ABSTRACT

MCKNIGHT, ALAYNE MARIE. Atrazine, Azoxystrobin and Imidacloprid Phytoremediation by Terrestrial and Wetland Plant Species. (Under the direction of Drs. Travis W. Gannon and Fred H. Yelverton).

Evaluating the pesticide phytoremediation capacity of plant species that are commonly used in landscaping equips land managers and producers with viable options to managing potential pesticide off-target movement. To build this list of phytoremediating plants, greenhouse research was conducted to characterize the ability of terrestrial plants to phytoremediate atrazine, azoxystrobin and imidacloprid; as well as the ability of wetland plants to phytoremediate azoxystrobin and imidacloprid.

Pesticides are useful to agricultural and urban land managers to control weeds, disease and insect damage to valuable crops and recreational areas such as athletic fields and golf courses. However, the same physicochemical component that makes pesticides mobile allows these chemicals to move off-target which could result in surface and/or groundwater contamination. This research aids in the understanding and utilization of terrestrial plants often established in landscaping as phytoremediators for commonly used pesticides: atrazine, azoxystrobin and imidacloprid. Three plant species blueflag iris (*Iris versicolor)*, broomsedge (*Andropogon virginicus)* and switchgrass (*Panicum virgatum)* were treated with one of the three pesticides and evaluated at 0, 28, 56 and 112 days after treatment (DAT) by analyzing pesticide residue from soil, above and below-ground vegetation using high-performance liquid chromatography (HPLC). *I. versicolor* was found to reduce greater atrazine in soil at 112 DAT by 58.7% when compared to non-planted containers. *I. versicolor* was also the most capable of reducing azoxystrobin by 86.9% from the soil at 112 DAT. At the same sampling time, *I.*

versicolor and *P. virgatum* reduced greater imidacloprid from soil by 62.5% and 64.3%, respectively.

If pesticides have reached a surface water source, aquatic plants have the ability to absorb and degrade the pesticide. Therefore, three wetland plant species phytoremediation capacity of azoxystrobin and imidacloprid from an aquatic system were evaluated. Softrush (*Juncus effuses)*, pickerelweed (*Pontederia cordata)* and arrowhead (*Sagittaria latifolia)* planted pots were treated with one of the two pesticides and evaluated at 0, 14, 28 and 56 DAT by analyzing water, soil, above and below ground vegetation using HPLC methodology. At 56 DAT, *P. cordata* reduced greater azoxystrobin from water (51.7% reduction) compared to *J. effuses* (24.9%) and *S. latifolia* (28.7%) compared to non-planted containers. *S. latifolia* reduced greater imidacloprid (79.3%) compared to *J. effuses* (36.0%) and *P. cordata* (37.1%) at 56 DAT. The evaluated landscaping plant species were reported to be promising atrazine, azoxystrobin and imidacloprid phytoremediators at differing capacities. When implemented in vegetative filter strips, diversity and abundance of plant species is recommended.

Atrazine, Azoxystrobin and Imidacloprid Phytoremediation by Terrestrial and Wetland Plant Species

by
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DEDICATION

This research is dedicated to my fiancé, family, friends and pets, Koda and Karma.

The betterment for tomorrow is continuous and hopeful.

BIOGRAPHY

Alayne M. McKnight was born and spent the first 18 years of her life in Concord, North Carolina, where her parents still reside. Adolescent years were joyfully lived on the athletic field or immersed in biology books. After attending Sea Turtle Camp sophomore year in high school, she found a passion for marine and environmental science with a special interest in saving the sea turtles. The desire to improve the health of the environment led her to pursue a B.S. degree in Biology with a concentration in Ecology, Evolution and Conservation at North Carolina State University, which was completed in 2017. While conquering biology and chemistry courses, Alayne enjoyed taking agroecology and agronomic classes as electives. She began working in Dr. Travis Gannon's research lab in 2014 as an assistant in projects focusing on pesticide environmental fate and behavior in turfgrass systems. After observing the impact of pesticides on agronomics and the environment, Alayne found the importance of balance in the needs of society and longevity of ecosystems. She decided to continue working in this field and began working towards Master of Science under the direction of Drs. Travis Gannon and Fred Yelverton at North Carolina State University in the Department of Crop and Soil Sciences, researching pesticide phytoremediation by terrestrial and wetland plant species. Alayne placed 1st in WSSA 2018 annual conference poster competition and 3rd in WSSA 2019 annual conference poster competition. Upon graduation, she hopes to find a career in pesticide ecological exposure research.

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A REVIEW OF THE LITERATURE

Pesticide Phytoremediation

Even with careful consideration of pesticidal chemical properties and the utilization of best management practices, scenarios unfold where pesticides move off-target via drift, runoff, or other means. (Nett et al. 2008). As pesticides move with water collection, concentrations tend to accumulate in sinks, also known as surface and ground water bodies. In a study conducted by the United States Geological Survey (2006), more than 90% of tested surface and ground water sites contained at least one pesticide or metabolite. Within these detections, 83% were reported to exceed the aquatic life benchmark for invertebrates (Gilliom et al. 2006). Pesticide exceedances of an aquatic life benchmark were found to be 25% greater in urban areas compared to agricultural, undeveloped or mixed-use sites (Gilliom et al. 2006).

A cost efficient and low maintenance method of preventing water contamination from pesticide runoff is by establishing a vegetative filter strip which is a physical barrier of plants between a pesticide treated area and the non-treated areas such as sensitive water bodies (Rai 2008). As pesticide contaminated water moves off-target via surface and subsurface flow, roots and stems of the established plants slow the water velocity and allow soil infiltration where water will meet the root system and can be absorbed by the plants. Through phytoremediation, the plant will absorb the pesticide and degrade it into simpler, less active compounds (Pilon-Smits 2005). Phytoremediation protects sensitive ecological areas through multiple processes such as rhizostabilization, rhizodegradation, phytotransformation/phytodegradation, phytoextraction and phytovolatilization (Hussain et al. 2009).

Many existing phytoremediation studies focus on the clean-up of contaminated metal sites with sunflowers used to remediate old army ammunition factories or coal ash spills (Doty et al. 2017; Vymazal 2014). Metal remediation typically revolves around the phytoextraction process, or the ability of the plant to uptake the chemical and stabilize it within plant tissue (Doty et al. 2017). In these cases, plant tissue must then be harvested and removed from the site. The purpose of pesticide phytoremediation is to establish a sustainable and continuously remediating system (Febriani 2018). To reach this goal, plants must be able to absorb the chemical and degrade it into simpler compounds before the tissue dies and returns to the ecological system.

Evaluated Pesticides

Synthetic pesticides are an integral part of agronomic production, as well as creating a safe and functional area in recreational landscapes (Aktar et al. 2009). The purpose of this research is to quantify the phytoremediation capacity of commonly used pesticides that have been found widely in surface and/or ground water and exhibit toxic characteristics which may be harmful to organisms.

Atrazine (1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) is a pre- and post-emergent triazine herbicide used to manage broadleaf and grassy weeds in crops such as maize, sorghum, soybeans, wheat, oats and sugarcane; and on turf, such as residential lawns, athletic fields and golf courses (USEPA 2016). Over 90% of atrazine is applied to corn; however, upwards of 65% of sorghum and sugarcane acres are also treated with atrazine (USEPA 2016). Atrazine is the most commonly detected pesticide in tested groundwater sites making it a pesticide of interest for phytoremediation research (Gilliom et al. 2006). Atrazine binds with a protein complex of photosystem II in chloroplast photosynthetic membranes which inhibits the transfer of electrons and in turn, the formation and release of oxygen (USEPA 2016). Atrazine is considered mobile and persistent in the environment with a low water solubility (K_S = 33 mg L^{-1}), moderate octanol-water partitioning coefficient (log K_{OW} = 2.6), and moderate soil half-life (soil $T_{1/2}$ = 13 - 261 days); however, it is very stable in water (groundwater $T_{1/2}$ = >200 days) (Schiavon 1988; USEPA 2016). The main degradation pathways for atrazine is through microbial degradation under aerobic conditions (USEPA 2016). The major degradates of ecological concern are deethylatrazine (DEA), deisopropylatrazine (DIA), diadealkylatrazine (DACT) and hydroxyatrazine (HA) (USEPA 2016). Atrazine is moderately toxic to freshwater and marine fish, highly toxic to freshwater aquatic invertebrates and very highly toxic to estuarine/marine aquatic invertebrates (USEPA 2006). Due to reproductive effects, freshwater fish have the most sensitive chronic endpoint (NOAEC = 5 µg a.i. L^{-1} water, LOAEC = 50 µg a.i. L^{-1} water) (USEPA 2006). The U.S. Environmental Protection Agency (2006) indicated a chronic aquatic life benchmark of 60 µg L^{-1} atrazine of freshwater for invertebrates, 5 µg L^{-1} for fish species and less than 1 µg L^{-1} for non-vascular plant species (USEPA 2006).

Azoxystrobin (methyl(E)-2-{2[6-(2-cyanophenoxy)pyrmidin-4-yloxy]phenyl}-3-methoxyacrylate) is a systemic strobilurin fungicide used to manage diseases commonly found in turfgrasses such as brown patch (*Rhizoctonia solani*) and gray leaf spot (*Pyriculuria grisea*) (USEPA 1997). Azoxystrobin is the largest gross selling fungicide, exceeding $1 billion annually (Lamberth 2016) and is registered for use on athletic fields, commercial/residential lawns, golf courses and parks. Azoxystrobin is also the most commonly detected fungicide in US surface waters making it of interest to phytoremediation studies (Gregoire et al. 2010). With a low water solubility (K_S = 6 mg L^{-1}), a moderate soil-organic carbon sorption coefficient (K_{OC} = 210 to 580 mL g^{-1}), and moderate soil half-life ($T_{1/2}$ = 72-164 days), azoxystrobin is very unlikely to move off-target (USEPA 1997). However, due to high use rates and the mobility of degradates, such as azoxystrobin acid, azoxystrobin has become a potential threat to water ecosystems near treated areas (Ghosh and Singh 2009). Azoxystrobin's biochemical mode of action is inhibition of electron transport in fungi (USEPA 1997). While azoxystrobin has low toxicity to mammals, birds, insects, and earthworms, it is very toxic to aquatic invertebrates with an established chronic benchmark of 44 µg per L of freshwater (USEPA 2006). Azoxystrobin is also highly toxic to freshwater fish [(*Oncorhynchus mykiss*) $LC_{50} = 0.26$ mg L^{-1}] and aquatic flora [(*Lemna minor*) $EC_{50} = 3.4$ mg L^{-1} and green algae (*Chlorophyta* spp.) $EC_{50} = 0.4$ mg L^{-1}] (Rodrigues et al. 2013; USEPA 1997; USEPA 2016).

Imidacloprid [N-[1-[(6-chloropyridin-3-yl)methyl]-4,5-dihydroimidazol-2-yl]nitramide] is a systemic neonicotinoid insecticide in the chloronicotinyl nitroguanidine chemical family applied to urban structures, crops, soil and as a seed treatment (Wismer and Plumlee 2004). Imidacloprid is used to manage sucking insects, some chewing insects such as termites, soil insects and fleas on pets (Tomlin 2006). Of 48 tested US surface water sites, 37% contained detectable imidacloprid which has been found to be the most detected neonicotinoid in US streams (Hladik et al. 2016). The majority of imidacloprid detections in surface waters were found to be in urban locations suggesting the application in lawns, gardens, parks and public spaces results in increased watershed contamination (Johnson and Pettis 2014). Imidacloprid has a moderate to high water solubility ($K_S = 514$ mg L^{-1}), moderate octanol-water partitioning coefficient (log $K_{ow} = 3.7$) and has a soil half-life of 26.4 to 229 days (US EPA 2016). This insecticide is effective by contact or ingestion and acts on several types of post-synaptic nicotinic acetylcholine receptors in the nervous system (Tomlin 2006). Following binding to the nicotinic receptor, nerve impulses are spontaneously discharged and then the neuron begins to fail to signal. The blood-brain barrier in vertebrates blocks access of imidacloprid to the central nervous system, reducing its toxicity (Sheets 2001). Imidacloprid is extremely toxic to invertebrates, with a very low chronic benchmark of 0.01 µg per L of freshwater. The US EPA

(2016) has indicated a much higher chronic benchmark for fish species at 9,000 μg L^{-1}; however, recent studies have found imidacloprid has resulted in sublethal effects including stunted growth, lesions, and deformities in the fish adolescents (Vignet et al. 2019). More specifically, reported imidacloprid may alter behaviors and neurological pathways in tadpoles (*Limnodynastes tasmaniensis*) (Rios et al. 2017).

Plant Selection

The ideal phytoremediating plant species would be native to the establishing area, contain large biomass and a fibrous root zone, require little to no maintenance, and tolerate the applied herbicide. Another key component for selecting the ideal phytoremediating plant species for these urban area vegetative filter strips and constructed wetlands is the aesthetic component to enhance landscape beautification and will be likely to be used by land managers, landscapers, and producers.

Previous research efforts have observed many different types of plant species phytoremediating multiple pesticides. Smith et al. (2008) reported *Iris versicolor*, *Tripsacum* dactyloides and *Andropogon gerardii* were best suited to remove chlorpyrifos (47 to 76% removal), chlorothalonil (91 to 95%), pendimethalin (17 to 48%) and propiconazole (22 to 33%) from soil over 7 months. Rice et al. (1997) reported *Ceratophyllum demersum*, *Elodea canadensis* and *L. minor* all reduced metolachlor and atrazine residues in water (38 to 60% less residue than non-planted containers). However, *C. demersum* and *E. canadensis* reduced residue in water (1 to 4% of applied remaining at 16 DAT) more than *L. minor* (23%). In contrast, *L. minor* was the best suited plant species (compared to *E. canadensis* and *Cabomba aquatica*) for removing copper sulfate, flazasulfuron and dimethomorph from water (Olette et al. 2008).

Wang et al. (2012) evaluated the phytoremediation capacity of sweet flag (*Acorus calamus*), yellow flag (*Iris pseudacorus*), and purple loosestrife (*Lythrum salicaria*) of atrazine in an aqueous environment and reported these species were able to reduce atrazine by 93.3, 97.2, and 94.4%, respectively, 20 DAT compared to the initial 4 mg L^{-1} dose. Qu (2017) studied atrazine contaminated lake sediment and phytoremediation by *Potamogeton crispus* and *Myriophyllum spicatum*. *P. crispus* reduced atrazine by 75.65% and *M. spicatum* reduced 76.15% compared to only 46.3% reduction in non-planted containers.

Plantago major, *Glycine max,* and *Helianthus annus* were reported by Romeh (2015) to efficiently reduce the amount of azoxystrobin from soil. After 14 days of azoxystrobin exposure, *P. major*, *G. max* and *H. annus* reduced 91.6%, 91.6% and 91.2%, respectively, compared to non-planted containers. Lv et al. (2016) reported fungicide phytoremediation efforts by *Juncus effuses*, *Typha latifolia*, *Phragmites australis*, and *Iris pseudacorus* in a hydroponic system. After 24 days, *J. effuses*, *T. latifolia*, *P. australis*, and *I. pseudacorus* reduced the amount of imazalil by 67%, 53%, 96% and 46%, respectively. Romeh (2014) conducted research on the phytoremediation capacity of imidacloprid by *P. major* after 10 days, which reported a 95.2% reduction from soil compared to 61.9% reduction in non-planted. Romeh (2017) also reported 2.51 µg g^{-1} in *P. major* above-ground biomass and 7.94 ug g^{-1} in below-ground vegetation after 10 days.

While many plant species in phytoremediation studies exhibit weed-like behaviors, it is imperative to select phytoremediating plant species that are not invasive or competitive with existing ecosystems to ensure a sustainable system. After a thorough literature review, as well as consulting local nurseries on low cost, easily established, low management, and aesthetically pleasing plant species, the chosen terrestrial plant species were: blueflag iris (*Iris versicolor*), broomsedge (*Andropogon virginicus)* and switchgrass (*Panicum virgatum*); and chosen wetland plant species were: softrush (*Juncus effuses*), pickerelweed (*Pontederia cordata*) and arrowhead (*Sagittaria latifolia*).

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Chapter 1: Evaluation of Atrazine, Azoxystrobin, and Imidacloprid Phytoremediation Capacity of Terrestrial Plant Species *I. versicolor*, *A. virginicus*, and *P. virgatum*

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Pesticides can move off-target due to many factors including improper application or unpredictable rainfall events, resulting in contamination of sensitive water bodies and causing adverse effects on inhabiting species. Through best management practices, such as the implementation of vegetative buffer strips, the off-target movement of pesticides can be decreased, and compound degradation can be increased via phytoremediation. In this study, blueflag iris (*Iris versicolor*), broomsedge (*Andropogon virginicus*) and switchgrass (*Panicum virgatum*) were planted in soil treated with one of the three commonly used pesticides, namely atrazine (herbicide), azoxystrobin (fungicide) or imidacloprid (insecticide). Non-planted pots and non-treated plants were included as controls. At 28, 56 and 112 days after treatment (DAT), plants were destructively harvested and analyzed for pesticide residue in the soil and in above-ground and below-ground vegetation using high-performance liquid chromatography (HPLC). Relative to the amount of pesticide found in planted pots compared to non-planted pots, *I. versicolor* was found to reduce greater atrazine in soil at 112 DAT by 58.7%. *I. versicolor* was also the most capable of reducing azoxystrobin, by 86.9%, from the soil at 112 DAT. At the same sampling time, *I. versicolor* and P. virgatum reduced greater imidacloprid from soil by

62.5% and 64.3%, respectively. This information supports the recommendation for establishment of diverse plant species for optimization of phytoremediation capacities.

Introduction

Synthetic pesticides are utilized to control potential interference, competition, and/or reduced aesthetics from weed plant species, disease, and insect damage in agronomic production. Pesticides are also used to maintain positive aesthetics and safety in urban turf areas. Since the environmental movement of the 1970s, regulation of pesticides have increased and research continues to engineer pesticides to meet higher efficiency standards with less toxic ingredients applied (Davis 2014). Recently innovated pesticides also encompass environmentally conscious characteristics such as decreasing off-target movement and bioaccumulation. However, even with favorable chemical properties, select pesticides can move off-target and potentially cause adverse effects in terrestrial, surface- and groundwater ecosystems (Arias-Estevez et al. 2007). Frequent pesticide use in agricultural, urban, and mixed land has led to at least one pesticide being detected in 90% of tested surface- and groundwater sites, according to the U.S. Geological Survey (USGS) in 2009 (Gilliom et al. 2006). Of the tested sites, 57% of agricultural and 83% of urban sites had detected a pesticide concentration that exceeded at least one aquatic life benchmark or the toxic concentration to aquatic organisms including fish, invertebrates, and plants.

The most commonly detected pesticide in US groundwater is atrazine, a photosynthesis inhibiting triazine herbicide commonly used in agronomic production and urban areas such as roadsides, lawns and recreational fields (Gilliom et al. 2006). Atrazine is not generally viewed as a mobile pesticide due to its low water solubility (K_S = 33 mg L^{-1}), moderate octanol-water partitioning coefficient (log K_{OW} = 2.6), and moderate soil half-life (soil $T_{1/2}$ = 13 - 261 days); however, it is very stable in water (groundwater $T_{1/2}$ = >200 days). It is widely used in corn, sugarcane, and sorghum production in North America and Australia because of its high efficacy and low cost (Schiavon 1988; USEPA 2016). Of the 186 tested USGS sites, five contained an atrazine concentration which exceeded the aquatic life benchmark (Gilliom et al. 2006). The U.S. Environmental Protection Agency (2006) indicated a chronic aquatic life benchmark of 60 µg L^{-1} atrazine of freshwater for invertebrates, 5 µg L^{-1} for fish species and less than 1 µg L^{-1} for non-vascular plant species. The US EPA (2006) mammalian studies have found atrazine to be an endocrine-disrupting chemical. Due to the potential risks of atrazine contamination there has been an increase in atrazine remediation methods such as the successful *Anthrobacter* bioremediation methods (Singh et al. 2004) and phytoremediation methods utilizing aquatic plant species pondweed (*Potamogeton crispus*) and watermilfoil (*Myriophyllum spicatum*) which can potentially slow atrazine movement and metabolize into less toxic compounds (Wang et al. 2013; Qu et al. 2017).

Another pesticide of interest is azoxystrobin, the highest grossing fungicide sales in agricultural and urban landscapes, and the most commonly detected fungicide in US surface waters (Battaglin et al. 2011; Gregoire et al. 2010). With a low water solubility (K_S = 6 mg L^{-1}), a moderate soil-organic carbon sorption coefficient (K_{OC} = 210 to 580 mL g^{-1}), and moderate soil half-life ($T_{1/2}$ = 72-164 days), azoxystrobin is very unlikely to move off-target (USEPA 2006). However, due to high use rates and the mobility of degradates, such as azoxystrobin acid, azoxystrobin has become a potential threat to water ecosystems near treated areas (Ghosh and Singh 2009). While azoxystrobin has low toxicity to mammals, birds, insects, and earthworms, it is very toxic to aquatic invertebrates with an established chronic benchmark of 44 µg per L of freshwater (USEPA 2006). Previous studies have found contaminated soil remediation methods to be successful by planting broadleaf plantain (*Plantago major* L.) and soybean (*Glycine max* L.) (Romeh 2017; Romeh 2015).

Another pesticide of interest with respect to water contamination is imidacloprid, a widely used insecticide to control insect damage in agronomic production and urban landscapes. Imidacloprid has a moderate to high water solubility (K_S = 514 mg L^{-1}), moderate octanol-water partitioning coefficient (log K_{ow} = 3.7) and has a soil half-life of 26.4 to 229 days (US EPA 2016). Imidacloprid is extremely toxic to invertebrates, with a very low chronic benchmark of 0.01 µg per L of freshwater. The US EPA has indicated a much higher chronic benchmark for fish species at 9,000 µg L^{-1}; however, recent studies have found imidacloprid has resulted in sublethal effects including stunted growth, lesions, and deformities in the fish adolescents (Vignet et al. 2019). More specifically, Siever et al. (2018) reported imidacloprid may alter behaviors and neurological pathways in tadpoles (*Limnodynastes tasmaniensis*) (Rios et al. 2017). Previous efforts to reduce imidacloprid contamination include testing the absorption of imidacloprid by *P. major* (Romeh 2014) and pumpkin (*Cucurbita pepo* L.) (Potur and Tiryaki 2019) which was found to phytoremediate a portion of applied imidacloprid. Every pesticide can be a threat to terrestrial, surface- and groundwater; therefore, it is imperative to take precaution and prepare for potential off-target movement.

While many of these events are accidental and difficult to retract, pesticide contamination to water bodies is reduced through best management strategies such as assessing soil type and topography, and monitoring weather patterns before and after application (Gaonkar et al. 2016). Other best management practices include adjusting spray carrier volume, nozzle type, and/or post application irrigation regimens. Another efficient and feasible mitigation practice to reduce pesticide off-target movement is the addition of vegetative buffer strips or constructed wetlands between the pesticide-treated area and sensitive off-target area (Moore et al. 2002). Vegetative buffer strips are typically a high-density planted area with varying plant species with the purpose to slow the surface and subsurface flow of water from a pesticide and/or nutrient treated area and function as a barrier to prevent contaminated water and soil from reaching water sources (Saleh et al. 2018). As water moves downhill, above-ground vegetation slows lateral movement and allows water to infiltrate soil where the subsurface water meets roots and can be absorbed by the root system (Syversen et al. 2004). Franco and Matamoros (2016) reported, vegetative filter strips decreased the amount of herbicide runoff and decrease their effects on phytoplankton by 99%.

A key component to vegetative buffer strips is phytoremediation which is the ability of a plant to uptake a compound, such as a pesticide, and metabolize into simpler and potentially less active compounds to reduce the presence of toxic chemicals in the environment (Stefani et al. 2011; Roongtanakiat 2007). Plant tissue absorption and metabolism is vital for the success of the phytoremediation system and ensuring the depletion of the toxic pollutant to the ecosystem. Many phytoremediation studies focus on the remediation of contaminating compounds such as metals, PFASs, and nutrients; however, pesticide phytoremediation studies remain scarce. Phytoremediation studies that focus on pesticides include the implementation of noxious weed and crop plant species. Attributes of an ideal phytoremediation species include non-invasive native species with high biomass and a fibrous root system. The objective of this study was to evaluate the pesticide-phytoremediation potential of three terrestrial riparian plant species to equip pesticide applicators with a constructive list of manageable phytoremediation plant species.

Materials and Methods

Experimental Conditions

Greenhouse research (NCSU Method Road Greenhouses, Raleigh, NC) was initiated September 1, 2017 to evaluate pesticide phytoremediation capabilities of three terrestrial plant species. Plant species included *Iris versicolor* (blueflag iris), *Panicum virgatum* (switchgrass), and *Andropogon virginicus* (broomsedge) which are perennial plants native to the southeastern US, tolerant to drought and flood conditions, tolerant to select herbicides, inexpensive to plant and manage, and often recommended for wetland mitigation and landscape beautification (Smith et al. 2008; Murphy and Coats 2011, Rossi et al. 2011). Plants were transplanted into unique 9 L (507 cm^2 surface area) containers in Candor sand (94% sand with 6% silt/clay and 2% organic matter w w^{-1}) with a pH of 6.1 and bulk density of 1.43 g cm^{-3}. Greenhouse conditions were set to provide 32/27˚C day/night with 14 h day length. Plants were grown for one month prior to initiation. Non-planted containers were filled with soil only and utilized to compare the pesticide bioremoval and degradation in planted vs non-planted pots.

Pesticides included atrazine (Aatrex 4L®, Syngenta Crop Protection, Inc., Greensboro, NC), azoxystrobin (Heritage TL®, Syngenta Crop Protection, Inc., Greensboro, NC), and imidacloprid (Merit®, Bayer Environmental Science, Research Triangle Park, NC) and were applied at the equivalent maximum single application rate (8.50 mg atrazine $container^{-1}$, 2.30 mg azoxystrobin $container^{-1}$, 1.70 mg imidacloprid $container^{-1}$) to observe maximum phytoremediation capabilities. Pesticides were applied to unique containers via 15 mL water stock solution syringed over the soil surface 2.5 cm away from stem of plant to prevent direct pesticide-plant contact. Containers were irrigated with 0.5 cm water immediately following application and an additional 2.5 cm weekly. At 0, 28, 56 and 112 days after treatment (DAT), soil samples were taken from both planted and non-planted containers at 2.5 cm increments up to 7.5 cm. At each collection timing, above- and below-ground vegetation was destructively harvested from the planted containers, stored temporarily on ice in the greenhouse and transferred to a freezer (-12˚C) within 2 h for subsequent residue analysis.

Residue Analysis

Soil samples were homogenized by adding 50 mL pulverized dry ice and sending through a soil processor (2.0 mm sieve screen, SA-45, Global Gilson, Lewis Center, OH). Plant tissue samples were homogenized by adding 100 mL of liquid nitrogen and sending through a food processor (3450 RPM, Blixer4, Robo Coupe, Kodak, TN).

Atrazine

Atrazine was extracted from soil by combining 15 g of processed soil with 25 mL of acetonitrile (Optima® LC/MS, Fisher Chemical, Fair Lawn, NJ) in a 225 mL high-density polyethylene container, shaken for 30 minutes (200 rpm, 30 mm orbital diameter, KS501 Digital®, IKA Works Inc., Wilmington, NC) and centrifuged for 10 minutes (3500 rpm, Allegra 6KR®, Beckman Coulter Inc., Indianapolis, IN) (Steinheimer 1993). Atrazine was extracted from plant tissue by combining 10 g of processed above- or below ground vegetation with 25 mL acetonitrile in a 225 mL high-density polyethylene container, shaken for 30 minutes and centrifuged for 10 minutes (Shin et al. 2011). Chlorophyll pigment was removed by vortex mixing 1.5 mL extracted sample with 2.5 mg graphitized carbon (QuECHERS, Bristol, PA) and centrifuged for 5 minutes. 1 mL of each extracted sample was filtered (13 mm syringe filter with 0.45 µm PTFE membrane, VWR International, Radnor, PA) and analyzed using high performance liquid chromatography–diode array detector methodology (Agilent-1260 Infinity; Agilent Technologies, Inc., Wilmington, DE). Analyte concentrations were quantified using peak area measurements (OpenLAB CDS ChemStation, version C.01.04; Agilent Technologies Inc., Wilmington, DE). Concentrations above the calibration curve were diluted and re-injected for analysis. Atrazine limits of quantification and detection for soil and plant tissue samples were 1.0 and 0.03 mg L^{-1}, respectively. Fortification recovery checks for soil and plant tissue samples ranged from 94-104% and 87-98%, respectively.

Azoxystrobin

Azoxystrobin was extracted from soil by combining 15 g of processed soil with 25 mL acetonitrile in a 225 mL high-density polyethylene container, shaken for 30 minutes and centrifuged for 10 minutes (Jeffries et al. 2016). Azoxystrobin was extracted from plant tissue by combining 10 g of processed above- or below ground vegetation with 30 mL acetonitrile in a 225 mL high-density polyethylene container, shaken for 30 minutes and centrifuged for 10 minutes (Jeffries et al. 2016). Chlorophyll pigment was removed by vortex mixing 1.5 mL extracted sample with 2.5 mg graphitized carbon and centrifuged. 1 mL of extracted sample was filtered and analyzed using HPLC-DAD methodology. Azoxystrobin limits of quantification and detection were 0.25 and 0.05 mg L^{-1}, respectively. Fortification recovery checks for soil and plant tissue samples ranged from 86-95% and 61-80%, respectively.

Imidacloprid

Imidacloprid was extracted from soil by combining 15 g of processed soil with 20 mL acetonitrile in a 225 mL high-density polyethylene container, shaken for 30 minutes and centrifuged for 10 minutes (Moghaddam et al. 2012). Imidacloprid was extracted from plant tissue by combining 10 g of processed above- or below ground vegetation with 20 mL acetonitrile in a 225 mL high-density polyethylene container, shaken for 30 minutes and centrifuged for 10 minutes (Ge et al. 2017). Chlorophyll pigment was removed by vortex mixing

1.5 mL extracted sample with 2.5 mg graphitized carbon and centrifuged. 1 mL of extracted sample was filtered and analyzed using HPLC-DAD methodology. Concentrations above the calibration curve were diluted and re-injected for analysis. Imidacloprid limits of quantification and detection were 0.05 and 0.01 mg L^{-1}, respectively. Fortification recovery checks for soil and plant tissue samples ranged from 89-104% and 89-102%, respectively.

Percent reduction of pesticide concentrations in water were utilized to to compare planted containers to non-planted containers

(Eq. 1):

Percent reduction (soil) = [1- (planted container soil (µg) / non-planted container soil (µg)) x 100]

Percent of recovered was calculated to quantify each matrix (soil, above-ground vegetation or below ground vegetation) compared to the total residue found within each unique container

(Eq. 2):

Percent of recovered = [(select matrix (µg) / total matrices (µg)) x 100]

Remaining reductions in residue recovery were found between total recovered in planted and non-planted pots (Eq. 3):

Percent reduction (total recovered) = {[(non-planted total (µg) – planted total (µg)) / non-planted total (µg)] x 100}

Experimental Design and Statistical Analysis

The experiment was conducted in a 3-by-3 factorial arrangement including three plant species (*I. versicolor, P. virgatum* and *A. virginicus*) and three pesticides (atrazine, azoxystrobin and imidacloprid). Three replicates of each plant and pesticide combination were arranged in a randomized complete block design and rotated weekly to account for greenhouse variability.

Plant species, sample timing, pesticide and sample matrices were considered fixed effects. Data were subjected to ANOVA ($\alpha < 0.05$) using MIXED procedure in SAS (Statistical Analysis Software®, Version 9.2, SAS Institute, Inc., Cary, NC). Means were separated using Fisher's Protected LSD ($\alpha = 0.05$).

Results and Discussion

Across all sample matrices and experimental runs, atrazine, azoxystrobin or imidacloprid were not detected in any control samples (i.e., no contamination of experimental units). ANOVA did not detect significant interactions between experimental run and pesticide ($P = 0.224$), DAT ($P = 0.268$), sample matrix ($P = 0.796$), or plant species ($P = 0.141$); therefore, data were pooled over runs for statistical analysis. The main effect of both sample matrix ($P \leq 0.001$) and DAT ($P \leq 0.0001$) as well as their interactions with all fixed effects ($P \leq 0.0002$) were significant; therefore, data were sorted by sample matrix and DAT and are presented accordingly.

Soil

A percent reduction in pesticide was calculated to compare each plant species to the corresponding non-planted container (Table 1). At 28 and 56 days after treatment (DAT), atrazine, azoxystrobin, and imidacloprid was not reduced when comparing *I. versicolor*, *A. virginicus*, and *P. virgatum* to non-planted containers. A significant plant-pesticide interaction was detected 112 DAT in soil residue samples relative to percent of applied. At 112 DAT, *I. versicolor* planted containers were reported to reduce greater atrazine (58.7% reduction) compared to *A. virginicus* (43.9%) and *P. virgatum* (41.9%). At the same sampling time, *I. versicolor* reduced greater azoxystrobin (86.9% reduction) than *A. virginicus* (40.8%) and both plant species reduced greater azoxystrobin compared to *P. virgatum* (25.2%). At 112 DAT, *I. versicolor* and *P. virgatum* planted containers reduced greater imidacloprid (62.5% and 64.3% reduction, respectively) compared to *A. virginicus* (34.5%). Even without statistical significances differentiating the plant species, a numerical trend shows the continual increase in pesticide reduction through 112 days. From this information, and data from Cardinali et al.'s (2017) study focusing on vegetative buffer strips, the selected plant species have the potential to reduce up to 99% more pesticide contamination, if planted in abundance and proximity.

Soil was also calculated relative to percent of total residue recovered to quantify the distribution of atrazine, azoxystrobin and imidacloprid through soil and plant tissues (Table 2). At 28 and 56 DAT, > 95.5% of recovered atrazine, azoxystrobin and imidacloprid was detected in the soil matrix and recoveries did not differentiate between plant species. At 112 DAT, atrazine recoveries ranked: P. virgatum (90.2% of recovered) > A. virginicus (86.4%) > I. versicolor (63.7%). At the same sample collection time, azoxystrobin recoveries ranked: *A. virginicus* (63.0% of recovered) > *P. virgatum* (46.1%) > *I. versicolor* (15.0%). At 112 DAT, imidacloprid recoveries ranked: *P. virgatum* (90.6% of recovered) > *I. versicolor* (87.4%) > *A. virginicus* (82.8%).

Wang et al. (2012) evaluated the phytoremediation capacity of sweet flag (*Acorus calamus*), yellow flag (*Iris pseudacorus*), and purple loosestrife (*Lythrum salicaria*) of atrazine in an aqueous environment and reported that these species reduced atrazine by 93.3, 97.2, and 94.4%, respectively, 20 DAT compared to the initial 4 mg L^{-1} dose. Atrazine was reduced by only 41.6% of the applied in non-planted containers. These percentages of reduction of atrazine are much larger than that found in *I. versicolor*, *A. virginicus*, and *P. virgatum*; however, the hydroponic study is expected to reduce more due to the absence of atrazine adsorption to soil and soil organic matter. Both studies concerning atrazine reduction over time can be comparable and give promise to the ability of plants to tolerate and phytoremediate atrazine. In a study (Qu 2017) that included atrazine contaminated lake sediment, curled pondweed (*Potamogeton* *crispus*) and spiked watermilfoil (*Myriophyllum spicatum*) were exposed to 0.10 mg atrazine kg^{-1} soil and reduced 75.65 and 76.15%, respectively, compared to 46.3% reduction of atrazine in the non-planted. These aquatic plant species did not reduce atrazine to the extent of the hydroponic study; however, reduced greater atrazine compared to *I. versicolor*, *A. virginicus*, and *P. virgatum*. These reductions in atrazine reduction between plant species from soil can be comparable because of reductions in soil type, as well as the presence of established microbial communities, which play a larger role in the degradation of atrazine in lake sediments.

Previous azoxystrobin phytoremediation studies (Romeh 2017) have reported *P. major* is able to reduce azoxystrobin by 87.4% of applied in planted containers compared to 51.7% in non-planted containers after 12 days of exposure. A similar study (Romeh 2014) reported *P. major* reduced up to 95.2% of imidacloprid compared to 61.9% reduction in non-planted containers 10 days after treatment. The reduction of azoxystrobin and imidacloprid by *P. major* was found to be higher compared to *I. versicolor*, *A. virginicus,* and *P. virgatum*; however, salinity and temperature control showed a trend that would affect the reduction of azoxystrobin between the four plant species. It is also imperative to note the natural behavior of weed-like species such as *P. major* to function under stressful conditions such as drought or nutrient deficiency; although, they are less likely to be planted in farming or urban landscapes in proximity to azoxystrobin and imidacloprid treated areas due to their appearance and invasive behavior.

Plant Tissue

Above-ground Tissue

Data suggest atrazine translocation increased and persistence decreased in above-ground biomass through 112 days after treatment in all plant species (Table 3). All data presented regarding pesticide residue in plant biomass is calculated as a percent recovery. At 28 DAT, *A. virginicus* and *P. virgatum* contained more atrazine (1.1 and 0.8% of recovered, respectively) than *I. versicolor* (0.3%). Percent of atrazine recovered in above-ground biomass increased through 56 days where *P. virgatum* containedm4.1% of recovered, *A. virginicus* contained 2.5% and *I. versicolor* contained 2.2%. At 112 DAT, the most atrazine was detected in *I. versicolor* above-ground biomass (36.2% of recovered) compared to *A. virginicus* (13.5%) and *P. virgatum* (9.6%). Wang et al. (2012) reported 0.22% of applied atrazine in *A. calamus* above-ground biomass, 0.29% in *I. pseudacorus*, and 0.37% in *L. salicaria* above-ground biomass after 20 days. Compared to this study, after 28 days, *A. virginicus* is more likely to absorb and translocate atrazine in above-ground tissue with *P. virgatum* least likely to perform these processes efficiently. Wang et al. (2012) explains the reason for low residue found in above-ground tissue may be due to the increased metabolism over time.

A similar trend was observed with azoxystrobin translocation and persistence in above-ground biomass (Table 3). At 28 DAT, greater azoxystrobin (3.9% of recovered) was detected in *P. virgatum* above-ground biomass compared to *A. virginicus* (1.8%) and *I. versicolor* (1.2%). Alternatively, at 56 DAT greater azoxystrobin was detected in *I. versicolor* (3.9% of recovered) compared to *A. virginicus* (2.3%) which was also greater compared to *P. virgatum* (1.1%). Of the evaluated plant species within this research, at 112 DAT, *I. versicolor* consistently yielded greater azoxystrobin in above-ground biomass (42.5% of recovered) compared to *A. virginicus* (13.6%) or *P. virgatum* (9.3%). Romeh (2017, 2015) reported 16.5% of applied azoxystrobin absorbed in *P. major* above-ground biomass in an aqueous solution after 12 days which is found to be more than *P. virgatum*, *A. virginicus* and *I. versicolor* at 28 DAT. Romeh also quantified azoxystrobin metabolite residue including azoxystrobinenol, azoxystrobin acid, azoxystrobin-pyOH, and azoxystrobin-benzoic under natural conditions after 12 days. From these data, the only metabolite detected after 12 days, azoxystrobin-pyOH, recovered 24.6 mg kg^{-1} which is numerically greater compared to 3.3 mg kg^{-1} parent compound azoxystrobin recovered at the same sample collection time. Further research on azoxystrobin dissipation and degradation in runoff and metabolite residue should be conducted to complete phytoremediation analyses and prevent azoxystrobin contamination.

Concentrations of imidacloprid detected in above-ground biomass were only found to be different between plant species at 28 and 56 days (Table 3). At 28 DAT, *I. versicolor* contained more imidacloprid (0.2% of recovered) than *A. virginicus* (0.1%) and *P. virgatum* (0.1%). Conversely at 56 days where greater imidacloprid was detected in *A. virginicus* above-ground biomass (1.4% of recovered) compared to *P. virgatum* (0.7%) or *I. versicolor* (0.7%). No reductions in imidacloprid residue were detected in above-ground biomass across plant species at 112 days (5.2 to 9.1% of recovered). Compare these data to Romeh (2017), who reported 2.51 µg g^{-1} in *P. major* above-ground biomass after 10 day exposure period which is more than the 0.98 µg g^{-1} recovered in *I. versicolor*, 0.21 µg g^{-1} in *A. virginicus,* and 0.04 µg g^{-1} in *P. virgatum* after 28 days in the presented research.

Below-ground Tissue

At 28 days, *A. virginicus* contained more atrazine (0.2% of recovered) than *I. versicolor* (0.1%) and *P. virgatum* (0.1%) (Table 4). Total atrazine recovered decreased at 112 days with greater levels in *P. virgatum* (0.2% of recovered) compared to *I. versicolor* (0.1%) or *A. virginicus* (0.1%). Compared to the other matrices (i.e., soil or above-ground biomass), greater atrazine was detected in soil or above-ground biomass with minimal amounts found in the below-ground biomass. This trend does not match that of azoxystrobin and imidacloprid where over time, the above-ground and below-ground biomass matrices display close to the same amount of pesticide. A study by Cecilia and Maggi (2017) suggests that atrazine persistence in roots may be due to the translocation to above-ground biomass or quick metabolism of a soluble compound.

The recovery of azoxystrobin in below-ground biomass increased from 28 DAT to 112 DAT (Table 4). After 28 days, *P. virgatum* recovered more azoxystrobin (6.8% of recovered) than *A. virginicus* (2.9%) and *I. versicolor* (1.6%). *A. virginicus* was found to recover less azoxystrobin at 56 days (5.1% of recovered) compared to *I. versicolor* (8.8%). Azoxystrobin recovery increased in below-ground biomass at 112 days where *P. virgatum* and *I. versicolor* recovered more (44.6 and 42.5% of recovered, respectively) compared to *A. virginicus* (23.4%). The study conducted by Romeh (2017) found more parent azoxystrobin was recovered (7.1 mg kg^{-1}) in below-ground biomass of *P. major* compared to azoxystrobin metabolite azoxystrobin acid (6.5 mg kg^{-1}) at 12 days.[13] Azoxystrobin recovered in *P. major* at 12 days was found to be greater than the recovered in *I. versicolor*, *A. virginicus*, and *P. virgatum* after 28 and 56 days; however, less than *I. versicolor* and *P. virgatum* through 112 days. This reduction at 112 DAT may be due to the increased metabolism of azoxystrobin in below-ground tissue in *P. major*.

Numerically, imidacloprid recovery in below-ground biomass was found to be more than atrazine and less than azoxystrobin. At 28 DAT, *P. virgatum* recovered more imidacloprid (1.1% of recovered) than *A. virginicus* (0.3%) while *I. versicolor* was not detected. At 56 days, imidacloprid recovery ranked *A. virginicus* (3.1% of recovered) > *P. virgatum* (1.8%) > *I. versicolor* (0.4%). Finally, at 112 days, *A. virginicus* and *I. versicolor* recovered more imidacloprid (8.1 and 7.4%, respectively) compared to *P. virgatum* (2.1%). Compared to a study conducted by Romeh (2014), *P. major* absorbed more imidacloprid in below-ground biomass

(7.94 ug g^{-1}) after 10 days than *I. versicolor* (0.00 µg g^{-1}), *A. virginicus* (0.04 µg g^{-1}), and *P. virgatum* (0.15 µg $g^{-}1$) at 28 days. This trend may be explained by increased translocation of imidacloprid (a systemic insecticide) into above-ground biomass and/or increased organic matter sorption to soil.

From these data, it is found that not one plant species remediates a compound more efficiently than the other plant species at any sampling time. It is imperative to recognize the importance in planting a diversity of plants in proximity to ensure the remediation of any compound that may move off-target (Cresswell et al. 2019). It is also important to note the lower recovery rates in biomass over time. In long-term exposure studies, atrazine's half-life can be decreased up to 95% with increasing root mass and rhizosphere microbial communities (Farrar et al. 2003; Kulikova and Perminova 2002; Ravit et al. 2005). While it is possible that the plant's ability to absorb and translocate the pesticide has decreased over time due to saturation, it is also possible that the plant has absorbed and quickly metabolized the pesticide which would result in lower concentrations recovered.

Additional Residue Reductions

Metabolism is an important contributing factor in phytoremediation because it degrades the compound into simpler and presumably less toxic compounds. While it can be difficult to quantify metabolites, the reductions between detections of non-planted pots and planted pots were calculated to determine additional degradation rates (Table 5). At 56 DAT, *I. versicolor* planted containers were found to degrade atrazine more (9.6% of reduction) compared to *P. virgatum* (5.3%) and *A. virginicus* (5.1%). Atrazine dissipation increased through 112 days where *A. virginicus* dissipated more (28.1% reduction) than *P. virgatum* (18.7%) and *I. versicolor* (17.3%).

A similar increasing trend from 28 to 112 DAT was found in azoxystrobin. At 28 days, *P. virgatum* differed from the non-planted containers more (8.2% reduction) than *A. virginicus* (3.7%) and *I. versicolor* (2.1%). At 56 days, *I. versicolor* increased to 11.48% reduction which is more than *A. virginicus* (6.9%) and *P. virgatum* (6.1%). At 112 days, *I. versicolor*'s reduction from non-planted containers dropped to 0.8% reduction, *P. virgatum* dropped to 2.9%, and *A. virginicus* spiked to 32.8%. From this information, it seems as though *P. virgatum* metabolizes azoxystrobin earlier in exposure and *A. virginicus* takes more time to translocate and metabolize. Planted containers treated with imidacloprid were not reported to have the same reductions compared to non-planted containers as seen in atrazine or azoxystrobin treated containers. At 28 days, *A. virginicus* planted containers treated with imidacloprid reported a greater reduction compared to non-planted containers (2.2% reduction) than *P. virgatum* (1.6%) and *I. versicolor* (0.1%). At 56 days, *A. virginicus* planted containers treated with imidacloprid reported a greater reduction compared to non-planted containers (5.3% reduction) than *P. virgatum* (2.9%) and *I. versicolor* (0.8%). Imidacloprid treated planted containers reduction from non-planted containers increased through 112 days where *P. virgatum* reported a greater reduction (15.5% reduction) than *I. versicolor* (9.0%). *A. virginicus* was not significantly different from either (12.6% reduction).

Pesticide recoveries in soil at each sample collection time were calculated to compare the half-life and degradation of atrazine, azoxystrobin and imidacloprid between plant species and non-planted containers (Table 6). Atrazine half-lives are ranked: *I. versicolor* (14.7 days) < *A. virginicus* (23.2 days) < *P. virgatum* (27.0 days) < non-planted (30.2 days). *I. versicolor* planted containers were reported the shortest azoxystrobin half-life (13.9 days) compared to *P. virgatum* (18.9 days) which was reported to have a shorter half-life than both *A. virginicus* (22.6) and non-planted (22.4) containers. *P. virgatum* was reported to have the shortest imidacloprid half-life (13.6 days) compared to *I. versicolor* (17.9 days), *A. virginicus* (20.9 days) and non-planted (21.2 days) containers. Romeh (2017) reported a reduction in azoxystrobin half-life when planted with *P. major* (8.5 days) compared to non-planted containers (11.6 days), as well as the effect of 20-100 mM NaCl on azoxystrobin phytoremediation (2.9-5.9 days). The decreases of pesticide half-life in the presence of a plant gives evidence towards the efficiency of phytodegradation within plant tissue and rhizodegradation.

As mentioned previously, *I. versicolor*, *A. virginicus* and *P. virgatum* all have the capabilities to absorb, translocate, and metabolize at least one of the evaluated pesticides. The rhizosphere and microbial communities it support also have the ability to remediate these pesticides (Ravit et al. 2005).   Metabolites should be fully quantified to understand the function of the entire planted system and its pesticide remediation ability compared to non-planted systems. It has been found that these plants operate at different rates and it is important to add diversity to vegetative buffer zones to ensure each pesticide is being absorbed and continues to be translocated and metabolized over time.

Plant Health

Plant health was recorded on a 0-100% scale, with 0% being complete plant death and 100% representing no injury. Most of the plants remained within 66.7-88.3%, on average (data not shown). The only significant reductions found were at 28 and 56 DAT. At 28 DAT, atrazine treated *I. versicolor* rated lower (70.0%) compared to *A. virginicus* (80.0%) and *P. virgatum* (88.3%). At 56 DAT, atrazine treated *I. versicolor* and rated lower (66.7%) than *A. virginicus* (70.0%) and *P. virgatum* (81.7%). With atrazine being an herbicide, it is not uncommon for plant injury or stunting to occur. Imidacloprid treated *A. virginicus* was reported to be lower

(68.3%) compared to *I. versicolor* (78.3%) and *P. virgatum* (83.3%) at 56 DAT. When choosing a phytoremediating species when the known herbicide applied is atrazine, *I. versicolor* would not be recommended as the only plant species due to the increased injury from herbicide exposure compared to the grasses. It was observed that even with stunted growth or leaf injury at any point of the study, the plants were able to produce more above-ground biomass to recover from herbicide exposure.

Conclusion

This research builds upon previous efforts intended to minimize off-target movement of pesticides through the implementation of vegetative buffer strips for phytoremediation. From the presented research, *I. versicolor, A. virginicus*, and *P. virgatum* have the capabilities to phytoremediate atrazine, azoxystrobin, and imidacloprid from soil. Relative to the amount of pesticide found in planted pots compared to non-planted pots, *I. versicolor* was found to reduce greater atrazine in soil at 112 DAT by 58.7%. *I. versicolor* was also the most capable of reducing azoxystrobin, by 86.9%, from the soil at 112 DAT. At the same sampling time, *I. versicolor* and P. virgatum reduced greater imidacloprid from soil by 62.5% and 64.3%, respectively. Therefore, this research supports the recommended practice of establishing diverse plant species in mitigation areas to optimize phytoremediation capacity of these systems and in turn lessen pesticide movement into non-target wildlife habitats. Measurements taken during this study also suggests the establishment of diverse plant species due to potential injury from atrazine. While plant death was not observed within this research, *I. versicolor* displayed visual injury following 56 days of atrazine exposure. Mature plants were able to recover and continue phytoremediating despite herbicidal effects; however, it may be beneficial to select plant species that are known to have increased levels of tolerance to routinely applied herbicides; however, additional research is needed to confirm. Future research should include observing plants of different physiology and their capabilities to phytoremediate varying types of commonly used pesticides in agricultural, urban and mixed landscapes.

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Tables

Table 1. Pesticide-by-plant species interaction on pesticide persistence in soil relative to non-planted containers at 28, 56 and 112 days after treatment.

	28 DAT			56 DAT			112 DAT		
Plant	ATRZ	AZOXY	IMID	ATRZ	AZOXY	IMID	ATRZ	AZOXY	IMID
	% reduction								
I. versicolor	23.9	20.5	13.9	39.9	28.9	27.8	58.7	86.9	62.5
A. virginicus	16.2	13.5	15.0	33.0	16.1	21.9	43.9	40.8	34.5
P. virgatum	16.4	11.5	21.4	34.3	16.0	28.9	41.9	25.2	64.3
Non-planted	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$LSD_{0.05}$	9.0			14.7			11.1		

Abbreviation: ATRZ, atrazine; AZOXY, azoxystrobin; DAT, days after treatment; IMID, imidacloprid; NS, non-significant.

Equation: Percent reduction = {[1 – (planted container soil residue / non-planted container soil residue)] x 100}

Table 2. Atrazine, azoxystrobin and imidacloprid persistence in soil relative to the total % of the recovered at each sampling day after treatment.

Plant	Atrazine			Azoxystrobin			Imidacloprid		
	28 DAT	56 DAT	112 DAT	28 DAT	56 DAT	112 DAT	28 DAT	56 DAT	112 DAT
	% of recovered								
I. versicolor	99.6	97.6	63.7	97.2	86.1	15.0	99.8	99.1	87.4
A. virginicus	98.7	97.1	86.4	95.3	92.6	63.0	99.6	95.5	82.8
P. virgatum	99.1	95.7	90.2	89.3	93.2	46.1	98.8	97.5	90.6
LSD	NS	NS	2.7	NS	NS	5.1	NS	NS	1.0

Abbreviations: DAT, days after treatment; NS, non-significant.

Equation: Percent recovered = [(soil residue / total residue recovered) x 100]

Table 3. Atrazine, azoxystrobin and imidacloprid persistence in above-ground biomass relative to the total % of the recovered at each sampling day after treatment.

	Atrazine			Azoxystrobin			Imidacloprid		
Plant	28 DAT	56 DAT	112 DAT	28 DAT	56 DAT	112 DAT	28 DAT	56 DAT	112 DAT
	% of recovered								
I. versicolor	0.3	2.2	36.2	1.2	3.9	42.5	0.2	0.5	5.2
A. virginicus	1.1	2.5	13.5	1.8	2.3	13.6	0.1	1.4	9.1
P. virgatum	0.8	4.1	9.6	3.9	1.1	9.3	0.1	0.7	7.3
$LSD_{0.05}$	0.5	1.3	11.4	1.9	1.0	8.4	0.1	0.7	NS

Abbreviation: DAT, days after treatment; NS, non-significant.

Equation: Percent recovered = [(above-ground vegetation residue / total residue recovered) x 100]

Table 4. Atrazine, azoxystrobin and imidacloprid persistence in below-ground biomass relative to the total % of the recovered at each sampling day after treatment.

	Atrazine			Azoxystrobin			Imidacloprid		
Plant	28 DAT	56 DAT	112 DAT	28 DAT	56 DAT	112 DAT	28 DAT	56 DAT	112 DAT
	% of recovered								
I. versicolor	0.1	0.2	0.1	1.6	8.8	42.5	0.0	0.4	7.4
A. virginicus	0.2	0.4	0.1	2.9	5.1	23.4	0.3	3.1	8.1
P. virgatum	0.1	0.2	0.2	6.8	5.7	44.6	1.1	1.8	2.1
$LSD_{0.05}$	0.1	NS	0.1	2.0	3.2	10.3	0.7	0.7	3.4

Abbreviation: DAT, days after treatment; NS, non-significant.

Equation: Percent recovered = [(below-ground vegetation residue / total residue recovered) x 100]

Table 5. Atrazine, azoxystrobin, and imidacloprid total recoveries in planted compared to non-planted containers in terms of % reduction at each sampling day after treatment.

	28 DAT[a]			56 DAT			112 DAT		
Plant	ATRZ	AZOXY	IMID	ATRZ	AZOXY	IMID	ATRZ	AZOXY	IMID
	% reduction								
I. versicolor	5.4	2.1	0.1	9.6	11.5	0.79	17.3	0.8	9.0
A. virginicus	2.2	3.7	2.2	5.1	6.9	5.3	28.1	32.8	12.6
P. virgatum	4.2	8.2	1.6	5.3	6.1	2.9	18.7	2.9	15.5
$LSD_{0.05}$	NS	NS	NS	NS	4.2	3.6	NS	15.7	NS

Abbreviation: ATRZ, atrazine; AZOXY, azoxystrobin; DAT, days after treatment; IMID, imidacloprid; NS, non-significant.

Equation: Percent reduction = {[(non-planted total residue – planted total residue) / non-planted total residue] x 100}

Table 6. Half-life of atrazine, azoxystrobin and imidacloprid in soil calculated from 0, 28, 56 and 112 DAT data.

Plant	Atrazine	Azoxystrobin	Imidacloprid
	————	days	————
I.versicolor	14.7	13.9	17.9
A. virginicus	23.2	22.6	20.9
P. virgatum	27.0	18.9	13.6
Non-planted	30.2	22.4	21.2
$LSD_{0.05}$	2.3	3.1	3.4

Abbreviations: DAT, days after treatment.

Chapter 2: Phytoremediation of Azoxystrobin and Imidacloprid by Wetland Plant Species *Juncus effuses*, *Pontederia cordata* and *Sagittaria latifolia*

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Azoxystrobin (strobilurin fungicide) and imidacloprid (neonicotinoid insecticide) have been detected in surface waters near treated agricultural, urban, and mixed landscapes. The hazards of pesticide runoff can be prevented through best management practices, including the establishment of diverse wetland plant barriers which can phytoremediate the chemicals in which they come into contact with. In this study, the wetland plant species softrush (*Juncus effuses*), pickerelweed (*Pontederia cordata*), and arrowhead (*Sagittaria latifolia)* were planted in sandy soil containers that were then placed in azoxystrobin or imidacloprid treated water. Every week for 2 months, water samples were collected for pesticide residue analysis using high-performance liquid chromatography (HPLC). At 14, 28, and 56 days after initiation, plants were destructively harvested and analyzed for pesticide residue in the soil, above-ground vegetation, and below-ground vegetation. Results from this study report *P. cordata* reduced greater azoxystrobin (51.7% reduction) compared to *J. effuses* and *S. latifolia* (24.9% and 28.7%) at 56 days. However, *S. latifolia* reduced greater imidacloprid (79.3%) compared to *J. effuses* and *P. cordata* (36.0% and 37.1%) at 56 days. All three plant species have been found to phytoremediate azoxystrobin and imidacloprid at varying rates, which supports the recommended establishment of diverse wetland species in aquatic areas that neighbor azoxystrobin or imidacloprid treated landscapes.

Keywords: phytoremediation; azoxystrobin; imidacloprid; pesticide remediation

Introduction

Pesticides are used worldwide in agronomic and urban landscapes to manage weed competition, disease, and insect damage. With the use of pesticides, production increases, quality increases, and in some cases, the amount of irrigation and fertilizer required for crop growth decreased by controlling competing weeds (Aktar et al. 2009). Pesticides are also widely used in recreational areas such as on athletic fields, golf courses, and roadsides to improve safety and aesthetics in these areas (Milesi 2005). The United States Environmental Protection Agency (US EPA) (2012) recorded the US applied 678 million pounds of pesticide active ingredient in 2012 and the world market applied 2.8 billion pounds. While pesticides have been engineered in recent decades to increase efficacy and decrease environmental exposure by reducing use rates, pesticides still have the potential to move off-target and have an adverse impact on ecosystems (Barbash et al. 2001). Some of the most sensitive ecosystems can be found within water bodies that provide habitat for aquatic organisms, a water source for terrestrial organisms, and support a breeding ground for many invertebrates and amphibians (Hladyz et al. 2011). According to the National Water-Quality Assessment conducted by the US Geological Survey, 57 percent of tested agricultural streams and 83 percent of tested urban streams were found to contain detectable concentrations of one or more pesticides which exceeded an aquatic-life benchmark (Gilliom et al. 2006).

Phytoremediation, the ability of a plant to absorb and degrade into simpler and potentially fewer toxic compounds, is a main function of constructed wetlands (Rai 2008). Pesticide off-target movement and runoff can be mitigated by best management practices such as monitoring weather patterns before and after application treatments, adjusting sprayer carrier volume, assessing soil volumetric water content, exchanging nozzle type (Knisel et al. 1995) and incorporating soil binding surfactants and slow release pesticide formulations (Shasha et al. 1976). Another line of defense is constructed wetlands, which have been found to be a low-cost, low-maintenance, and efficient solution to other pollution problems such as industrial wastewater and landfill leachate (Vymazal 2014). Constructed wetlands, which are physical barriers made of plant species established between a treated area and a sensitive aquatic area, have also been implemented in combatting nutrient runoff from managed landscapes. These systems are comprised of three main components that function in unison: soil, vegetation, and microbial communities. With increasing organic material within these aquatic systems, pesticides have additional binding sites and are less likely to move (Rai 2008). With proper microbial and phytoremediating activity, pesticides may be degraded.

While many phytoremediation studies involve the clean-up of industrial waste and leaching metals such as those in superfund sites (Doty et al. 2017), phytoremediation has also been explored to prevent pesticide off-target movement (Licht and Isebrands 2005). The primary reduction between phytoremediation in superfund clean-up sites and constructed wetlands is the sustainability and plant health factors. As opposed to the one time use and harvest of plants in superfund sites, the purpose of implementing constructed wetlands is to create a vegetation barrier that is tolerant to the pesticide and will continuously absorb and degrade routinely applied pesticides (Febriani 2018). Continuous phytoremediation may be hindered if the plant is not tolerant and is damaged by exposure to an herbicide or the plant becomes saturated by the pesticide and only remediates for a limited concentration or time.

Azoxystrobin is highest gross selling fungicide (>$1 billion) across agricultural and urban landscapes (Lamberth 2016). Azoxystrobin is effective in managing many common diseases such as brown patch (*Rhizoctonia solani*) and pythium root rot (*Pythium spp*.). As evidence of its widespread use, Battaglin et al. (2011) reported detectable azoxystrobin in 45 percent of tested US streams, making it the most commonly detected fungicide in surface waters. Physicochemical properties specific to azoxystrobin's off-target transport include a low water solubility (K_S = 6 mg L^{-1}), a moderate soil-organic carbon sorption coefficient (K_{OC} = 210 to 580 mL g^{-1}), and moderate soil half-life ($T_{1/2}$ = 72-164 days) (O'Rouke 2000). Azoxystrobin is not very toxic to mammals, birds, insects, or earthworms; however, it is moderately to highly toxic to aquatic invertebrates with an established chronic benchmark of 44 µg L^{-1} of freshwater (Carey and Wolf 2009). Previous azoxystrobin remediation studies include bioremediation and terrestrial phytoremediation such as the implementation of *Plantago major, Glycine Max*, and *Helianthus annus* L. in soil after 14 days of exposure compared to non-planted containers (azoxystrobin reduction in soil 91.6%, 91.6%, and 91.15%, respectively) (Romeh 2015). It has also been observed that during soil applied phytoremediation studies, azoxystrobin accumulates more in root tissue compared to leaf tissue (Bouldin et al. 2006).

Imidacloprid is a systemic neonicotinoid insecticide applied to soil, seeds and foliage to prevent insect damage in US agronomic production and urban landscapes (Sappington 2016). Of 48 tested US surface water sites, 37% contained detectable imidacloprid which has been found to be the most detected neonicotinoid in US streams (Hladik et al. 2016). The majority of imidacloprid detections in surface waters were found to be in urban locations suggesting the application in lawns, gardens, parks and public spaces results in increased watershed contamination (Johnson and Pettis 2014). Physicochemical properties specific to imidacloprid's increased efficacy include moderate to high water solubility (K_S = 580-610 mg L^{-1}), moderate soil-organic carbon sorption coefficient (K_{OC} = 132-310 mL g^{-1}) and has a soil half-life of 26.4 to 229 days. Imidacloprid's main degradation pathway is photolysis in water ($T_{1/2}$ = 1-4 hours)

(Sappington et al. 2016). Imidacloprid is classified by the US EPA as extremely toxic to invertebrates and established a low chronic benchmark for aquatic invertebrates (0.01 µg L^{-1} of freshwater). Previous research evaluating remediation methods have reported *P. major* to be an efficient phytoremediating plant species (Romeh 2010). *P. major* planted containers filled with water reduced imidacloprid residues by 55.8-95.2% as compared to non-planted containers which reduced imidacloprid residues by 13.7-61.9% after 10 days (Romeh 2010).

Previous research quantifying the ability of plants to remove azoxystrobin and imidacloprid pesticides is limited. As mentioned, *P. major* has been found phytoremediate azoxystrobin and imidacloprid in aquatic areas; however, *P. major* is less likely to be established due to its weedy growth habit characteristics in some areas (Romeh 2010, Romeh 2015). Romeh (2015) reported *G. max* and *H. annus* can efficiently absorb and translocate azoxystrobin from soil to the leaves of the plants. *Eichhornia crassipes*, an invasive aquatic plant to the southern US, is commonly used in phytoremediation studies due to its ability to efficiently absorb compounds (Mercado-Borrayo et al. 2015, Anudachakul et al. 2015, Chen et al. 2019). Other phytoremediation studies observing shoreline plants have been successful such as *Acorus calamus* phytoremediating chlorpyrifos (Wang 2016) and *Phragmites*, *Iris pseudacorus*, and *Juncus effuses* phytoremediating imazalil and tebuconazole (Lv et al. 2016) which are all native to the southeastern US, have high biomass, and are feasibly managed in agricultural and urban landscapes. The purpose of this research was to quantify azoxystrobin and imidacloprid pesticide phytoremediation by three aquatic plant species (*Juncus effuses*, *Pontederia cordata*, and *Sagittaria latifolia*) that could be easily implemented and managed by producers and landscapers to build a constructed wetland and prevent pesticide off-target movement.

Materials and Methods

Experimental Conditions

Greenhouse research (NCSU Method Road Greenhouses, Raleigh, NC) was initiated December 1, 2018 to evaluate pesticide phytoremediation potential of three aquatic plant species. Plant species included *Juncus effuses*, *Pontederia cordata*, and *Sagittaria latifolia* which are perennial plants native to the southeastern US, inexpensive to plant and manage, and often recommended for wetland mitigation and landscape beautification. Plants were transplanted into unique 64 cm^3 containers in Candor sand (94% sand with 6% silt/clay and 2% organic matter $w\ w^{-1}$) with a pH of 6.1 and bulk density of 1.43 g cm^{-3}. Greenhouse conditions were set to provide 32/27˚C day/night with 14 hour day length. Plants were fertilized (NPK 20/20/20) and grown for one month prior to initiation. Non-planted containers were filled with soil only and utilized to compare the pesticide bioremoval and degradation in planted vs non-planted pots. Planted and non-planted pots were placed 9 L pots which were lined with 2 black trash bags (3 Mil) and filled with 6 L of pond water (Pond #1, NCSU, Raleigh, US) with a pH of 6.4 (Figure 1).

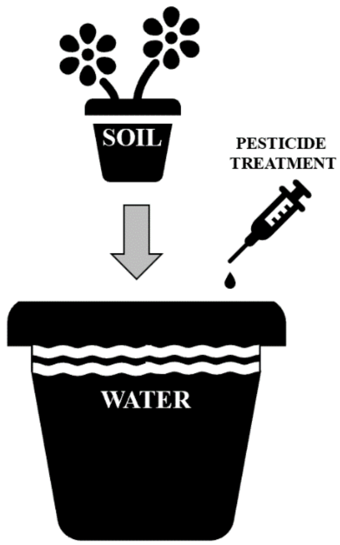

Figure 1. 9 L pots were filled with 6 L of pond water and treated with pesticide. Planted soil containers were then placed in the water and covered with a black trash bag liner.

Azoxystrobin (Heritage TL®, Syngenta Crop Protection, Inc., Greensboro, NC) and imidacloprid (Merit®, Bayer Environmental Science, Research Triangle Park, NC) were applied at the aquatic benchmark of 1 mg L^{-1} active ingredient to water. Pesticides were applied to unique containers via 15 mL water stock solution syringed over the water surface prevent direct pesticide-plant contact. Water was stirred for 30 seconds to evenly distribute the pesticide. Water containers were covered with black trash bag (3 Mil) lining to decrease photolysis and algae growth. At 14, 28, and 42 days after initiation (DAI), water volume remaining in containers was measured, poured out, and replenished with 6 L of fresh pong water and new initial concentration of pesticide. 50 mL water samples were collected weekly from each container and stored temporarily on ice in the greenhouse and then transferred to a refrigerator (2˚C) within 2 h. At 0, 14, 28 and 56 DAI, soil samples were taken from both planted and non-planted containers 10 cm. At each collection timing, above- and below-ground vegetation was destructively harvested from the planted containers, stored temporarily on ice in the greenhouse and then transferred to a freezer (-12˚C) within 2 h for subsequent residue analysis.

Residue Analysis

Soil samples were homogenized by adding 50 mL pulverized dry ice and sending through a soil processor (SA-45, Global Gilson, Lewis Center, OH). Plant tissue samples were homogenized by adding 100 mL of liquid nitrogen and sending through a food processor (R2B, Robot Coupe USA Inc., Ridgeland, MS).

Azoxystrobin

Azoxystrobin was analyzed from water by vortexing for 15 seconds (230 V Vortex Mixer, VWR International, Radnor, PA), filtering (13 mm syringe filter with 0.45 µm PTFE membrane, VWR International, Radnor, PA) 1 mL of water sample and analyzing using high performance liquid chromatography-diode array detector (Agilent-1260 Infinity; Agilent Technologies, Inc., Wilmington, DE) (Jeffries et al. 2017). Azoxystrobin was extracted from soil by combining 15 g of processed above- or below ground vegetation with 25 mL acetonitrile (Optima® LC/MS, Fisher Chemical, Fair Lawn, NJ) in a 225 mL high-density polyethylene container, shaken for 30 minutes (200 rpm, 30 mm orbital diameter, KS501 Digital®, IKA Works Inc., Wilmington, NC) and centrifuged for 10 minutes (3500 rpm, Allegra 6KR®, Beckman Coulter Inc., Indianapolis, IN). Azoxystrobin was extracted from plant tissue by combining 10 g of processed above- or below ground vegetation with 30 mL acetonitrile in a 225 mL high-density polyethylene container, shaken for 30 minutes and centrifuged for 10 minutes. Chlorophyll pigment was removed by vortex mixing 1.5 mL extracted sample with 2.5 mg graphitized carbon (QuECHERS, Bristol, PA) and centrifuging. 1 mL of extracted sample was filtered and analyzed using HPLC-DAD methodology. Concentrations above the calibration curve were diluted and re-injected for analysis. Limits of quantification and detection were 0.25

and 0.05 mg L^{-1}, respectively. Fortification recovery checks for water, soil and plant tissue samples ranged from 94-108%, 98-105%, and 101-105%, respectively.

Imidacloprid

Imidacloprid was extracted from water by vortexing for 15 seconds, filtering 1 mL of water sample and analyzing using high performance liquid chromatography-diode array detector. Imidacloprid was extracted from soil by combining 15 g of processed above- or below ground vegetation with 20 mL acetonitrile in a 225 mL high-density polyethylene container, shaken for 30 minutes and centrifuged for 10 minutes (Moghaddam et al. 2012). Imidacloprid was extracted from plant tissue by combining 10 g of processed above- or below ground vegetation with 20 mL acetonitrile in a 225 mL high-density polyethylene container, shaken for 30 minutes and centrifuged for 10 minutes (Ge et al. 2017). Chlorophyll pigment was removed by vortex mixing 1.5 mL extracted sample with 2.5 mg graphitized carbon (QuECHERS, Bristol, PA) and centrifuging. 1 mL of extracted sample was filtered and analyzed using HPLC-DAD methodology. Concentrations above the calibration curve were diluted and re-injected for analysis. Limits of quantification and detection were 0.05 and 0.01 mg L^{-1}, respectively. Fortification recovery checks for water, soil and plant tissue samples ranged from 101-113%, 94-100%, and 89-97%.

Percent of applied values were also converted into percent reduction to compare planted containers to non-planted containers

(Eq. 1):

Percent reduction (soil) = [1- (planted container soil (µg) / non-planted container soil (µg)) x 100]

Percent of recovered was calculated to quantify each matrix (water, soil, above-ground vegetation or below ground vegetation) compared to the total residue detected within each container

(Eq. 2):

$$\text{Percent of recovered} = [(\text{select matrix } (\mu g) / \text{total matrices } (\mu g)) \times 100]$$

Remaining reductions in residue recovery were found between total pesticide recovered in planted and non-planted pots

(Eq. 3):

$$\text{Percent reduction (total recovered)} = \{[(\text{non-planted total } (\mu g) - \text{planted total } (\mu g)) / \text{non-planted total } (\mu g)] \times 100\}$$

Experimental Design and Statistical Analysis

The experiment was conducted in a 3-by-2 factorial arrangement including three plant species (*J. effuses, P. cordata, and S. latifolia*) and two pesticides (azoxystrobin and imidacloprid). Three replicates of each plant and pesticide combination were arranged in a randomized complete block design and rotated weekly to minimize greenhouse variability. Data were subjected to ANOVA ($\alpha < 0.05$) using MIXED procedure in SAS (Statistical Analysis Software®, Version 9.2, SAS Institute, Inc., Cary, NC). Fixed effects included pesticide, plant species and sample collection timing. Means were separated using Fisher's Protected LSD ($\alpha = 0.05$).

Results and Discussion

Control samples across all sample matrices and experimental runs did not detect azoxystrobin or imidacloprid (i.e., no contamination of experimental units). Data was pooled for statistical analysis due to no detection of significant interactions between experimental run and pesticide (P

= 0.198), DAT (P = 0.254), sample matrix (P = 0.565), or plant species (P = 0.243). The main effect of DAT ($P \leq 0.0001$), run ($P \leq 0.0001$) and sample matrix ($P \leq 0.0001$) as well as interactions with all fixed effects ($P \leq 0.0001$) were significant; therefore, data were sorted by sample matrix then DAT and presented accordingly. There were no visible or measurable indicators of plant injury between pesticide treatments and non-treated plants (data not shown).

Water

When comparing planted containers to their assigned non-planted containers, percent reduction of pesticide was calculated and found significantly different for azoxystrobin and imidacloprid after 56 days (Table 1). At 56 DAI, *P. cordata* was reported to reduce the amount of azoxystrobin more (51.7% reduction) than *J. effuses* and *S. latifolia* (24.9 and 28.7%). *S. latifolia* reduced more imidacloprid (79.3% reduction) compared to *J. effuses* (36.0%) and *P. cordata* (37.1%) at 56 DAT. Azoxystrobin and imidacloprid in water was quantified as a percent of recovery compared to total pesticide recovery in each container (Table 2). At 14 DAT, azoxystrobin recovery in water ranked: *J. effuses* (86.7% of recovered) > *S. latifolia* (81.9%) > *P. cordata* (77.9%). At 28 DAT, *S. latifolia* planted containers detected more azoxystrobin (74.2% of recovered) compared to *J. effuses* (65.3%) and *P. cordata* (64.4%). At 56 DAT, *S. latifolia* planted containers detected more azoxystrobin (73.5% of recovered) compared to *J. effuses* (40.3%) and *P. cordata* (34.3%). At 14 DAT, imidacloprid recoveries in water ranked: *P. cordata* (88.0% of recovered) > *J. effuses* (86.4%) > *S. latifolia* (83.4%). At 28 DAT, imidaclopried recoveries ranked: *P. cordata* (87.0% of recovered) > *S. latifolia* (77.6%) > *J. effuses* (72.4%). At 56 DAT, imidacloprid recoveries ranked: *S. latifolia* (74.3% of recovered) > *P. cordata* (61.5%) > *J. effuses* (44.0%). Lv et al. (2016) reported greater reductions of fungicides imazalil and tebuconazole in hydroponic solutions by four plant species: *Juncus* *effuses*, *Typha latifolia*, *Phragmites australis*, and *Iris pseudacorus*, which reduced imazalil by 67, 53, 96, and 46%, respectively, after 24 days. While these macrophytes were able to reduce the amount of other fungicides in half the amount of time, it is important to note the higher active ingredient added to the hydroponic system (5 mg a.i. 500 mL^{-1} solution). It is also imperative to mention the different behaviors of these pesticides in hydroponic vs aquatic systems with soil. Fungicides, such as azoxystrobin, imazalil, and tebuconazole, are typically bind more tightly to soils and decrease movement, which can be seen in these chemical's organic carbon-water partition coefficient (K_{OC}): 395 ml g^{-1}, 4,324 mL g^{-1}, 1251 mL g^{-1}, respectively (O'Rourke 1997, Shackleford 2002, Montague et al. 2000). With the addition of soil, pesticides bind more to soil particles, are less available to plant tissue absorption and therefore phytoremediation capabilities are hindered. Comparing this study to Lv. et al.'s and adjusting for soil sorption, *J. effuses*, *P. cordata*, *S. latifolia*, *T. latifolia*, *P. australis*, and *I. pseudacoru*s are able to phytoremediate azoxystrobin, imazalil, and tebuconazole; with *P. australis* and *J. effuses* leading remediation capabilities. Similarly, Riaz et al. (2017) reported plant species *E. crassipes* and *Pistia strateotes*, as well as algal species *Chaetomorpha sutoria*, bioaccumulate more organochlorine and pyrethroid insecticides compared to sterilized soil. *E. crassipes* removed 60% of organochlorine and 68% of pyrethroid pesticides, *P. stratiotes* removed 62% and 76%, and *C. sutoria* removed 58% and 70%, respectively, after 7 days as compared to controls which did not contain a plant or algae. Riaz et al. treated the pots with 1 mg a.i. L^{-1} solution and replenished with distilled water to account for transpiration. After adjusting for more transpiration occurring within planted pots compared to non-planted pots, *E. crassipes*, *P. strateotes* and *C. sutoria* are found to have the same remediating trend as *J. effuses*, *P. cordata*, and *S. latifolia* remediating imidacloprid after 56 days.

Soil

Azoxystrobin recoveries in soil were quantified as a percent of recovery compared to total pesticide recovery in each container (Table 3). At 14, 28 and 56 DAT, *J. effuses* contained greater azoxystrobin (8.5%, 26.9% and 36.7% of recovered, respectively) compared to *P. cordata* (5.5%, 10.1%, and 12.6%, respectively) and *S. latifolia* (4.1%, 7.7%, and 15.1%, respectively). At 14 DAT, *J. effuses* contained more imidacloprid (12.4% of recovered) compared to *P. cordata* (8.9%) and *S. latifolia* (8.1%). At 28 DAT, imidacloprid recovery in soil ranked: *J. effuses* (21.7% of recovered) > *S. latifolia* (12.5%) > *P. cordata* (6.3%). At 56 DAT, imidacloprid recovery in soil ranked: *J. effuses* (42.1% of recovered) > *P. cordata* (29.0%) > *S. latifolia* (19.9%). Soil accumulated azoxystrobin and imidacloprid over time due to the compounding addition of pesticide during water replenishments.

Plant Tissue

Above-ground Vegetation

At 14 DAI, azoxystrobin residue was consistent between plant species (ranging from 1.6 to 6.8% of recovered, respectively) (Table 4). Azoxystrobin residue in above-ground vegetation was found significantly different among plant species after 28 and 56 days. At 28 DAI, *J. effuses* contained less azoxystrobin (2.9% of recovered) compared to *P. cordata* (8.1%) and *S. latifolia* (8.3%). At 56 DAI, *J. effuses* and *S. latifolia* contained less azoxystrobin (6.1 and 5.0% of recovered) compared to *P. cordata* (13.3). Imidacloprid residue in above-ground vegetation was reported consistent at each sample collection time throughout the study. At 14 DAI, imidacloprid detection in above-ground vegetation in all three plant species ranged from 0.8 to 5.4% of recovered. At 28 DAI, above-ground vegetation ranged from 2.3 to 4.5% of recovered imidacloprid. At 56 DAI, above-ground vegetation ranged from 2.7 and 5.7% of recovered imidacloprid. Bouldin et al. (2006) reported a translocation increase of 32.0% of 0.028 kg a.i. lambda-cyhalothrin ha^{-1} application and 25.4% of 2.23 kg a.i. atrazine ha^{-1} application into *J. effuses* above-ground biomass after 8 days.

Below-ground Vegetation

Azoxystrobin residue detected in below-ground vegetation was significantly different at 14 and 56 DAI (Table 5). At 14 DAI, *J. effuses* contained less azoxystrobin (3.2% of recovered) in below-ground vegetation compared to *P. cordata* (13.4%); however, *S. latifolia* was not found significantly different (7.2%). At 28 DAI, azoxystrobin detection was not found significantly different among the three plant species and ranged from 4.9 to 17.4% of recovered. At 56 DAI, *J. effuses* and *S. latifolia* contained less azoxystrobin (16.9 and 6.4% of recovered, respectively) in below-ground vegetation compared to *P. cordata* (39.8%). Imidacloprid residue detection in below-ground vegetation was found significantly different only after 56 days. At 14 DAI, imidacloprid detection in below-ground vegetation was consistent in all three plant species and ranged from 0.4 to 3.1% of recovered. At 28 DAI, imidacloprid was also reported to be consistent through each plant species and ranged from 2.2 to 5.5% of recovered. However, at 56 DAI, *P. cordata* and *S. latifolia* contained less imidacloprid (3.8 and 3.1% of recovered, respectively) in below-ground vegetation compared to *J. effuses* (9.8%).

Compared to Romeh's (2015) study quantifying the phytoremediation of azoxystrobin in soil by *P. major*, *H. annus*, and *G. max*, it was reported that azoxystrobin is absorbed by the roots and translocated to above-ground vegetation; however, majority was detected in the root mass. After 10 days of pesticide exposure, *P. major, H. annus*, and *G. max* accumulated 20.62 mg kg^{-1}, 18.29 mg kg^{-1}, and 25.32 mg kg^{-1}, respectively, in root tissue, and accumulated less in leaf tissue: 15.03 mg kg^{-1}, 9.8 mg kg^{-1}, and 3.12 mg kg^{-1}, respectively. Similarly, Potur et al. (2019)

reported a greater accumulation of imidacloprid from soil in root tissue compared to leaves and fruit of *Cucurbita pepo*. After 14 days, imidacloprid concentrations varied between 217.0-491.3 mg kg in *C. pepo* roots, 191.0-475.0 mg kg in leaves and 128.0-246.3 mg kg in stems. Bouldin et al. (2016) also reported the majority of atrazine (32% of applied) and lambda-cyhalothrin (72.1%) was detected in root tissue after 8 days.

Accelerated Degradation

A key component of phytoremediation is the ability of a plant to degrade, metabolize and/or detoxify the contaminating compound via phytotransformation, phytodegradation, and/or rhizoremediation (Lewandowski et al. 2006; Licht and Isebrands 2005). Throughout the study, planted pots were found to have a greater decrease of pesticide residue detection compared to non-planted pots after combing the recoveries from all matrices (Table 6). This reduction is due to either phytotransformation, phytodegradation, phytovolatilization, rhizoremediation, and/or the reaction with added fertilizer. Because of azoxystrobin and imidacloprid's low vapor pressure at room temperature, volatilization is not likely (Spencer 1982); therefore, the reduction in recovered pesticide residue is a result from the presence of plant metabolism, the microbial activity which is supported by the rhizosphere, and/or the addition of fertilizer prior to the study (Xia and Ma 2006).

Pesticide total residue detected in each container was compared between planted and non-planted containers to quantify the effect of vegetation on increased dissipation and was put into terms of "% reduction". At 14 DAI, all three plant species were consistent in % reduction of azoxystrobin residue in non-planted containers, ranging from 1.3 to 2.5% reduction (Table 6). Similarly, % reduction among plant species remained consistent at 28 DAI, ranging from 7.2 to 11.3%. However, at 56 DAI, *J. effuses* and *S. latifolia* were reported to have a greater %

reduction (26.9 and 42.4%, respectively) compared to *P. cordata* (4.5%). At 14 DAI, the three plant species were consistent in % reduction ranging from 16.0 to 23.9%. At 28 DAI, the three plants remained consistent in % reduction ranging from 21.6 to 45.7%. At 56 DAI, *S. latifolia* reported a greater % reduction (73.7%) compared to *J. effuses* (28.7%) which was also found significantly greater than *P. cordata* (1.3%). These trends give promise to *J. effuses*, *P. cordata* and *S. latifolia* efficiently remediating imidacloprid; however, with *J. effuses's* consistent increased degradation of azoxystrobin, it is recommended to establish this plant species near azoxystrobin treated areas. Residue reductions may have been altered due to phytoextraction, a process of phytoremediation which stabilizes contaminants in vacuoles, such as sunflowers are utilized to uptake and accumulate metals and then must be harvested (Hussain et al. 2009).

Romeh (2017) reported the decrease of the half-life of azoxystrobin under varying conditions including soil only ($T_{1/2}$ = 11.6 days), soil planted with P. major (8.5 days), and soil planted with *P.major* and treated with 20-100 mM NaCl (2.9-5.9 days). This half-life decrease in the presence of a known phytoremediating plant species is supporting evidence that plants provide increase degradation. Xia and Ma (2006) reported a decreasing rate of ethion by *E. crassipes* by 54.7-90.9% in shoots and 73.5-86.4% in roots after 1 week of incubation in a nutrient solution containing 1 mg L^{-1} ethion. While both of these studies have found that *P. major* and *E. crassipes* are very efficient phytoremediators and have decreased pesticide half-lives and reduced concentrations in hydroponic studies, it is imperative for further research to quantify pesticide phytoremediating capabilities in wetland plants that can be easily established and managed by landscapers, land managers and producers; as well as support the surrounding ecosystem.

Conclusion

From this research, data support the use of *J. effuses*, *P. cordata*, and *S. latifolia* as azoxystrobin and imidacloprid phytoremediating plant species in wetland areas. Relative to the concentration of pesticide found in planted pots compared to non-planted pots, *P. cordata* was found to reduce the amount of azoxystrobin (51.7% reduction) greater than *J. effuses* (24.9%) and *S. latifolia* (28.7%) from treated water after 56 days. In imidacloprid treated containers, *S. latifolia* reduced more pesticide (79.3% reduction) than *J. effuses* (36.0%) and *P. cordata* (37.1%) from treated water after 56 days. Plant tissue was analyzed and found 4.3% of applied azoxystrobin detected in above-ground vegetation and 8.6% in below-ground vegetation after 56 days. At the same sample collection time, 1.5% of applied imidacloprid was detected in each above-ground vegetation and below-ground vegetation. These data support the recommended practice of establishing wetland buffer systems to encapsulate and metabolize pesticide runoff to protect ecological health. Further research is recommended to quantify the phytoremediation of more commonly used pesticides, as well as the establishment of diverse plant species that can be utilized in urban, agricultural, and mixed landscapes.

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Tables

Table 1. Azoxystrobin and imidacloprid residue reduction in water compared to non-planted containers.

	Azoxystrobin					Imidacloprid				
	7 DAI	14 DAI	21 DAI	28 DAI	56 DAI	7 DAI	14 DAI	21 DAI	28 DAI	56 DAI
Plant	7 DAR	14 DAR	7 DAR	14 DAR	14 DAR	7 DAR	14 DAR	7 DAR	14 DAR	14 DAR
	% reduction									
J. effuses	16.6	31.6	11.8	12.9	24.9	8.1	26.8	51.1	38.8	36.0
P. cordata	6.4	17.1	14.3	21.1	51.7	6.6	25.5	34.1	36.3	37.1
S. latifolia	12.5	14.3	11.7	10.8	28.7	16.5	34.0	39.1	55.6	79.3
Non-planted	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$LSD_{0.05}$	10.5	5.2	4.4	12.0	23.9	5.6	11.7	13.4	19.8	21.7

Abbreviations: DAI, days after initiation; DAR, days after replenishment; NS, non-significant.

Equation: Percent reduction = [1- (planted container soil (µg) / non-planted container soil (µg)) x 100]

Table 2. Azoxystrobin and imidacloprid quantification in water relative to the total % of the recovered at each sampling day after treatment.

	Azoxystrobin			Imidacloprid		
Plant	14 DAI	28 DAI	56 DAI	14 DAI	28 DAI	56 DAI
	% of recovered					
J. effuses	86.7	65.3	40.3	86.4	72.4	44.0
P. cordata	77.9	64.4	34.3	88.0	87.0	61.5
S. latifolia	81.9	74.2	73.5	83.4	77.6	74.3
$LSD_{0.05}$	2.1	3.5	7.5	1.2	3.0	5.6

Abbreviations: DAI, days after initiation; NS, non-significant.
Equation: Percent recovered = [(water (µg) / total matrices (µg)) x 100]

Table 3. Azoxystrobin and imidacloprid quanitification in soil relative to the total % of the recovered at each sampling day after treatment.

	Azoxystrobin			Imidacloprid		
Plant	14 DAI	28 DAI	56 DAI	14 DAI	28 DAI	56 DAI
	% of recovered					
J. effuses	8.5	26.9	36.7	12.4	21.7	42.1
P. cordata	5.5	10.1	12.6	8.9	6.3	29.0
S. latifolia	4.1	7.7	15.1	8.1	12.5	19.9
$LSD_{0.05}$	1.8	3.3	4.3	2.2	2.0	5.1

Abbreviations: DAI, days after initiation; NS, non-significant.
Equation: Percent recovered = [(soil (µg) / total matrices (µg)) x 100]

Table 4. Azoxystrobin and imidacloprid quantification in above-ground biomass relative to the total % of the recovered at each sampling day after treatment.

	Azoxystrobin			Imidacloprid		
Plant	14 DAI	28 DAI	56 DAI	14 DAI	28 DAI	56 DAI
	% of recovered					
J. effuses	1.6	2.9	6.1	0.8	2.3	4.1
P. cordata	3.2	8.1	13.3	1.6	4.5	5.7
S. latifolia	6.8	8.3	5.0	5.4	4.4	2.7
$LSD_{0.05}$	NS	3.3	4.3	NS	NS	NS

Abbreviations: DAI, days after initiation; NS, non-significant.
Equation: Percent recovered = [(above-ground vegetation (µg) / total matrices (µg)) x 100]

Table 5. Azoxystrobin and imidacloprid quantification in below-ground biomass relative to the total % of the recovered at each sampling day after treatment.

	———Azoxystrobin———			———Imidacloprid———		
Plant	14 DAI	28 DAI	56 DAI	14 DAI	28 DAI	56 DAI
	——————————% o recovered——————————					
J. effuses	3.2	4.9	16.9	0.4	3.6	9.8
P. cordata	13.4	17.4	39.8	1.5	2.2	3.8
S. latifolia	7.2	9.8	6.4	3.1	5.5	3.1
$LSD_{0.05}$	7.8	NS	15.5	NS	NS	5.4

Abbreviations: DAI, days after initiation; NS, non-significant.
Equation: Percent recovered = [(below-ground vegetation (µg) / total matrices (µg)) x 100]

Table 6. Azoxystrobin and imidacloprid residue reductions compared between total % of the recovered in planted and non-planted containers.

	Azoxystrobin			Imidacloprid		
Plant	14 DAI	28 DAI	56 DAI	14 DAI	28 DAI	56 DAI
	% reduction					
J. effuses	2.5	7.2	26.9	16.0	21.6	28.7
P. cordata	2.3	11.3	4.5	16.1	29.2	1.3
S. latifolia	1.3	10.6	42.4	23.9	45.7	73.7
$LSD_{0.05}$	NS	NS	19.0	NS	NS	22.3

Abbreviations: DAI, days after initiation; NS, non-significant.
Equation: Percent reduction = {[(non-planted total (µg) – planted total (µg)) / non-planted total (µg)] x 100}