal emergence was also observed in some herbivorous insects, though this may be the result of other stressors, such as decreased abundance of food, and not due to the toxicity or presence of atrazine [15]. Some bioaccumulation of atrazine has been observed in *Lumbriculus variegates* [16].

Atrazine has been shown to have numerous effects on amphibians. Lower than normal body weights, decreases in fat body size, and decreases in liver weights have been observed in *Xenopus laevis* tadpoles at high atrazine concentrations, 200-400 ug/L, but no effect on feeding behavior was observed [17]. These weight changes were observed early in the exposure, leading the authors to suggest that even short term exposures can have significant physiological effects [17]. Another study observed effects of lower atrazine concentrations (0.1, 1, 10, and 25 ug/L) on *X. laevis* reared from the larval stage to metamorphosis [18]. At these doses, the authors found numerous gonadal abnormalities, including multiple gonads in individual animals or hermaphroditic animals; these abnormalities were not observed in any of their controls [18]. A continuation of this work revealed that atrazine can reduce spermatogenesis and decrease fertility in male frogs [19]. Lowered testosterone levels and smaller breeding gland size were also reported, suggesting atrazine can cause feminization in male frogs [19]. In fact, 10% of frogs treated with atrazine
developed female morphological features, and some specimens were capable of breeding with untreated males, resulting in the production of viable eggs [19]. Salamanders have also been shown to be vulnerable to atrazine. Experiments with spotted salamander embryos, Ambystoma maculatum, and their symbiotic algae, Oophila amblystomatis, showed that atrazine could prevent algal growth in the egg mass and thus potentially limit the amount of oxygen available to the developing embryo [20]. This inhibition of algal growth led to an increase in embryonic death and significantly reduced successful egg hatching [20]. Even the lowest concentration of atrazine tested, 50 ug/L, was shown to reduce hatching success by more than 50% [20].

In fish, reduced growth rates have been observed in brook trout, Salvelinus fontinalis [15]. Behavioral changes, including preferring habitats with dark substratum, have been observed in zebra fish [21]. Detrimental effects observed in humans include chromosomal abnormalities in lymphocytes of farm workers and interference with estradiol metabolism which can trigger breast cancer [22-23]. Atrazine has also been linked to various other cancers in laboratory animals, including brain, ovarian, stomach, and testicular cancer, although these effects are usually observed at doses that are considerably higher than those found in the environment [24].

Remediation Strategies

Due to their toxic properties and detrimental effects on ecosystems, several strategies have been developed to remove contaminants from the environment. Conventional remediation technologies include isolation and containment, air venting, and excavation and disposal of soil in a landfill [25]. However, these techniques can be very expensive, costing,
at minimum, a quarter-million dollars per acre [26]. As a result, researchers have been exploring more cost-effective remediation techniques. One technique that has seen a large increase in research has been phytoremediation. Phytoremediation is the use of plants to degrade, sequester, or otherwise neutralize organic and inorganic contaminants in soil and water. Phytoremediation is a broad research field that encompasses phytofiltration, phytoextraction, phytoimmobilization, phytostabilization and phytodegradation [25].

**Phytoremediation Strategies**

In phytofiltration, plants remove contaminants from water by incorporating them into the biomass [25]. Some aquatic plants have been shown to be capable of remediating selenium contamination in industrial wastewater through phytofiltration [27]. Alfalfa (*Medicago sativa*) can be used to remove metals, including lead, zinc, copper, nickel and gold from aqueous solutions [28]. Chromium can be removed from water and reduced to a nontoxic form by water hyacinth (*Eichhornia crassipes*) [29].

Phytoimmobilization also removes contaminants through uptake and harvest. However, after harvest, the plant tissues are allowed to decompose, and the contaminants are released and become immobilized in geomats or mineral-amended soils [25]. Sometimes contaminants cannot be removed from soils. In these cases remediation through phytostabilization is possible. Phytostabilization complexes metal contaminants with plants, reducing the hazard of the metal contaminant, while not removing the contaminant from the soil [25].

Phytoextraction is the use of plants to remove inorganic compounds, usually metals, from contaminated soils [30]. In that technique, plants grown on the contaminated soils are
harvested at maturity [30]. The plants then undergo a post-harvest processing step to reduce biomass volume to allow for easier disposal [26]. Alternatively, plants accumulating high-value metals, such as nickel and copper, can undergo a post-harvest processing step designed to recover the metal from the plant biomass [26]. The success of phytoextraction techniques depends on three factors: the degree of metal contamination, the degree of metal bioavailability, and the capacity of the plants to accumulate metals [31]. The bioavailability is controlled by several aspects. Physical aspects include the amount of organic material available in the soil, the amount of water available in the soil and soil structure and type [31]. Chemical aspects include soil pH, oxygen status, and metal speciation [31]. Biological aspects, such as bacteria, fungi, plants, and all the enzymes associated with these organisms, are perhaps most important as they can greatly affect the all the chemical and physical aspects listed above [31]. The third factor, capacity of plants to accumulate metals, can be controlled through plant selection. Typically, hyperaccumulators are used for phytoextraction; the amount of metals these hyperaccumulators can take up can be up to 100 times greater than levels found in nonaccumulator plants [30]. One type of fern (*Pteris vitatta*) can accumulate up to 14,500 parts per million of arsenic with no adverse effects observed [32]. *Thlaspi caerulescens* is a well-known hyperaccumulator of zinc and cadmium [30]. Other effective hyperaccumulators include *Ipomea alpina* and *Haumaniastrum robertii* for copper and cobalt respectively [30].

**Phytodegradation**

For organic contaminants, degradation by plants is also possible. This is known as phytodegradation. Phytodegradation occurs when a plant takes a contaminant up into its
tissues and the contaminant is then broken down, or degraded by plant enzymes [25]. These products are known as metabolites of the parent compound. The metabolites can then be further degraded by the plant enzymes, stored within plant tissues, or released as exudates via the roots [25]. It is important to understand these degradation processes as the transformation that occurs from the parent compound to metabolite can affect the compound’s toxicity and bioavailability to other organisms [25]. Generally, the metabolites are less toxic than the parent compounds; however, this is not true for all chemicals [25]. Many different plants have been shown to be capable of degrading a wide range of organic contaminants. Some aquatic plants are capable of taking up and degrading various explosives such as TNT and RDX [33-34]. Some native prairie grasses, such as big bluestem and yellow Indian grass, have been shown to be capable of degrading various herbicides [35].

Atrazine Phytoremediation

Atrazine has been the subject of much phytoremediation research because of its prevalence in the environment. Several grasses have been studied to determine their efficiency of degradating atrazine. Native prairie grasses are a prevalent choice for phytoremediation work. They have an extensive fibrous root system that produces a root surface area greater than any other vegetation and can penetrate up to ten feet below the surface [36]. Several studies have shown that prairie grasses, such as big bluestem and switchgrass, are able to phytoremediate atrazine and several other agrochemicals. Belden and Coats [37] found that a mix of three different prairie grasses reduced atrazine in leachate by 43% and that it was degraded more quickly in vegetated soils than unvegetated soil; mineralization was increased 260% in vegetated soils. Another study found that the amount
of degradation occurring in a mix of three prairie grasses can be affected by the concentration of atrazine in the soil before the addition of vegetation and by the length of time the grasses are allowed to grow [38]. A mass balance study reported that atrazine and its metabolites were equally distributed between the leaf and root tissue of very young switchgrass plants, though in minute amounts [2]. Switchgrass can provide significant reduction of atrazine toxicity due to its ability to form hydroxylated metabolites that are considered to be significantly less toxic than the chlorinated parent compound and chlorinated metabolites [39]. Switchgrass can also promote degradation in the rhizosphere. This study also showed that after 25 days, more than 80% of the atrazine in a soil planted with switchgrass is degraded to less toxic metabolites, and 47% of these residues are hydroxylated metabolites that are considerably less mobile [39].

More recent research sought to determine the ability of switchgrass to degrade atrazine in both laboratory and simulated phytoremediation settings. One study showed that in a microbe-free environment, switchgrass could take up and degrade atrazine into the metabolites DEA, DIA, and DDA; however a combination of switchgrass and soil microbes proved to be most effective in the degradation of atrazine, with no atrazine being detectable in treated sand after seven days [40]. The authors also determined that the degradation of atrazine in switchgrass systems was significantly increased compared to naturally occurring chemical degradation, suggesting that switchgrass could be employed in as a phytoremediation technology [40]. Interestingly, they also reported that higher concentrations of the metabolite DEA were found in the autoclaved sand of switchgrass plants than in autoclaved sand with no plants; this suggests that switchgrass plants may exude
or diffuse the atrazine metabolites in to the soil after degradation, but further research must be performed to confirm this [40].

Another recent study looked at the effects of mature switchgrass on atrazine degradation in a greenhouse phytoremediation setting with simulated surface water runoff [Murphy, I.J. 2009. M.S. thesis. Iowa State University, Ames, IA, USA]. The authors determined that atrazine was taken up, degraded, and completely absent from switchgrass in less than 21 days; atrazine and its metabolites DEA, DIA, and DDA, were not detected in soil or samples or leaf tissue from switchgrass three weeks after the first high-concentration runoff event [Murphy, I.J. 2009. M.S. thesis. Iowa State University, Ames, IA, USA]. Additionally, the authors showed that switchgrass may have a threshold for how much atrazine can be taken up. The levels of atrazine in the switch grass peaked after the second high concentration runoff event and continued to decline after the application of the third and final runoff even, even though there was an ample amount of atrazine available in the soil [Murphy, I.J. 2009. M.S. thesis. Iowa State University, Ames, IA, USA].

Research Interest

The goal of this research is to further understand the degradation of atrazine by switchgrass and its potential to benefit water quality through in-field phytoremediation of atrazine in surface water runoff. The first objective was to determine the ability of switchgrass to exude metabolites of atrazine after uptake and degradation. The proposed hypothesis was that switchgrass will exude metabolites of atrazine after uptake and degradation of the parent compound. The second objective was to determine metabolite formation in the leaf biomass of switchgrass through the use of radiolabeled atrazine in a
greenhouse study under simulated runoff conditions. The proposed hypothesis was that previously undetectable metabolites, such as hydroxyatrazine and cyanuric acid, will be identified within switchgrass leaf biomass. The information obtained from these experiments will further the knowledge on the phytoremediation capabilities of switchgrass with the expectation that this technology will eventually be implemented on a watershed scale to reduce atrazine in surface water runoff.

THESIS ORGANIZATION

My thesis is organized into four chapters. Two chapters describe research projects performed by myself during my graduate education at Iowa State University. There is also a literature review chapter (Chapter 1) and a general conclusions chapter (Chapter 4). Chapters 2 and 3 are written in a journal paper format following guidelines laid out by Environmental Toxicology and Chemistry with the intention that both chapters will be submitted to this journal. Chapter 2 investigates the possibility of exudation of atrazine metabolites following uptake and degradation by switchgrass. Prior research in our laboratory suggested that exudation may occur, and this chapter seeks to answer that question. Chapter 3 seeks to investigate in further detail, the degradation of atrazine in switchgrass leaf biomass by utilizing radiolabeled [\(^{14}\text{C}\)]atrazine, including tracking metabolites like hydroxyatrazine which were undetectable by Murphy’s methods. Both chapters are responsible for an equal contribution to my Master’s research.

My major professor, Joel Coats, also appears as an author on both papers. He provided invaluable support and consultation for both experiments and for serving as the primary editor for both papers.
REFERENCES


CHAPTER 2. EXUDATION OF ATRAZINE METABOLITES FOLLOWING UPTAKE AND DEGRADATION OF ATRAZINE BY SWITCHGRASS

Vurtice C. Albright III and Joel R. Coats

A paper to be submitted to Environmental Toxicology and Chemistry

ABSTRACT

Extensive use of the agricultural herbicide atrazine has led to contamination of numerous ground and surface water bodies. Research has shown that it can have a variety of negative impacts on numerous non-target organisms in the environment. Phytoremediation is one strategy that has been studied to remove atrazine contamination. This paper investigates the hypothesis that switchgrass (Panicum virgatum) can exude metabolites of atrazine after uptake and degradation, which has been suggested by prior research. Pots planted with switchgrass were treated with a 4 ppm solution of atrazine spiked with [14C]atrazine. After 4 days, switchgrass plants were transplanted to new pots with fresh sand. Four days later, the pots were sacrificed, and sand and plant samples were extracted. Plant and sand samples were analyzed for the presence of atrazine and its major metabolites. The percentage of atrazine present was observed to decrease over the course of the study while the metabolites were observed to increase. The presence of the metabolite cyanuric acid in a phytoremediation system is reported for the first time.

INTRODUCTION

The triazine herbicide atrazine has been one of the most widely used herbicides in the agricultural industry for control broadleaf weeds, mainly in corn, sorghum, and sugarcane
crops by a reversible inhibition of photosynthesis [1-3]. In 2010, 51 million pounds of atrazine were applied across 18 states, with 6.8 million pounds applied in Iowa alone [http://quickstats.nass.usda.gov/]. As a result of widespread usage, atrazine and its metabolites are common contaminants of both ground water and surface water sources. The United States Environmental Protection Agency (EPA) estimated that atrazine is present in 1,570 community water source wells and 70,800 private wells, but these numbers may be as high as 2,700 and 214,000 wells respectively [4]. Battaglin et al. [5] found atrazine was present in 100% of 129 samples from 75 Midwestern rivers and streams in 1998.

The presence of atrazine in these bodies of water has been shown to have many detrimental effects on organisms in an ecosystem. Inhibition of photosynthesis in algae, phytoplankton, and macrophytes as a result of exposure to atrazine has been observed [6]. Numerous detrimental effects have been observed in amphibians including decreases in fat body size and liver weights in *Xenopus laevis* tadpoles, decreased spermatogenesis and fertility in male *X. laevis*, and reduced successful egg hatching in spotted salamander embryos, *Ambystoma maculatum* [7-9]. In humans, atrazine has been liked to various cancers including breast, brain, ovarian, stomach, and testicular cancer [10,11].

One major source of atrazine contamination is non-point source runoff from agricultural fields. In fact, 2-5% of the atrazine applied to a field may be lost to water runoff; this number may be even higher if a storm event occurs immediately after application [12]. As a result, several strategies have been developed in an effort to mitigate the amount of atrazine that reaches surface bodies of water. One prominent method is phytoremediation, the
use of plants to degrade, sequester, or otherwise neutralize organic and inorganic contaminants in soil and water.

Native prairie grasses are a popular choice for phytoremediation strategies as they have an extensive fibrous root system that produces a root surface area greater than any other vegetation and can penetrate as much as ten feet below the surface [13]. Prior research in our lab has considered the use of several native prairie grasses in phytoremediation strategies. Belden and Coats [14] found that a mix of three different prairie grasses reduced atrazine in leachate by 43% and that it was degraded more quickly in vegetated soils than unvegetated soil. Leaf and root tissue have been shown to be equally capable of taking up and degrading atrazine [2].

The purpose of this research is to examine movement of atrazine and its metabolites in a vegetative system. Preliminary research indicated that switchgrass may exude or diffuse the atrazine metabolites into the soil after degradation or that they may diffuse out of the plant [15]. To test this hypothesis, radiolabeled [14C]atrazine was utilized to track uptake and degradation of atrazine and possible exudation of atrazine metabolites into virgin soil.

METHODS AND MATERIALS

Experimental setup

Approximately 22.7 kg of commercial sand (Lowe’s) was obtained, was sifted to remove particulate matter, and was washed with water to remove dust (pH 8.15, 0.3% organic matter; soil composition: sand 98.27%, clay 1.65%, silt 0.08%). The sand was allowed to dry in a greenhouse (16 h light: 8 h dark schedule at 27°C/day and 22°C/night) for five days. Seven days prior to the start of the study, 18 pots (8.5 cm x 8.5 cm x 10 cm), with
a hollow propylene tube (9.5 cm x 2.7 cm) placed in the center, were filled with 400 g of sand. All 18 pots were autoclaved for one hour at 121°C once a day for three consecutive days.

Switchgrass preparation

Switchgrass plants (Cave-in-rock variety) utilized in this experiment were planted in Sunshine® Professional Growing Mix potting soil (Sun Gro Horticulture, Bellevue, WA, USA) in March of 2010 and grown throughout 2010 in a greenhouse on a 16 h light: 8 h dark schedule at 27°C/day and 22°C/night. In the fall of 2010, the greenhouse temperature was slowly lowered to 4.5°C day and night over eight weeks at a rate of 2.8°C per week. At the end of the ramp down, the day: night cycle was changed to 12 h light: 12 h dark. Switchgrass plants were allowed to senesce over winter. The temperature was slowly ramped back up and the day: night cycle changed back to 16 h light: 8 h dark in the spring of 2011, and the plants were allowed to grow for four months before being utilized in this study. Plants were watered 3 times a week during the growing season and once every two weeks during the winter.

Experimental conditions and time frame

Four days prior to the start of the study (Day -4), switchgrass plants were removed from their growing medium and stored in Hoagland’s solution for 30 minutes [16]. The plants then had their roots dipped in 10% hydrogen peroxide for 10 seconds to destroy any microbes present on the roots and then placed in each of the 20 pots. After a four-day acclimation period, four pots were sacrificed, and the sand as well as the plant material (including roots) was extracted to show that no background radioactivity was present. The remaining 14 pots were treated with 50 mL of a 4 µg/ml (parts per million) solution of
atrazine spiked with approximately $3 \times 10^6$ disintegrations per minute (dpm) of $[^{14}\text{C}]$atrazine (specific activity = 28.9 $\mu$Ci/mg) that was applied to the surface of each pot. This represents Day 0 of the study. One day after the start of the study, an additional 10 pots were prepared as previously described and autoclaved for one hour at 121°C once a day for three consecutive days. Four days after the start of the study (Day 4), four pots were sacrificed, and the sand, as well as the plant material, including roots, was extracted to show that atrazine has been taken up into the plant. The 10 remaining pots had their plants and roots extracted and placed in a Hoagland’s solution to rinse off any adhered sand. The switchgrass plants then had their roots dipped in 10% hydrogen peroxide and then were placed in each of the 12 recently autoclaved pots. Four days after transplantation (Day 8), the switchgrass was removed from the pots. Sand from each of the pots was extracted and analyzed for the presence of atrazine and/or metabolites. Each pot was watered with 50 mL autoclaved Hoagland’s solution on Days -4, -2, 2, 4, and 6. All treatment groups were kept in a greenhouse for a total of 12 days on a 16h light: 8h dark schedule at 27°C/day and 22°C/night.

*Extraction of sand*

Sand was homogenized by placing in a Mason jar and shaken for 5 minutes. Twenty grams of sand were weighed and placed in a glass French square bottle with 40 mL of ethyl acetate and mechanically shaken horizontally at 300 rpm’s for 20 minutes. The 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.
Extraction of switchgrass material

Roots were teased out of the sand. Roots of each sample of switchgrass were rinsed with water to remove any adhered sand. Entire plants were weighed and then cut into pieces that were approximately one-half inch in length. The sample was then homogenized with a mortar and pestle using 30 mL of ethyl acetate. The solvent was decanted off through a filter containing 15 g of anhydrous granular sodium sulfate to absorb any water contained in the sample. This procedure was repeated for a total of three times.

Concentration of switchgrass and sand samples

All solvent extractions were placed in a N-evaporator. Samples were dried down with nitrogen gas to approximately 1 mL. The extracts were quantitatively transferred 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 vial to a total volume of approximately 10 mL. The vials were then blown down with a N-evaporator and reconstituted with methanol to 1 ml and placed in an HPLC vial.

Analysis of samples

A 100 µl sample of both plant and sand extracts were analyzed using high-performance liquid chromatography (HPLC). A Hewlett-Packard 1100 series HPLC equipped with an autosampler was used to separate the metabolites and parent compound. An Atlantis dC18 5µm, 4.6x250 mm column (Waters Corporation, Milford, MA, USA) was used with a mobile phase as described in Table 1. Flow rate was 1 ml/min; column temperature was maintained at 30°C. A Model 2B beta-ram detector and Laura Lite 3
radiochromatography software (IN/US Systems, Inc., Tampa, FL, USA) connected to the HPLC was used in identification and quantification of the metabolites. After entering the beta-ram detector, the elutant from the HPLC was mixed with Flow Logic ES liquid scintillation cocktail (LabLogic, Brandon, FL, USA) at a ratio of 3:1. Non-radioactive analytical standards and radioactive standards were used to determine retention times of the compounds on the HPLC and the beta-ram. All radioactive standards and non-radioactive atrazine, deethylatrazine, deisopropylatrazine, and didealkylatrazine standards were obtained from CIBA-GEIGY (Syngenta), Greensboro, North Carolina, USA. Non-radioactive hydroxyatrazine was obtained from Sigma-Aldrich (St. Louis, MO, USA), and non-radioactive cyanuric acid was obtained from Fluka (St. Louis, MO, USA).

Statistical analysis

An analysis of variance (p < 0.05) was used to test the null hypothesis that switchgrass does not detoxify atrazine and release metabolites from its roots. Means are expressed with standard errors. Statistical calculations were performed using SAS (SAS Institute Inc., Cary, NC, USA).

RESULTS

Sand Samples

No radioactivity was detected in any of the Day 0 sand samples (data not shown). After four days, approximately 73% of the radioactivity extracted from the sand samples remained in the form of the parent compound atrazine (Table 2). Deethylatrazine (DEA) accounted for approximately 19% of the extractable radioactivity, and deisopropylatrazine
(DIA) accounted for approximately 8. No radioactivity was detected in the Day 8 sand samples.

**Plant Samples**

Plant material was extracted after it was determined there was no radioactivity in the sand samples. No radioactivity was detected in any of the Day 0 plant samples. Atrazine comprised 43.7% of the extractable radioactivity and was present at significantly higher concentrations than the six metabolites in Day 4 plant samples. Metabolites comprised of 8.8% to 20.0% of the extractable radioactivity (Table 3). In Day 8 plant samples, 52.8% of the extractable radioactivity that remained was in the form of didealkylatrazine (DDA). Cyanuric acid, atrazine, and DEA accounted for 22.7%, 16.5%, and 8.0% of the extractable radioactivity, respectively.

**DISCUSSION**

The main objective of this research was to study the potential of atrazine metabolites to diffuse or be exuded from roots after uptake and degradation by switchgrass. This was suggested in preliminary research by Murphy and Coats [15]. In that study, the authors compared degradation of atrazine in pots of autoclaved sand without switchgrass against degradation of atrazine in pots of autoclaved sand planted with switchgrass. Atrazine, DIA, and DDA were found in both sand samples, while DEA was found only in pots planted with switchgrass. This led the authors to propose two possible scenarios: (1) plant enzymes were released into the sand and removed the ethyl group of atrazine or (2) atrazine was taken up by the plant, degraded to DEA, and then released back into the sand.
The current study shows that no metabolites were detected in the second set of sand samples four days after being planted with switchgrass that were exposed to atrazine. In the original extractions, only 20 grams of sand was extracted. After running the samples on the HPLC and observing no metabolites, the decision was made to extract more sand in an effort to concentrate any metabolites that may be present. The remaining sand in three pots was split into two portions and extracted by mechanical shaker three times with 300 ml of ethyl acetate for 30 min at 300 rpm; sand weights in these extractions ranged from 186 g to 190 g. The new samples were then analyzed on the HPLC, and no metabolites of atrazine were detected. This leads us to conclude that after uptake and degradation of atrazine by switchgrass plants, no metabolites were diffused or exuded.

The use of radiolabeled \([^{14}C]\)atrazine in this study allowed better identification and tracking of atrazine metabolites and degradation pathways. It also allowed the detection and tracking of atrazine metabolites, such as hydroxyatrazine and cyanuric acid, which were not previously detectable with gas chromatography methods. Uptake and degradation of atrazine was observed as evidenced by the Day 4 and Day 8 plant samples. The presence of the metabolites DEA, DIA, and DDA in plant (Day 4 and Day 8) and sand (Day 4) samples is consistent with prior research [1, 2, 15].

One difference from prior research is the present study found no hydroxyatrazine present in either the sand or the plant tissues. This is in contrast to Lin et al. [17] who detected hydroxyatrazine present in switchgrass tissues. The major difference between that study and the present study was the growing media utilized in the research. In Lin et al. [17], the switchgrass plants were grown in sandy loam soil and no attempt was made to sterilize or
otherwise reduce the microbe population present in the soil. In the present study, sand was autoclaved for 1 hour at 121°C on three consecutive days which has been shown to significantly reduce the number of microbes present [15]. Therefore, it is possible that the presence of hydroxyatrazine observed by Lin et al. [17] in switchgrass tissues is a result of atrazine degradation to hydroxyatrazine by microbes in the rhizosphere followed by uptake by the switchgrass plants.

The current study also reports the presence of cyanuric acid in switchgrass tissues. This compound was previously undetectable in using gas chromatography methods. The use of radiolabeled [14C]atrazine allowed cyanuric acid to be detected and quantified, since the ring structure, which contains the radiolabel, was not compromised in the degradation process. Cyanuric acid made up 9% of the extractable radioactivity in the Day 4 samples. This was higher in the Day 8 samples where cyanuric acid accounted for 23% of the extractable radioactivity. To our knowledge, this is the first time this compound has been reported to be detected in switchgrass tissues.

Formation and detection of the metabolites of atrazine is important as they are generally considered to be less toxic than the parent compound. DEA and DIA have been shown to be significantly less toxic than atrazine to some amphipods and algae, as well as to some bacteria and plants [18-20]. However, these chlorinated metabolites have still been included in regulatory limits for atrazine in drinking water in the past. Hydroxylated metabolites, such as hydroxyatrazine and cyanuric acid, may be even less toxic as they do not contain the chlorine atom. In fact, hydroxyatrazine has been shown to be nontoxic to green algae and cyanobacteria that have their growth and photosynthetic processes inhibited by
atrazine [21]. Thus, by degrading atrazine to its metabolites, the risks to organisms in aquatic ecosystems can be reduced.

The final objective of this course of research is to integrate switchgrass, alone or in combination with other native prairie grasses, into buffer strips between agricultural fields and waterways and around existing tile line intakes. The findings reported here are very significant in advancing knowledge toward that goal. By showing that metabolites of atrazine are not exuded, we show that atrazine can safely remove atrazine and its metabolites from surface water runoff. The metabolites DEA and DIA have also been shown to be toxic to plants, but less so than atrazine [20]. Thus, if exudation of atrazine metabolites was observed, there would be concerns over the toxicity of these compounds. However, because exudation does not occur, these compounds remain in the plant, eliminating any concern about them reaching a body of water.

ACKNOWLEDGEMENT

Funding for this project was provided by the Iowa State University Toxicology Graduate Program and Iowa Agriculture and Home Economics Experiment Station, Projects 5075 and 5088.
### Table 1. High performance liquid chromatography conditions for compound separation and determination.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time</th>
<th>Column Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td></td>
<td>Isocratic 75:25 water:acetonitrile (0-3 min)</td>
</tr>
<tr>
<td>Cyanuric Acid</td>
<td>6.6</td>
<td>Linear Gradient from 75:25 water:acetonitrile to 25:75 water:acetonitrile (3-11 min)</td>
</tr>
<tr>
<td>Didealkylatrazine</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>Deisopropylatrazine</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>Deethylatrazine</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>Hydroxyatrazine</td>
<td>11.5</td>
<td>Linear Gradient from 25:75 water:acetonitrile to 75:25 water:acetonitrile (11-16 min)</td>
</tr>
<tr>
<td>Atrazine</td>
<td>12.6</td>
<td>75:25 water:acetonitrile (11-16 min)</td>
</tr>
<tr>
<td>Blank</td>
<td></td>
<td>Isocratic 75:25 water:acetonitrile (16-20 min)</td>
</tr>
</tbody>
</table>
Table 2. Percentages of atrazine and metabolite radioactive extracted from sand samples.

<table>
<thead>
<tr>
<th></th>
<th>% of extractable radioactivity</th>
<th>SE(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atrazine</strong></td>
<td>73.23(^{A1}) ± 1.10</td>
<td></td>
</tr>
<tr>
<td><strong>Deethylatrazine</strong> (DEA)</td>
<td>18.51(^{B}) ± 0.99</td>
<td></td>
</tr>
<tr>
<td><strong>Deisopropylatrazine</strong> (DIA)</td>
<td>8.26(^{C}) ± 0.62</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>% of extractable radioactivity</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Atrazine</strong></td>
<td>0.00 ± -</td>
<td></td>
</tr>
<tr>
<td><strong>Deethylatrazine</strong> (DEA)</td>
<td>0.00 ± -</td>
<td></td>
</tr>
<tr>
<td><strong>Deisopropylatrazine</strong> (DIA)</td>
<td>0.00 ± -</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Letters indicate significance within each day.
\(^2\) Standard Error (SE).
Table 3. Percentages of atrazine and metabolite radioactive extracted from plant samples.

<table>
<thead>
<tr>
<th></th>
<th>% of extractable radioactivity</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 4 Plant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrazine</td>
<td>43.71A</td>
<td>± 4.46</td>
</tr>
<tr>
<td>Deethylatrazine</td>
<td>13.57B</td>
<td>± 4.57</td>
</tr>
<tr>
<td>Deisopropylatrazine</td>
<td>13.85B</td>
<td>± 2.53</td>
</tr>
<tr>
<td>Didealkylatrazine</td>
<td>20.03B</td>
<td>± 4.99</td>
</tr>
<tr>
<td>Cyanuric Acid</td>
<td>8.84B</td>
<td>± 4.06</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>% of extractable radioactivity</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 8 Plant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrazine</td>
<td>16.47A</td>
<td>± 1.73</td>
</tr>
<tr>
<td>Deethylatrazine</td>
<td>8.03B</td>
<td>± 1.12</td>
</tr>
<tr>
<td>Didealkylatrazine</td>
<td>52.77C</td>
<td>± 1.91</td>
</tr>
<tr>
<td>Cyanuric Acid</td>
<td>22.72D</td>
<td>± 0.87</td>
</tr>
<tr>
<td>Deisopropylatrazine</td>
<td>0.00E</td>
<td>±  -</td>
</tr>
</tbody>
</table>

1 Letters indicate significance within each day.
2 Standard Error (SE).
REFERENCES


CHAPTER 3. STUDY ON THE PHYTOREMEDIATION CAPABILITIES OF SWITCHGRASS USING $^{14}$CATRAZINE

Vurtice C. Albright III and Joel R. Coats

A paper to be submitted to Environmental Toxicology and Chemistry

ABSTRACT

Atrazine, a broad-leaf herbicide, has been widely used to control weeds in corn and other crops for several decades. This has led to widespread contamination of soils and water bodies. Many remediation strategies have been employed to lessen the impact of atrazine in the environment. Phytoremediation is one strategy that has been studied to remove atrazine contamination. This study utilized $^{14}$C-atrazine to study uptake and degradation of atrazine in soil columns with well-established switchgrass (Panicum virgatum) stands. Atrazine and two metabolites were detected as soon as one day following treatment. The percentage of atrazine was observed to decrease over the course of the study while the percentages of the metabolites present increased. The presence of the metabolite cyanuric acid in switchgrass leaf biomass is reported here for the first time.

INTRODUCTION

Control of broadleaf weeds in corn, sorghum, and sugarcane crops can be achieved through a reversible inhibition of photosystem II by the triazine herbicide atrazine [1-3]. With 51 million pounds of atrazine applied across 18 states in 2010 and 6.8 million pounds applied in Iowa alone, it is one of the most widely used herbicides in the agricultural industry (http://quickstats.nass.usda.gov/). Widespread usage has resulted in contamination of both ground water and surface water sources by atrazine and its metabolites. It has been estimated
that as many as 2,700 community water source wells and 214,000 private wells are contaminated with atrazine, though those are conservative estimates and the actual numbers may be closer to 1,570 community wells and 70,800 private wells [4]. In 1998, atrazine was found to be present in 100% of 129 samples from 75 Midwestern rivers and streams [5].

When atrazine enters bodies of water, it can have many detrimental effects on the ecosystem. Atrazine has been shown to inhibit photosynthesis in algae, phytoplankton, and macrophytes [6]. *Xenopus laevis* tadpoles exhibit decreases in fat body size and liver weights, while decreased spermatogenesis and fertility are observed in male *X. laevis* [7-8]. Another detrimental effect observed is reduced success of egg hatching in embryos of the spotted salamander, *Ambystoma maculatum* [9]. Breast, brain, ovarian, stomach, and testicular cancer are a few of the many human cancers that have been linked to atrazine [10-11].

Agricultural fields are a major source of atrazine contamination. As much as 5% of the applied atrazine may be lost from a field through surface water runoff, although this can be greatly affected by the occurrence of a storm event following atrazine application [12]. Many strategies have been employed in an attempt to mitigate the concentration of atrazine reaching ground and surface water bodies. Phytoremediation, a well-researched method, uses plants to degrade, sequester, or otherwise neutralize organic and inorganic contaminants in soil and water.

Native prairie grasses are commonly used in phytoremediation strategies. Their extensive fibrous root system can penetrate up to ten feet below the surface and can result in a greater surface area than other vegetation [13]. Mixtures of native prairie grasses have been
used in previous research in our lab as phytoremediation strategies. A mixture of three
different prairie grasses was shown by Belden and Coats [14] to reduce atrazine in leachate
by 43%; degradation was also observed to occur more quickly in vegetated soils than
unvegetated soils. Henderson et al. [2] determined that atrazine and its metabolites are
equally distributed between the leaf and root tissues of prairie grasses following uptake and
degradation.

Recent research indicated that atrazine was taken up, degraded, and completely
absent from mature switchgrass stands in less than 21 days (Murphy, I.J. 2009. M.S. thesis.
Iowa State University, Ames, IA, USA). In that research, atrazine was detected in above
ground switchgrass biomass one day following application (Murphy, I.J. 2009. M.S. thesis.
Iowa State University, Ames, IA, USA). The atrazine metabolites, deethylatrazine
deisopropylatrazine, and didealkylatrazine were not detected until four days following
application, at the earliest, and only intermittent peaks were observed (Murphy, I.J. 2009.
M.S. thesis. Iowa State University, Ames, IA, USA).

The purpose of this research is to expand upon that study by using radiolabeled
\[^{14}\text{C}]\)atrazine in simulated surface water runoff to track atrazine as it is degraded into a
variety of metabolites with more accuracy than was previously possible in an attempt to
observe trends in the fate of atrazine instead of only observing intermittent peaks of
metabolites. Additionally, this study will look for the presence of hydroxyatrazine which was
not detected in the above study, but has been detected in switchgrass residues in other studies
[2,15].
METHODS AND MATERIALS

Experimental setup

Five previously constructed columns were utilized in this experiment. These columns were constructed in 2006 by collecting soil from an agricultural field in Clarke County, Iowa, and amending it with potting soil. The pH and organic matter of the amended soil were determined to be 7.20 and 9.6%, respectively and soil composition was determined to be: sand 39.90%, silt 37.19%, clay 22.91%. The soil was then placed in a polyvinyl chloride pipe (76 cm x 20 cm) in a vertical position in a greenhouse. The columns were then planted with switchgrass seed (Cave-in-rock variety) with approximately 10 plants per cm² soil surface initially; currently there are 20 to 30 switchgrass stems per column. Plants were grown in a greenhouse with conditions varied to simulate the seasons: 16 h light: 8 h dark schedule at 27°C/day and 22°C/night in the summer growing season and 12 h light: 12 h dark at 4.5°C day and night during the winter senescence period. Prior to application of [14C]atrazine, switchgrass plants were grown for a period of five years.

Treatment and sample collection

On Day 0, columns were treated with 200 ml of 4 µg/ml (parts per million) spiked with approximately 18 million disintegrations per minute (dpm) of [14C]atrazine to each of 4 columns (specific activity = 21,806 dpm/1 µg atrazine). One column was treated with only 200 ml of distilled water to act as a negative control. On Days 1, 3, 5, and 7 following treatment with atrazine, two above-ground biomass samples per column were collected for extraction by cutting the stem off at the soil level. Columns were watered with 200 ml each of distilled water on Days 5 and 7.
Extraction of switchgrass material

Switchgrass biomass samples were weighed and then cut into pieces that were approximately one-half inch in length. The sample was then homogenized with a mortar and pestle using 30 mL of ethyl acetate. The solvent was decanted off through a filter containing 15 g of anhydrous granular sodium sulfate to absorb any water contained in the sample. This procedure was repeated for a total of three times.

Concentration of switchgrass samples

All solvent extractions were placed in an N-evaporator. Samples were dried down with nitrogen gas to approximately 1 mL. The extracts were quantitatively transferred 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 vial to a total of approximately 10 mL. The vials were then blown down with a N-evaporator and reconcentrated with methanol to 1 mL and placed in an HPLC vial.

Analysis of samples

A 100 µl sample of both plant and sand extracts were analyzed using high-performance liquid chromatography (HPLC). A Hewlett-Packard 1100 series HPLC equipped with an autosampler was used to separate the metabolites and parent compound, and the corresponding fractions were collected (Table 1). An Atlantis dC18 5µm, 4.6x250mm column (Waters Corporation, Milford, MA, USA) was used with a mobile phase as described in Table 1. Flow rate was 1 ml/min; column temperature was maintained at 30°C. The fractions were then analyzed for radioactivity on a Tri-Carb 2900TR Liquid
Scintillation Analyzer (Packard BioScience Company, Meriden, CT, USA). Non-radioactive analytical standards were used to determine retention times of the compounds on the HPLC and the beta-ram. Non-radioactive atrazine, deethylatrazine, deisopropylatrazine, and didealkylatrazine standards were obtained from CIBA-GEIGY (Syngenta), Greensboro, North Carolina, USA. Non-radioactive hydroxyatrazine was obtained from Sigma-Aldrich (St. Louis, MO, USA), and non-radioactive cyanuric acid was obtained from Fluka (St. Louis, MO, USA).

Statistical analysis

An analysis of variance (p < 0.05) was used to test the null hypothesis that switchgrass does not take up and degrade atrazine. Statistical calculations were performed using SAS (SAS Institute Inc., Cary, NC, USA).

RESULTS

One day after treatment, atrazine accounted for approximately 42% of the radioactivity recovered in the plants (Figure 1). This was significantly more than either deethylatrazine (DEA) or deisopropylatrazine (DIA), which accounted from 28% and 20% of the radioactivity recovered, respectively. No other metabolites were observed in significant amounts. By Day 3, four metabolites were observed to be above background; DIA (36% of recovered radioactivity), DEA (25%), cyanuric acid (8%), and didealkylatrazine (DDA) (7%). Atrazine still accounted for 20% of the recovered radioactivity. Significant differences existed between all chemicals and the background, except for between cyanuric acid and DDA. By Day 7, DIA accounted from 43% of the recovered radioactivity, more than double the next most prevalent metabolite, DEA (20%). Cyanuric acid (13%), DDA (13%), and
atrazine (8%) were also detected in significant amounts. Hydroxyatrazine was not observed at any of the time points over the course of the study.

Over the duration of the study, the percentage of atrazine radioactivity decreased steadily; the only significant decrease was observed between Day 1 and Day 3 (Figure 2). DIA percentage increased significantly between Day 1 and Day 3 (Figure 3). Significant increases were observed for DDA between Day 1 and Day 5 (Figure 4) and for cyanuric acid at Day 3 and Day 7 (Figure 5). No significant increases were observed for DEA (Figure 6). No radioactivity was observed in any of the negative controls. Recovery efficiency for this extraction method was determined to be 97.5%.

DISCUSSION

The purpose of this experiment was to allow for more sensitive analysis of atrazine and its metabolites in leaf biomass of switchgrass. Previously, the earliest that atrazine had been detected in switchgrass leaf biomass was two days after treatment (Murphy, I.J. 2009. Master’s thesis. Iowa State University, Ames, IA, USA). The first metabolites were not detected until three days after treatment [16]. In contrast to what was reported earlier, in this study, atrazine and two metabolites were detected only one day after treatment (Figure 1). Detection of the metabolites also differed. In Murphy and Coats [16], DEA and DDA were detected three days after treatment and DIA was not detected until seven days after treatment. In the other study, DEA, DDA, and DIA were first detected four, seven, and 14 days after treatment, respectively (Murphy, I.J. 2009. Master’s thesis. Iowa State University, Ames, IA, USA). Both of these studies were in contrast with the current study where DEA and DIA were detected one day after treatment, while DDA was first detected three days after
treatment (Figure 1). Thus, the use of radiolabeled atrazine allowed for more sensitive
detection and quantification of atrazine and its metabolites in switchgrass leaf biomass.

Another objective of this study was to track the presence of hydroxyatrazine in
switchgrass leaf biomass over the course of the study, however, no significant amounts of
hydroxyatrazine were detected at any time point in the study (Figure 1). This is in contrast to
earlier reports which showed that hydroxyatrazine was detected in switchgrass [2,15]. In
Henderson et al. [2] the authors detected hydroxyatrazine in leaf biomass; however, they
used a mixture of switchgrass, yellow indiangrass, and big bluestem and extracted all the
grass tissue together. Thus, the presence of hydroxyatrazine in that study cannot be
definitively attributed to degradation by switchgrass. Lin et al. [15] detected the presence of
hydroxyatrazine in switchgrass leaf biomass; however, they could not determine if the
presence of hydroxyatrazine was due to degradation by switchgrass or uptake from the soil.
The latter may be the more likely the case, as no hydroxyatrazine was detected in this study.
Therefore, one probable explanation is that different microbes present in the two soils
account for the degradation of atrazine to hydroxyatrazine, which was observed in Lin et al.
[15] and not the current study. Another possible explanation is that chemical transformation
of atrazine to hydroxyatrazine occurred in the Lin et al. soil. The hydroxyatrazine could have
then been taken up into the plant following the transformation. The soil used in the current
study may not have transformed atrazine to hydroxyatrazine. Lastly, the extraction method
used in this study may have not been optimal for the recovery of hydroxyatrazine from
switchgrass biomass.
The current study also reports the presence of cyanuric acid in switchgrass tissues. To our knowledge, this is the first time this compound has been reported to be detectable in switchgrass tissues. This compound was previously undetectable by using gas chromatography methods. The use of radiolabeled $^{14}$C-atrazine allowed cyanuric acid to be detected, as the ring structure, which contains the radiolabel, was still intact through much of the degradation process. Cyanuric acid made up 9% of the extractable radioactivity in the Day 4 samples respectively. This increased in the Day 8 samples where cyanuric acid accounted for 23% of the extractable radioactivity. It is interesting to observe the presence of cyanuric acid while not observing the presence of hydroxyatrazine, since both metabolites are de-chlorinated and hydroxylated (Figure 7). However, it is possible that cyanuric acid is formed by a de-chlorination of DDA. One potential explanation for this is that the enzymes present in the plant that catalyze the de-chlorination reaction may be unable to hydroxylate atrazine due to the presence of the ethyl and isopropyl groups and thus, the de-chlorination reaction could only occur after the removal of these groups.

The total percentage of radiotracer recovered (i.e. a mass balance) could not be determined as there were 20-25 switchgrass stems per column, resulting in several unused plants. Likewise, root material was not extracted, so no information on the amount of atrazine retained in the root tissues can be determined. Also, it cannot be determined from this study if the presence of metabolites in the plant were the result of plant degradation or degradation in the soil followed by uptake. Prior research, however, supports the former. Murphy and Coats [16] studied uptake and degradation of atrazine using switchgrass planted in autoclaved soil. The authors showed that in a microbe-free setting, switchgrass could take
up and degrade atrazine into metabolites DEA, DIA, DDA. This suggests that at least a portion of the metabolites present are a result of uptake and degradation of atrazine by switchgrass; however in the current study, it cannot be determined what proportion that might be.

The metabolites of atrazine are generally considered to be less toxic than the parent compound, but their formation and detection is of great significance. Several acute toxicity studies have shown that the metabolites DEA and DIA are significantly less toxic than atrazine to algae, amphipods, bacteria, and plants [17-19]. However, in the past, these chlorinated metabolites have still been included in regulatory limits for atrazine in drinking water. Hydroxyatrazine and cyanuric acid, two hydroxylated metabolites, do not contain the chlorine and, thus, they may be even less toxic. One study showed that hydroxyatrazine is not toxic to green algae and cyanobacteria compared to atrazine, which can inhibit their growth and photosynthesis [20]. Thus, degradation of atrazine to its metabolites results in reduced risks to organisms in aquatic ecosystems.

The average total concentration of atrazine and metabolites (as atrazine equivalents) present in the mature switchgrass plant was 27.0, 73.3, 76.0, and 79.6 ng/g on Days 1, 3, 5, 7, respectively (Table 2). All of these concentrations are well below the EPA’s established tolerance of 4 µg/g for range grasses (http://www.epa.gov/oppsrrd1/REDs/atrazine_ired.pdf). Thus, the presence of atrazine or its metabolites in switchgrass used along and in corn fields should be of no consequence to animals grazing on switchgrass or switchgrass residues.
ACKNOWLEDGEMENT

Funding for this project was provided by the Iowa State University Toxicology Graduate Program and Iowa Agriculture and Home Economics Experiment Station, Projects 5075 and 5088.
Figure 1. Recovery percentages of atrazine and its metabolites from switchgrass above ground biomass over the course of the study.

Letters indicate significance between chemicals within days $\alpha = 0.05$
Letter 1 indicates significance between days \( \alpha = 0.05 \)

Figure 2. Recovery percentage of atrazine over time from switchgrass.
Letters indicate significance between days $\alpha = 0.05$

Figure 3. Recovery percentage of deisopropylatrazine over time from switchgrass.
Letters indicate significance between days $\alpha = 0.05$

Figure 4. Recovery percentage of didealkylatrazine over time from switchgrass.
Letters indicate significance between days $\alpha = 0.05$

Figure 5. Recovery percentage of cyanuric acid over time from switchgrass.
Letters indicate significance between days $\alpha = 0.05$

Figure 6. Recovery percentage of deethylatrazine over time from switchgrass.
Figure 7. Atrazine pathway of degradation. Atrazine parent compound is highlighted.
Table 1. High performance liquid chromatography (HPLC) conditions for separation and determination of atrazine and metabolites.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Retention Time</th>
<th>Column Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td></td>
<td>Isocratic 75:25 water:acetonitrile (0-3 min)</td>
</tr>
<tr>
<td>Cyanuric Acid</td>
<td>6.6</td>
<td>Linear Gradient from 75:25 water:acetonitrile to 25:75 water:acetonitrile (3-11 min)</td>
</tr>
<tr>
<td>Didealkylatrazine</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>Deisopropylatrazine</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>Deethylatrazine</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>Hydroxyatrazine</td>
<td>11.5</td>
<td>Linear Gradient from 25:75 water:acetonitrile to 75:25 water:acetonitrile (11-16 min)</td>
</tr>
<tr>
<td>Atrazine</td>
<td>12.6</td>
<td>75:25 water:acetonitrile (11-16 min)</td>
</tr>
<tr>
<td>Blank</td>
<td></td>
<td>Isocratic 75:25 water:acetonitrile (16-20 min)</td>
</tr>
</tbody>
</table>
Table 2. Concentration of atrazine and metabolites in switchgrass over the course of the study.

<table>
<thead>
<tr>
<th></th>
<th>Concentration (ng/g) in plant biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
</tr>
<tr>
<td>Atrazine</td>
<td>11.6</td>
</tr>
<tr>
<td>HYA</td>
<td>0.2</td>
</tr>
<tr>
<td>DEA</td>
<td>7.8</td>
</tr>
<tr>
<td>DIA</td>
<td>5.6</td>
</tr>
<tr>
<td>DDA</td>
<td>0.7</td>
</tr>
<tr>
<td>Cyanuric Acid</td>
<td>0.042</td>
</tr>
<tr>
<td>Total</td>
<td>27.0</td>
</tr>
</tbody>
</table>

ng/g atrazine equivalents based on wet weight of plant biomass
REFERENCES


CHAPTER 4. GENERAL CONCLUSIONS

The purpose of this research was to further the knowledge of atrazine biodegradation by switchgrass. Research was undertaken to investigate two objectives. The first objective was to determine if switchgrass could exude metabolites of atrazine after degradation. The second objective was to study the degradation of atrazine in mature switchgrass leaf biomass in further detail using radiolabeled atrazine.

The exudation of atrazine metabolites after degradation was investigated in Chapter 2. Switchgrass was shown to take up and degrade atrazine, however, no metabolites or parent compound were observed in the sand samples after transplantation. This suggests that switchgrass does not exude metabolites after degradation. Instead, the metabolites and any remaining parent compound are retained within the plant biomass, which prevents them from entering a body of water. The presence of cyanuric acid, a previously undetected metabolite of atrazine, was also observed.

In Chapter 3, $[^{14}\text{C}]$atrazine was utilized to study the degradation of atrazine in leaf biomass of switchgrass. Specifically, the presence of metabolites not detected in prior research was investigated. Atrazine and two metabolites were detected in switchgrass leaf biomass as soon as one day after treatment. The percentage of atrazine radioactivity recovered declined over the course of the study, while the percentages for DIA, DDA, and cyanuric acid increased. Concentrations of DEA did not change significantly over time. Hydroxyatrazine, detected in a few prior studies, was not detected at all. Cyanuric acid presence was reported for what is believed to be the first time.

The findings reported in the previous two chapters, along with the multitude of prior research, identify switchgrass as a good candidate for field trials. In these trials, switchgrass,
alone or in combination with other native prairie grasses, would be planted in buffer strips between agricultural fields and waterways and around existing tile-line intakes. Inflow and outflow water samples, soil samples, and downstream water samples would be taken to determine the effect switchgrass would have on reducing the amount of atrazine and metabolites reaching the surface water bodies. The expectation is that by integrating this strategy into current buffer strips, the overall amount of atrazine in surface waters across the Midwest can be reduced.
ACKNOWLEDGEMENT

I would like to thank Keri Carstens and Dingfei Hu for their technical assistance on high performance liquid chromatography. I would like to thank my fellow ENT-TOX laboratory members for their help and support. I also would like to thank my major professor, Joel Coats, for his support and guidance throughout my M.S. course of study. Finally, I would like to thank my family for their patience, support, and understanding.