TOXICITY OF STROBILURIN FUNGICIDES TO *HYALELLA AZTECA*

By

SHANE A. MORRISON

Bachelor of Science in Chemistry

Saginaw Valley State University

University Center, Michigan

2010

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
MASTER OF SCIENCE
December, 2012

TOXICITY OF STROBILURIN FUNGICIDES TO

HYALELLA AZTECA

Thesis Approved:

Dr. Jason Belden

Thesis Adviser

Dr. Scott McMurry

Dr. Loren Smith

Dr. Tracy Quan

ACKNOWLEDGEMENTS

Partial funding for this project was provided by the U.S. Department of Agriculture and the Oklahoma State University Department of Zoology. Special thanks to Jessica M. Morrison and Kristal K. Sieve for their contributions with sample extraction, elution and evaporation processes.

Name: SHANE A. MORRISON

Date of Degree: DECEMBER, 2012

Title of Study: TOXICITY OF STROBILURIN FUNGICIDES TO *HYALELLA AZTECA*

Major Field: ZOOLOGY

Abstract:

Fungicide application rates on row crop agriculture have increased across the United States. As a result, contamination of adjacent aquatic systems can potentially occur through spray drift, unintentional direct spraying, or field runoff. To investigate the potential toxicity of the fungicides, the epibenthic aquatic amphipod *Hyalella azteca* was exposed to two common fungicide formulations Headline® and Stratego® and their individual active strobilurin ingredients. Water-only exposure studies for both formulations and active strobilurin ingredients resulted in LC_{50} values of 21 (18-25) for Headline®, 25 (21-30) for pyraclostrobin, 20 (19-22) for Stratego®, and 25 (18-38) µg/L for trifloxystrobin, suggesting that toxicity is primarily due to the active strobilurin ingredient. When the fungicides were added to the overlying water of sediment/water microcosms, toxicity was reduced by 500% for Headline® and 160% for Stratego® as compared to water-only exposures, based on the total amount of fungicide added to the systems. Additionally, when fungicides were added to the sediment 24 h prior to the addition of water and amphipods, the reduction in toxicity was even greater, with no toxicity occurring at levels that would be environmentally relevant. Differences in toxicity among exposure groups were explained by dissipation from water as toxicity values based on measured water concentrations were within 20% between all systems. Although aquatic based LC_{50} values are below environmental concentrations that would likely occur following a direct overspray event into water (150 and 74 µg/L for Headline® and Stratego® respectively), the presence of sediment in the system is likely to ameliorate some of the toxicity of fungicide formulations, especially if exposure to the wetland occurs prior to an inundation event.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

Figure Page

CHAPTER I

TOXICITY OF STROBILURIN FUNGICIDES TO *HYALELLA AZTECA*

Introduction

Fungicide application rates have increased on corn and other row-crops planted in the United States from less than 2% of the planted area in 2004 – 2005 (National Agricultural Statistics Service; http://www.pestmanagement.info/nass/app_usage.cfm) to approximately 25 – 30 % in 2009 (Letter from Universities Regarding the Strobilurin, Pyraclostrobin, Supplemental Label, 2009, http://www.epa.gov/pesticides/regulating/headlineletter.pdf). With greater percentages of crops seeing fungicide applications, total fungicide mass per hectare has increased significantly for corn, soybean, and winter wheat in the last decade (See Table 1) (National Agricultural Statistics Service; http://quickstats.nass.usda.gov). Typical cultural practices of varying planting date, row width, and crop rotation have been ineffective at combating continuing outbreaks of soybean rust (*Phakopsora pachyrhizi*), leaving strobilurin fungicides as the only effective means of controlling the disease [1, 2]. Additionally, two of the most prominently used fungicides, Headline® (BASF) and Stratego® (Bayer CropScience), have had recent supplement label changes to include statement claims of increased nutrient uptake, general plant health and production even in the absence of fungal disease [3-5]. Collectively, these reasons are contributing factors to the recent increase fungicide use. Environmental impacts may be of rising concern in the future due to increased fungicide application rates in response to continuing disease pressures and recent label changes allowing for prophylactic use [1].

Typically, these fungicides are aerially applied to crops resulting in the distribution of the chemicals across the crop and the possibility of direct exposure to adjacent or imbedded aquatic environments [3, 5]. Because most wetlands within the mid-continent of North America are located near areas used for row crop agriculture [6], wetland organisms are at risk for exposure through spray drift or unintentional direct spraying. This is especially true for depressional playa wetlands, the dominant aquatic feature of the Great Plains [7], where crop cultivation often extends to the edge or through non-inundated wetlands [8]. These imbedded cropland playa wetlands are often shallow (< 2 m) and have shortened hydroperiods [7]. Croplands are a major influencing factor on imbedded playa wetlands because they are depressional recharge wetlands that drain catchments (e.g., are at the lowest elevation in the watershed). The primary hydrologic inputs to playas are precipitation and overland runoff, while outputs are evapotranspiration and groundwater recharge [7]. Many other midcontinental prairie and steppe basin wetlands share the same drainage, recharge and habitat use characteristics as playa wetlands such as the prairie potholes of the Upper Midwest and the Rainwater Basin and Sandhills wetlands of central Nebraska [9].

Recent studies have shown that agricultural fungicides Headline® and Stratego® have toxic effects to non-target animals following aquatic exposures. Both active strobilurin and azole fungicide ingredients of these formulations cause acute toxicity to water fleas (*Daphnia magna*) at concentrations below 500 µg/L [10]. Pyraclostrobin, the active strobilurin fungicide ingredient of Headline®, has been shown to be toxic to freshwater mussels (*Lampsilis siliquoidea*) at median effective concentrations (EC_{50}) below 50 µg/L [11]. Pyraclostrobin is also toxic to bluegill sunfish (*Lepomis macrochirus*) with a median lethal concentration (LC_{50}) of 11 µg/L [12]. Trifloxystrobin, the active strobilurin ingredient in Stratego®, has a LC_{50} value of 54 µg/L for bluegill whereas propiconazole, the second active ingredient in Stratego®, was less toxic with a LC_{50} of 4,500 µg/L [13]. Belden et al. (2010) showed that both formulations caused acute

toxicity to the Great Plains Toad (*Bufo cognatus*) tadpoles and juvenile toads at environmentally relevant concentrations [14]. Hooser et al. (2012) also tested fungicide toxicity to *B. cognatus* tadpoles and found no differences in toxicity between Stratego® formulation and trifloxystrobin whereas, the Headline® formulation was slightly more toxic than pyraclostrobin alone; nonetheless, both Headline® and pyraclostrobin resulted in LC_{50} values of 3.7 µg/L and 10.0 µg/L, respectively [15]. However, these studies were performed using water-only exposures and limited toxicological data is available in peer-reviewed literature for aquatic invertebrates.

Within a wetland, we would expect these fungicides to dissipate from water through degradation processes and/or adsorption to sediment, organic matter and suspended particulates as the active ingredients for Headline® (pyraclostrobin) and Stratego® (trifloxystrobin and propiconazole) have soil sorption constants (log K_{oc}) of 4.04, 3.39, and 3.26, respectively [16, 17]. However, it is unclear the extent to which these losses would be protective of aquatic life. Previous work by Belden et al. (2010) has found acute toxicity occurs within hours of exposure; therefore, toxicity may occur before the fungicides partition to the sediment. Furthermore, exposure may continue to occur to some benthic or epibenthic aquatic organisms, albeit from a different less bioavailable route of sediment desorption. For instance, invertebrate detritivores such as amphipods forage on leaf detritus and other litter deposited above the sediment and are therefore important species for sediment associated compounds because they inhabit the sediment-water interface where exposure will be greatest via both water and sediment [18]. *Hyalella azteca* is probably the best studied epibenthic aquatic amphipod in regard to toxicity of pesticides and frequently serves as a model organism in aquatic toxicity tests for a broad range of environmental contaminants [18]. *H. azteca* were chosen for this study because they live at the water-sediment interface where exposure to fungicide formulations are likely to occur due to the relatively high log K_{oc} values of the active strobilurin ingredients.

The first objective of this study was to evaluate the toxicity of the fungicide formulations Headline® and Stratego® as compared to the individual active strobilurin ingredients to *H. azteca* in a water-only exposure. The second objective was to investigate the toxicity of Headline® and Stratego® in sediment/water microcosms to determine how exposure routes influence toxicity in sediment/water systems. Specifically, we compared toxicity between microcosms where the overlying water was treated to those where the sediment was treated prior to the addition of water.

Methods and Materials

Test Chemicals

Two fungicide formulations commonly applied aerially were acquired from a local distributor: Headline® Fungicide (U.S. Environmental Protection Agency [U.S. EPA] Reg. 7969-186, BASF), Stratego® Fungicide (U.S. EPA Reg. 264-779, Bayer Crop Science). Formulation concentrations were mixed with dechlorinated water to replicate expected environmental concentrations based on maximum label application rates for corn determined by Belden et al. (2010). Concentrations of the formulation represented the concentration of the strobilurin active ingredient it contained. Analytical grade pesticides, pyraclostrobin (99.9% purity) and trifloxystrobin (99.5% purity), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solutions containing the individual active strobilurin ingredients were dissolved in acetone from neat material to achieve the same concentration of active ingredient in the formulation concentrations based on percentages of the active ingredients in the formulations. Analytical grade deuterated PAHs were purchased from Accustandard (New Haven, CT, USA) as internal standards. All solvents and reagents used in reagent make-up or sample preparations were at least pesticide grade. Dechlorinated water was obtained by carbon filtration of Oklahoma State University tap water (pH: 7.5 – 7.7, Hardness: 80 – 100 mg calcium carbonate/L, and DO: 6.7 –

9.0 mg/L) and was used to carry out all toxicity tests. Sediment was collected from a depressional playa wetland from the Rainwater Basin of Central Nebraska. The wetland was dry at the time of sediment collection and was imbedded in a cropland that had been cultivated the previous year; however, no standing crop vegetation was present at the time of collection. Sediment was sieved to a 2 mm particle size to remove excess organic debris and obtain a uniform particle size. The textural class was a silty clay loam consisting of 16.2% sand, 50% silt and 33.8% clay with an overall organic matter content of 6.78%. Oklahoma State University's Soil, Water and Forage Analytical Laboratory (Stillwater, OK) determined the composition and classification of the sieved sediment. The sediment was analyzed for a suite of pesticides based on Belden et al. (2012) and no detectable pesticides were found (less than 10 ppb including many current use insecticides and herbicides, including atrazine) [19].

Test Organisms

Juvenile *H. azteca* were obtained from pre-existing cultures housed at Oklahoma State University. Cultures were kept in a flow-through system with dechlorinated water and coarse sand substrate. Organisms were fed a combination of Hikari Tropical algae wafers (Kyorin USA Inc., Teaneck, NJ) and Tetramin® (Blacksburg, VA) fish food every other day until collected for aquatic toxicity testing. Juvenile amphipods were haphazardly collected for aquatic toxicity testing using stainless steel sieves and held for 7 days; individuals between the sieve size 250 and 500 μm were used in this study. Based on unpublished data presented in US EPA (2000), this method should provide amphipods that are approximately 12 days old at the start of the test.

Overall Experimental Design

Each experiment was setup with five concentrations and a control with either formulation or the active strobilurin ingredient applied to the experimental unit. Each experimental unit contained 10 juvenile amphipods and a known concentration of active strobilurin ingredient or

formulation. Concentrations corresponding to formulations represented the concentration of the active strobilurin ingredient it contained, not the concentration of the formulation as a whole. The active strobilurin ingredients (pyraclostrobin or trifloxystrobin) were introduced with 100 µL of acetone as a solvent carrier and the corresponding control organisms received 100 µL of pure acetone. Formulations suspended in dechlorinated water were added to experimental units to achieve the same concentration of active ingredient as the non-formulation units. Water quality measurements were taken every 24 h for the duration of the experiments with dissolved oxygen concentrations ranging from 3.2 – 9.0 mg/L, temperature 23 (± 1)°C and pH 7.2 – 7.5 with a 16:8 h light:dark cycle using low indirect light. Organisms were fed daily by adding 1.0 mL of 1800 mg/L stock solution of ground Tetramin® fish food into each experimental unit for all experiments [18].

Water-only Toxicity Tests

Water-only exposures were conducted in experimental units consisting of 600 mL glass beakers containing 500 mL dechlorinated water and one small piece of nylon mesh screen. Four replicates were performed for each treatment. Concentrations for Headline® and pyraclostrobin were 150, 70, 30, 12 and 5 µg/L. Concentrations for Stratego® and trifloxystrobin were 150, 74, 37, 18 and 9 µg/L. Although Stratego® contains both trifloxystrobin and propiconazole fungicides, Hooser et al. (2012) showed that propiconazole did not contribute to the overall toxicity; therefore, we did not conduct an exposure scenario with propiconazole alone. Mortalities were checked six hours and every 12 h after the start of the exposure for 10 d. Organisms found dead during any time check were removed from the units. Organisms that were found to be non-responsive to gentle prodding with a glass rod were considered dead and removed from the experimental unit. Analyte concentrations were measured at multiple time points throughout the toxicity tests. Aliquots (10 mL) from each experimental unit were sampled and pooled with other samples from the same treatment concentration.

Microcosm Toxicity Tests

Microcosm exposures were conducted in glass jars consisting of 800 mL dechlorinated water and 100 g of sediment. Experimental units were treated with fungicide formulations in two ways: 1) fungicide formulation diluted in water was added directly to the overlying water and gently stirred into solution; and 2) sediment was sprayed using a MADomizer® (Wolfe Tory Medical) to achieve an even distribution across the sediment surface 24 h prior to the addition of overlying water. Sediment treated microcosms were also gently stirred following the addition of water for consistency across treatments. Six replicates were performed for each treatment. Amphipods were added to water treated microcosms prior to treating the overlying water and were added to sediment treated microcosms immediately following the addition of water to the system. Concentrations applied per sediment /water system for Headline® were 284, 107, 39, 14, and 5 µg/L. Concentrations applied per sediment /water system for Stratego® were 293, 103, 39, 14, and 6 µg/L. Concentrations listed for each formulation represent the concentration based on full water incorporation into the 800 mL of water. Although it was expected that partitioning between matrices would occur, concentrations are expressed based on full water incorporation as a mechanism to contrast routes of entry into the microcosm and compare results to previously published work that utilized water concentrations based on the worst-case scenario of full water incorporation [14, 15]. Mortalities were assessed at 168 h by passing the contents of each jar through 500 and 250 µm stainless steel sieves and individually removing surviving amphipods from the sediment surface [18]. The microcosm toxicity tests were ended at seven days because no additional toxicity was observed in the water-only toxicity tests after 168 h, despite exposure lasting for 10 d. Analyte concentrations were monitored throughout the experiment by combining 5 mL aliquots removed from each of three replicates 2 cm above the sediment for each concentration to obtain two duplicate samples for analysis from the six replicate experimental units.

Microcosm Fungicide Fate Tests

A secondary test was conducted to directly determine the degree of fungicide partitioning to the sediment within the microcosms because mortalities of *H. azteca* were scored at the end of the toxicity tests and the sediment sampling method required the destructive take-down of the experimental units. Microcosm treatments were setup using the same methods as the toxicity tests to include both sediment and water treated pathways except only three replicates were used. The amount of fungicide applied to the sediment/water system corresponded to a water concentration of 300 µg/L (assuming full water incorporation) or a sediment concentration of 2300 µg/kg sediment concentration (assuming complete adsorption to the sediment) for both for both Headline® and Stratego®. Three replicates and a control were destructively taken down for each treatment at the following time points: 3, 12, 48, 96, and 168 h. Water column analyte concentrations were monitored by removing 15 mL aliquots from each replicate 2 cm above the sediment for each take-down time point. Sediment bound analyte concentrations were monitored by decanting the overlying water, homogenizing the sediment and extracting 1.5 g of sediment from a subsample representing the entire homogenized sample within the microcosm.

Analytical determination of fungicides in water and sediment samples

Acquired water samples were passed through 1000 mg C18 SampliQ® SPE cartridges (Agilent Technologies, Santa Clara, CA, USA). Cartridges were conditioned with 5 mL methanol and 10 mL distilled water and samples were extracted at a rate of approximately 3.5 mL/minute. Water extraction cartridges were centrifuged at 4000 rpm for 4 min. to removed any excess water and kept frozen at -20°C until further use. Fungicides were eluted from the columns with 8 mL of ethyl acetate. Collected effluents were also stored at -20°C until evaporated to dryness under a gentle stream of nitrogen and brought to a final volume of 500 µL with isooctane.

Sediment samples were centrifuged at 4000 rpm for 4 min. to separate excess water which was then removed by pipette. Samples were then stored frozen at -20°C until further use. Percent solids were determined for each sediment sample by drying 5.0 g sediment overnight at 100°C. Sediment dispersion was performed by combining 1.5 g sediment with 750 mg diatomaceous earth, 900 mg florisil, and 50 mg primary secondary amine (PSA) bounded silica in a mortar and grind together until homogenized. Analyte extraction was conducted by passing 15 mL solvent solution of 1:2 hexane:ethyl ether through an extraction column assembled from the bottom up with 500 mg activated silica gel, 1000 mg sodium sulfate, and finally the dispersed sediment, collecting the effluent. Collected effluents were also stored at -20°C until evaporated to dryness under a stream of nitrogen and brought to a final volume of 500 µL in isooctane.

Analysis of extracts performed using gas chromatography/mass spectrometry (GC/MS) (Agilent 5975c, Santa Clara, CA, USA). The GC inlet temperature was set at 240°C and the oven program started at 70°C and increased over 30 minutes to 290°C. Select ion monitoring (SIM) was utilized with the following quantitation:qualifier ions pyraclostrobin (132:133, 164), trifloxystrobin (116:206, 222), propiconazole (259:173, 261). Chrysene D12 (240:241) and perylene D12 (264:265) were used as internal standards for calibration. Method precision and accuracy was monitored by spiking distilled water and sediment blanks with individual fungicide ingredients (n=4 for each matrix). Percent extraction efficiencies (± standard error) for the active ingredients spiked in water were 83% (±4), 72% (±10), and 100% (±10) for pyraclostrobin, trifloxystrobin, and propiconazole, respectively. Efficiencies for the active ingredients spiked on sediment were 129% (±9), 63% (±2), and 83% (±6) for pyraclostrobin, trifloxystrobin, and propiconazole, respectively. Due to decreased quantitative capabilities, sample trifloxystrobin concentrations were adjusted based on relative extraction efficiencies.

Statistical analysis

Differences in mortalities between formulation and active strobilurin ingredients during water-only exposures and mortality differences for water verses sediment treated microcosm exposures were determined using IBM SPSS Statistics Data Editor® (Armonk, NY, USA). A univariate general linear model was implemented to determine analysis of variance (ANOVA) and a Tukey's HSD (α=0.05) was used for post-hoc tests. Percent mortalities were determined by averaging replicates for the corresponding treatment concentrations.

Water concentrations representing the average measured concentrations were calculated from the start of the experiment to 96 h. Most of the toxicity occurred within 48 – 96 h; therefore, the concentrations within this time period are likely the most relevant to the observed toxicity. Pyraclostrobin and propiconazole proved to be extremely stable during the water-only concentrations, staying within 15% of the initial concentration over 96 h; therefore, 96 h average concentrations were calculated by averaging measurements from 6 to 96 h. Trifloxystrobin water-only concentrations and all fungicide concentrations in the microcosm test declined throughout the exposures, following a first order exponential decay function. Therefore, weighted water concentration averages were calculated by fitting an equation to the average concentrations over time and taking the average of the curve using the equation:

$$\text{Weighted Water Conc.} = \frac{\int_{\text{a}}^{\text{b}} f(x)dx}{(\text{b} - \text{a})}$$

where a = 0 h, b = 96 h and $f(x)$ = a first order exponential decay function . Lethal concentration values (LC_{50} and LC_{10}) and confidence limits were calculated using a log probit analysis of mortalities and the average water concentrations using IBM SPSS Statistics Data Editor®.

Results

Water-only toxicity of formulations and active strobilurin ingredients

Control mortality was < 5% for all experiments. Measured water concentrations for pyraclostrobin, trifloxystrobin and propiconazole were 122(±28)%, 78(±32)% and 136(±38)%, respectively, of targeted concentrations over the entire test. On average, measured water concentrations declined 13(±9)%, 59(±12)% and 17(±15)% for pyraclostrobin, trifloxystrobin and propiconazole, respectively, from the initial concentrations over 168 h.

Exposure to Headline® resulted in a lowest effective concentration (LOEC) of 12 µg/L ($p < 0.001$) whereas pyraclostrobin the LOEC was 30 µg/L ($p < 0.001$). There was a significant difference, albeit slight, between mortalities between Headline® and pyraclostrobin ($p=0.035$; Fig. 1). However, the LC_{50} and LC_{10} values were similar, and 95% confidence intervals overlapped (Table 2). Exposure to Stratego® formulation and trifloxystrobin resulted in LOECs of 37 µg/L ($p < 0.001$); however, there was no significant difference between the two treatments ($p=0.271$; Fig. 1). This is supported by overlapping LC_{50} and LC_{10} confidence intervals for the Stratego® and trifloxystrobin (Table 2). All calculated LC_{50} values were well below environmental concentrations expected from a direct overspray event and complete partitioning to water for both Headline® (150 µg/L) and Stratego® (74 µg/L) (Table 2) based on calculations made by Belden et al. (2010) for the maximum application rate of corn (800 mL/ha for Headline® and Stratego®) and water depth of 16 cm.

Toxicity of Headline® in sediment/water microcosms

Control mortality was < 10% across all experiments. Pyraclostrobin rapidly dissipated from the water following application of Headline® to the overlying water within the microcosms. Pyraclostrobin water concentrations across all treatment concentrations were 28% (±2) of that expected based on full water incorporation at the first measured time point (4 h) during microcosm toxicity tests (Fig. 2). Water concentrations continued to decline throughout the test with 13% (±8) remaining at 96 h and 9% (±7) after 168 h (Fig. 2). Based on the parallel

microcosm experiment conducted to study the fate of pyraclostrobin following the application of Headline®, pyraclostrobin water concentrations declined 69% whereas sediment concentrations increased 56% from initial measurements by 168 h (Fig. 3). However, no differences were observed in the total mass balance of pyraclostrobin in system from beginning (59% [±6]) to the end (62% [±4]) due to the dissipation from the water column. Thus, partitioning of pyraclostrobin into the sediment accounts for the majority of the declining water concentrations with other dissipation processes contributing to the 30-40% being lost from the system.

Observed toxicity was lower when Headline® was applied to the overlying water in the microcosm as compared to the aquatic water-only toxicity tests. Sediment/water microcosm systems reduced the toxicity by 500% of the observed toxicity in water-only tests when the overlying water was treated, based on the total amount of fungicide applied to the system (LC_{50} values of 85.5 and 17.0 µg/L, respectively; Table 2 and Table 3). However, LC_{50} (18.3 and 85.5 µg/L) and LC_{10} (3.9 and 20.0 µg/L) values for Headline® were lower (non-overlapping 95% confidence intervals) when calculated using average measured water concentrations as compared to concentrations based on full water incorporation of the total fungicide applied to the sediment/water system (Table 3). LC_{50} values for Headline® water-only toxicity tests and water treated microcosms were similar when calculated using measured water concentrations (21.0 and 18.3 µg/L, respectively; Table 2 and Table 3). However, water-only LC_{10} values were higher (non-overlapping 95% confidence intervals) as compared to corresponding values in microcosms receiving water applications of Headline® (9.8 and 3.9 µg/L, respectively; Table 2 and Table 3).

Application of Headline® to the sediment 24 h prior to adding water resulted in minimal partitioning of pyraclostrobin from the sediment into the water column. The highest pyraclostrobin water concentrations across all treatments (2% [±3]) were observed at the first measurement (4 h), with concentrations decreasing to < 1% of the total expected from full water incorporation for the remainder of the microcosm toxicity tests (Fig. 2). Measured water and

sediment concentrations declined to 65% and 45% of the initial measured concentrations respectively by 168 h based on the parallel fate experiment (Fig. 3). There was a pronounced loss of pyraclostrobin from the system when Headline® was applied to the sediment with total mass balance recoveries declining from 81% (±10) to 43% (±9) at 168 h. When Headline® was applied to sediment, pyraclostrobin was not readily released into the overlying water; therefore, association with the sediment and degradation mechanisms are responsible for the loss of detectable pyraclostrobin from the system.

No toxicity was observed in microcosms where sediment was treated with Headline® under the conditions tested (Fig. 4). Pyraclostrobin water concentrations approached LC_{10} levels (based on water-only exposures) at the highest treatment level (284 µg/L); however, no significant differences were observed in mortality between the controls and sediment treated microcosms for Headline® at any treatment concentration ($p=1.00$; Fig. 4 and Table 3). Due to the lack of toxicity, lethal concentration values could not be estimated other than expressed as exceeding the measured water concentrations (Table 3). Water verses sediment treatment applications of Headline® resulted in significantly different mortalities at the two highest treatment concentrations 107 µg/L and 284 µg/L ($p<0.001$) and non-overlapping LC_{50} and LC_{10} values between treatments (Fig. 4 and Table 3).

Toxicity of Stratego® in sediment-water microcosms

Control mortality was < 10% across all of the experiments. Trifloxystrobin rapidly dissipated from the water following application of Stratego® to the overlying water within the microcosms. Trifloxystrobin concentrations across all treatment concentrations were 73% (±11) of that expected based on full water incorporation at the initial time point (4 h) during microcosm toxicity tests (Fig. 2). Water concentrations continued to decline throughout the test with 25% (±2) remaining after 96 h and 13% (±3) 168 h (Fig. 2). Based on the parallel microcosm

experiment conducted to study the fate of trifloxystrobin following the application of Stratego® to the overlying water, trifloxystrobin water concentrations declined by 84% and sediment concentrations declined 73% over the duration of the experiment (Fig 3). The consistent loss of trifloxystrobin from both water and sediment coincided with a mass balance decrease of trifloxystrobin in Stratego® water treated microcosms from 86 (±14) to 16% (±3) at 168 h. The total percentage of trifloxystrobin lost from Stratego® water treated microcosms was 30% greater than the observed loss in water-only exposures. Thus, partitioning of trifloxystrobin into the sediment contributes to the declining water concentrations; however, the majority of the losses occur due to other dissipation processes. Based on the parallel microcosm experiment conducted to study the fate of propiconazole (the second fungicide ingredient in Stratego®), water concentrations declined 49% and sediment concentrations increased 24% from the initial measurements by 168 h (Fig. 3). The mass balance of propiconazole in Stratego® water treated microcosms decreased from 71 (±5) to 58% (±7) by 168 h. Thus, dissipation from the water column can be partially contributed to partitioning to the sediment with the remaining 40-50% being completely lost from the system due to other dissipation processes.

Similar to Headline® toxicity trends, observed toxicity was much lower when Stratego® was applied to the overlying water in the microcosm as compared to the aquatic water-only toxicity tests. The microcosm sediment/water system reduced toxicity by 160% as compared to water-only tests when the overlying water was treated, based on the total amount of fungicide applied to the system (LC_{50} values of 25.8 and 43.1 µg/L, respectively; Table 2 and Table 3). However, LC_{50} (16.3 and 43.1 µg/L) and LC_{10} (4.0 and 13.8 µg/L) values for Stratego® were lower (non-overlapping 95% confidence intervals) when calculated based on measured water concentrations as compared to concentrations based on full water incorporation of the total fungicide applied to the sediment/water system (Table 3). LC_{50} values for Stratego® water-only toxicity tests and water treated microcosms were similar when calculated using measured water

concentrations (20.4 and 17.8 µg/L, respectively; Table 2 and Table 3). However, water-only LC_{10} values were higher (non-overlapping 95% confidence intervals) as compared to corresponding values in microcosms receiving water applications of Stratego® (14.2 and 4.0 µg/L, respectively; Table 2 and Table 3).

Application of Stratego® to the sediment 24 h prior to adding water resulted in minimal partitioning of trifloxystrobin from the sediment into the overlying water, with the exception of the highest treatment concentration. During microcosm toxicity tests, highest trifloxystrobin water concentrations across all treatment concentrations (8% [±2]) were observed at the first measurement (4 h), with concentrations falling to 5% (±2) by 96 h and continuing to fall to 3% (±1) that of the total expected from full water incorporation by 168 h (Fig. 2). Measured water and sediment concentrations declined to 75 and 90% that of the initial measured concentrations respectively by 168 h based on the parallel fate experiment (Fig. 3). Trifloxystrobin was immediately lost from the system when Stratego® was applied to the sediment with total mass balance recoveries declining from 55% (±25) at 4 h to 8% (±2) at 168 h. Sediment application of Stratego® resulted in higher rates of dissipation of trifloxystrobin as compared to water-only exposures; however, dissipation processes other than sediment association are responsible for these losses. Based on the parallel microcosm experiment conducted to study the fate of propiconazole, water and sediment concentrations declined 20% and 5%, respectively from the initial measurements by 168 h (Fig. 3). Propiconazole was immediately lost from the system when Stratego® was applied to the sediment with an initial (4 h) mass balance of 57% (±25) but remained relatively constant by the conclusion of the fate study with a mass balance of 50% (±8). Although propiconazole concentrations remained relatively constant throughout the test, sediment association or other dissipation mechanisms are responsible for the immediate loss from the system.

Only the highest application of Stratego® to the sediment was significantly more toxic as compared to controls. No statistical differences in mortality were observed between controls and microcosms receiving sediment treated with Stratego® ($p=1.00$) with the exception of the highest treatment concentration ($p<0.001$) (Fig. 4). The sediment/water microcosm system reduced toxicity 1000% as compared to water-only tests when the overlying water was treated based on the total amount of fungicide applied to the system (LC_{50} values of 284 and 25.8 µg/L, respectively; Table 2 and Table 3). However, LC_{50} (17.8 and 284 µg/L) and LC_{10} (2.6 and 32.4 µg/L) values for Stratego® were lower (non-overlapping 95% confidence intervals) when calculated based on measured water concentrations as compared to concentrations based on full water incorporation of the total fungicide applied to the sediment/water system (Table 3). LC_{50} values for Stratego® water-only toxicity tests and sediment treated microcosms were similar when calculated based on measured water concentrations (20.4 and 17.8 µg/L, respectively; Table 2 and Table 3). However, sediment treated microcosms had lower LC_{10} values as compared to water-only exposures treated with Stratego® (2.6 and 14.2 µg/L, respectively; Table 2 and Table 3). Application of Stratego® to the overlying water in sediment/microcosm systems resulted in a lower LOEC of 39 µg/L ($p<0.001$) than the sediment application LOEC of 293 µg/L ($p< 0.001$) (Fig. 3). LC_{50} and LC_{10} values between treatments with non-overlapping 95% confidence intervals for all values with the exception of slight overlapping of LC_{50} values calculated based on measured water concentrations (Table 3).

Discussion

Both Headline® and Stratego® are acutely toxic to *H. azteca* with LC_{50} values of 25 µg/L and 20 µg/L respectively. High toxicity of the active strobilurin ingredients (pyraclostrobin and trifloxystrobin) in water-only exposures suggests that these active ingredients are responsible for the majority of the toxicity. Although Headline® resulted in greater toxicity than pyraclostrobin alone (Fig. 1), the LC_{50} and LC_{10} values were not statistically different (Table 2). For Stratego®,

the results show that neither propiconazole nor the other formulation adjuvants significantly contribute to the overall toxicity for *H. azteca* to the extent that trifloxystrobin does (Fig. 1 and Table 3). Hooser et al. (2012) tested fungicide toxicity to *B. cognatus* tadpoles in a water-only system and found no differences in toxicity between Stratego® formulation and trifloxystrobin, whereas, Headline® formulation was slightly more toxic than pyraclostrobin alone. Their results suggest that differences in toxicity between Headline® formulation and pyraclostrobin indicate that the formulation's proprietary adjuvant ingredients may play a role in the observed toxicity. For instance, naphthalene and 2-methylnapthalene were detected at concentrations representing 6.2 and 13.7% of the total formulation [15], and naphthalene may constitute up to 8% of the overall formulation according to the label [3]. Despite these slight differences in toxicity, pyraclostrobin appears to be the major environmental hazard.

Most notably, all of the LC_{50} values from this study were well below relevant environmental concentrations as determined by Belden et al. (2010) based on a direct overspray of 16 cm of water with maximum label rate for corn (Table 2, Table 3). This worst case scenario exposure would result in expected environmental concentrations of the individual active ingredients of 150 and 74 µg/L for Headline® (pyraclostrobin) and Stratego® (trifloxystrobin and propiconazole), respectively, based on full water incorporation. Accounting for label application variables, the spray drift model (AgDrift®) predicts that up to 10% of the fraction of agrochemicals applied aerially can be detected 100 m downwind from the application event given the right circumstances [20]. However, 10% of the application rate hitting the overlying water would still be higher than the LC_{10} for Headline® exposure to *H. azteca*. Furthermore, embedded cropland playas are likely to receive a direct spraying event because cultivation often extends up to the wetland boundary or even through dry wetlands [8]. Based on sediment/water microcosm LC_{50} values and expected environmental concentrations from a direct overspray event at maximum label rate for corn over 16 cm of water, even a partial overspray event would be

sufficient to cause significant toxicity. For instance, only a 57% overspray event would be required to achieve a LC_{50} concentration of 85.5 µg/L for Headline® and only 12% overspray to reach 18.3 µg/L at maximum label rate (150 µg/L). Similarly for Stratego®, a 58% overspray event would be sufficient to reach LC_{50} concentrations of 43.1 µg/L and only 22% to reach 16.3 µg/L at maximum label rate (74 µg/L).

Beyond spray drift or accidental direct spraying, contamination through runoff may also carry fungicides into a wetland. Deb et al. (2010) modeled environmentally relevant concentrations of agricultural chemicals occurring in runoff at the edge of cultivated fields, with maximum annual average inputs of 13.7, 2.1 and 42.3 µg/L for pyraclostrobin, trifloxystrobin and propiconazole respectively. Our experimental LC_{50} values for Headline® were slightly above the modeled concentrations; however, 13.7 µg/L is within the 95% confidence limits of LC_{50} values calculated from sediment treated microcosm exposures Headline®. Experimental LC_{10} values from microcosm exposures were less than the modeled concentration for pyraclostrobin and significant mortality was observed at 12 µg/L for Headline® in water-only exposures. Experimental LC_{50} and LC_{10} values for Stratego® were both greater than the modeled runoff concentration for trifloxystrobin; which suggests that trifloxystrobin in unlikely to reach toxic levels in the environment. However, it should be noted that concentrations of pesticides in surface water runoff provided by Deb et al. (2010) were generated using the National Agricultural Pesticide Risk Analysis (NAPRA) program which provides conservative using simulations provided by the Groundwater Loading Effects of Agricultural Management Systems (GLEAMS) model. Although these concentrations may be environmentally relevant in surface waters at the edge of fields, the values do not account for subsequent dilutions into adjacent water bodies or losses due to matrix partitioning. Nonetheless, the surface water concentrations could be relevant to embedded wetlands in the Southern High Plains that frequently go dry between precipitation events thus resulting catchment fungicide concentrations matching those in the surface waters.

Even if this scenario occurred, our results indicate that concentrations would decline 72%, 27%, and 20% of the initial concentrations within 4 h for pyraclostrobin, trifloxystrobin and propiconazole, respectively.

Incorporation of sediment in our aquatic systems demonstrated that partitioning reduces the aquatic toxicity of fungicides. Sediment used in our system had relatively high organic matter content (6.78%) and high organic material content is known to increase sorption to sediment for hydrophobic organic contaminants. For example, Tiwari and Guha (2012) demonstrated increased sediment adsorption of endosulfan and chlorpyrifos with increased levels of organic carbon [21]. Delle Site (2000) also demonstrated in a review of factors effecting sorption of organic contaminants in sediment/water systems that organic matter concentrations and sorption are directly related [22]. Therefore, the degree of partitioning and thus the extent that toxicity would be reduced would vary among wetland types with varying amounts of organic matter. For example, the organic matter content range of the sediment in playas from the High Plains of the United States is typically in the range of 1.2-1.7% in the top 15 cm [23]. Thus, we would expect playas with different land use histories, sediment composition and inputs to have varying capacities to reduce toxicity based on the amount of organic carbon sequestered in the wetland basin.

Due to confounding environmental factors that could influence partitioning (e.g. organic matter content, particle size, adsorption/desorption equilibriums, etc.), the question becomes whether soil sorption coefficients (K_d) estimated based on log K_{oc} values obtained from literature and the unique organic matter content of our model sediment could have predicted toxicity. Expected literature derived K_d values for pyraclostrobin, trifloxystrobin and propiconazole were 74, 17 and 12 mL/g, respectively. Literature derived values over predicted sediment partitioning in microcosms where the overlying water was treated based on experimental 96 h K_d values of 31, 4, 4 mL/g, respectively, for pyraclostrobin, trifloxystrobin and propiconazole. However,

literature values were comparable when the sediment was treated based on experimental 96 h K_d values of 78, 8 and 12 mL/g, respectively, for pyraclostrobin, trifloxystrobin and propiconazole. The loss of trifloxystrobin from our system is likely the contributing factor for the experimental values being a factor of two lower for the sediment treated K_d values. Therefore, literature K_d values were only useful in predicting water concentrations and thus toxicity when sediment was treated and would have grossly under predicted toxicity in water treated microcosms. Differences between literature and experimental K_d values can be attributed to our systems not being in complete equilibrium which would have resulted from more vigorous mixing/shaking of our units. However, we wanted to observe how partitioning would occur in the absence of vigorous shaking in the laboratory to match more closely what would happen in the environment.

Interestingly, there were large differences between toxicity of fungicides when added to the sediment as compared to direct addition to overlying water. Specifically, observed toxicity was reduced in microcosms where sediments were treated with fungicides as compared to microcosms where the overlying water was treated. Limited toxicity was the result of stronger partitioning to the sediment and higher dissipation rates of the fungicides in the sediment, following sediment application. This may have been a consequence of changes in availability of the fungicides once they have sorbed to sediment and undergo chemical aging processes. For example, desorption hysteresis (i.e., the reluctance of some fraction of the contaminant to readily desorb from sediment back to the aqueous phase) frequently occurs [24-26]. The driving concept behind hysteresis is that organic contaminants are not in total equilibrium with environmental matrices such as sediment, particulate detritus material and water due to partial irreversible sorption to environmental matrices or extremely slow desorption kinetics due to entrapment by the characteristics of the matrices [24-26]. For example, studies have shown that the relationship between hysteresis is correlated with increased concentrations of organic matter that induces rapid sorption of the contaminant and simultaneously retards desorption mechanisms [24-26].

Because the extent of hysteresis increases with concentration and contact time, we would expect that aquatic organisms would be more protected in playa wetlands receiving a direct spray event prior to wetland inundation as compared to post inundation due to chemical aging mechanisms like hysteresis.

Our results showed that LC_{50} values calculated based on overlying water concentrations in sediment/water microcosms were very similar to LC_{50} values of the water-only exposures for both fungicide formulations. Similarly, Suedel et al. (1993) reported fluoranthene EC_{50} values for *H. azteca* exposed using three different sediments with wide variations based on porewater (46 – 237 µg/L), dry sediment (0.002 – 0.007 µg/kg) and organic carbon-normalized sediment concentrations (0.5 – 1.5 µg/kg). However, the water-only EC_{50} value (45 µg/L) was predictive of the EC_{50} values based on overlying water concentrations (32 – 54 µg/L) which were also less variable than the other measured matrices [27]. This suggests, at least for some compounds, that water concentrations are the most important factor in determining toxicity in sediment/water systems for *H. azteca*. Lack of availability from the sediment and the behavior of the organism or both could potentially explain this. Despite the fact that US EPA (2000) suggests that *H. azteca* are epibenthic detritivores that burrow into the sediment surface, it has been shown that although they prefer to scurry along the sediment surface and occasionally side-swim through the water column, they only burrow into the sediment when disturbed [28]. Therefore, the majority of the exposure would be from the water column, not from the sediment surface.

The objective of sediment/water microcosm toxicity exposures is to assess the biological effects of contaminants in conditions similar to those found in nature. Microcosm toxicity exposures conducted in this study were designed to investigate the extent of toxicity reduction that sediment could provide in wetland systems such as small crop embedded wetlands such as those found in the High Plains and Rainwater Basin of the United States. Typical sediment toxicity tests for *H. azteca*, as outlined by US EPA (2000), utilize a large volume of homogenized

contaminated sediment (artificially or obtained from a field site) to relatively little overlying water (< 1:2) and this water is frequently renewed. Typically, this method is employed to investigate the toxicity of contaminants associated with sediment such as persistent organic pollutants (e.g. DDT, dioxins, PCBs, etc.), pyrethroid insecticides or heavy metals. Because most exposure scenarios involving these contaminants will occur through sediment desorption, this method is environmentally relevant; however, it may not be appropriate for current use pesticides that are less persistent, have lower K_d values and can enter aquatic systems directly through the aqueous phase. Organization for Economic Co-operation and Development (OECD) guidelines for the testing of chemicals may be more appropriate for testing of current use pesticides. OECD guideline 233 acknowledges the possibility of spray drift contamination of surface waters and field run-off contributions to sediment toxicity; therefore, both water and sediment spiking methods are outlined using chironomids [29]. This allows us to make better predictions on how fungicides will behave in the environment under different entry routes, thus enabling us to better understand how pathways of chemical dissipation such as partitioning and biodegradation can affect bioavailability.

Conclusion:

This study adds to the growing body of literature on the toxicity of fungicides to aquatic organisms. The continuing increase of fungicide application rates in the United States is cause for concern because of the growing evidence of acute toxicity to both invertebrate [10, 11] and vertebrate [12-15] species. This study reinforces previous studies that the Headline® and Stratego® are toxic to non-target aquatic organisms at environmentally relevant concentrations; however, the active strobilurin ingredients are responsible for the majority of observed toxicity [14, 15]. Additionally, sediment reduces toxicity by lowering water concentrations through partitioning and other dissipation mechanisms in aquatic systems. However, due to the rapid toxicity observed in this study and others [14, 15], significant toxicity may still occur prior to

sediment sorption. Further investigation of passive sampling techniques to measure pulsed doses of surface water run-off and controlled field spraying events over wetlands are required to understand the risks posed to embedded wetlands or other adjacent water bodies by fungicides.

REFERENCES

1. Deb, D.; Engel, B. A.; Harbor, J.; Hahn, L.; Lim, K. J.; Zhai, T. 2010. Investigating Potential Water Quality Impacts of Fungicides Used to Combat Soybean Rust in Indiana. *Water Air Soil Poll*, 207:273-288.

2. Shaner, G. E. A., C.; Christmas, E.; Conley, S.P.; Dobbins, C.L.; Hurt, C.A.; Patrick, G.F.; Rane, K.K.; Ruhl, G.E. 2005. Preparing for Asian Soybean Rust. Purdue Extension, http://www.extension.purdue.edu/extmedia/ID/ID-324.pdf. Accessed 24 April 2012.

3. BASF Corporation. 2008. Headline® Fungicide Label. NVA 2008-04-088-0262. Research Triangle Park, NC, USA.

4. BASF Corporation. 2008. Headline® Fungicide Supplemental Label. NVA 2008-04-088-0327. Research Triangle Park, NC, USA.

5. Bayer CropScience. 2008. Stratego® Fungicide Label. EPA Reg. No. 264-779. Research Triangle Park, NC, USA.

6. Smith, L. M.; Euliss, N. H.; Wilcox, D. A.; Brinson, M. M. 2008. Application of a geomorphic and temporal perspective to wetland management in North America. *Wetlands,* 28:563-577.

7. Smith, L. M. 2003. Playas of the Great Plains. 1st ed.; University of Texas Press: Austin.

8. O'Connell, J. L.; Johnson, L. A.; Smith, L. M.; McMurry, S. T.; Haukos, D. A. 2012. Influence of land-use and conservation programs on wetland plant communities of the semiarid United States Great Plains. *Biol Conserv*, 146:108-115.

9. Tiner, R. W. 2003. Geographically isolated wetlands of the United States. *Wetlands*, 23:494-516.

10. Ochoa-Acuna, H. B., W; Yale, G.; Hahn, L. 2008. Toxicity of soybean rust fungicides to freshwater algae and Daphnia magna. *Ecotoxicology*, 18:440-446.

11. Bringolf, R. B. C., W. G.; Eads, C. B.; Lazaro, P. R.; Barnhart, M. C.; Shea, D. 2007. Acute and chronic toxicity of technical-grade pesticides to glochidia and juveniles of freshwater mussels (*Unionidae*). *Environ Toxicol Chem,* 26:2086-2093.

12. BASF Corporation. 2008. Headline® Fungicide MSDS. Research Triangle Park, NC, USA.

13. U.S. Environmental Protection Agency. 2007. ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. Washington, DC.

14. Belden, J. B.; McMurry, S.; Smith, L. M.; Reilley, P. 2010. Acute toxicity of fungicide formulations to amphibians at environmental relevant concentrations. *Environ. Toxicol. Chem*, 29:2470-2480.

15. Hooser, E. B.; Belden, J.B.; Smith, L. M.; McMurry, S. T. 2012. Acute toxicity of three strobilurin fungicide formulations and their active ingredients to tadpoles. *Ecotoxicology*, 21:1458-1464.

16. Rose, G.; Lane, S.; Jordan, R. 2009. The fate of fungicide and insecticide residues in Australian wine grape by-products following field application. *Food Chem*, 117: 634-640.

17. Tomlin, C. 2000. The pesticide manual: a world compendium. 12th ed.; British Crop Protection Council: Farnham.

18. US Environmental Protection Agency. 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. 2nd ed., (EPA-600/R-99/064).

19. Belden, J. B.; Hanson, B. R.; McMurry, S. T.; Smith, L. M.; Haukos, D. A. 2012. Assessment of the Effects of Farming and Conservation Programs on Pesticide Deposition in High Plains Wetlands. *Environ Sci & Tech*, 46:3424-3432.

20 Teske, M. E. B., A. L.; Esterly, D. M.; Curbishley, T. B.; Ray, S. L.; Perry, S. G. 2002. AgDRIFT(R): a model for estimating near-field spray drift from aerial applications. *Environ Toxicol Chem*. 21:659-671.

21. Tiwari, M. K.; Guha, S. 2012. Role of soil organic matter on the sorption and cosorption of endosulfan and chlorpyrifos on agricultural soils. *J Environ Eng-Asce*, 138:426-435.

22. Delle Site, A. 2001. Factors affecting sorption of organic compounds in natural sorbent/water systems and sorption coefficients for selected pollutants. A review. *J. Phys. Chem. Ref. Data*, 30:187-439.

23. Lou, H. R. 1994. The effects of land use on sediment deposition in playas. Master's thesis. Texas Tech University, Lubbuk, TX.

24. Weber, W. J.; Lueking, A. D.; Huang, W. L.; Soderstrom-Schwarz, S.; Kim, M. S. 2000. Relationship of soil organic matter characteristics to organic contaminant sequestration and bioavailability. *J Environ Qual*, 29:317-323.

25. Kan, A. T.; Fu, G. M.; Tomson, M. B. 1994. Adsorption-desorption hysteresis in organic pollutant and soil sediment interaction. *Environ Sci & Tech,* 28:859-867.

26. Braida, W. J.; White, J. C.; Zhao, D. Y.; Ferrandino, F. J.; Pignatello, J. J. 2002. Concentration-dependent kinetics of pollutant desorption from soils. *Environ Toxicol Chem,* 21:2573-2580.

27. Suedel, B. C.; Rodgers, J. H.; Clifford, P. A. 1993. Bioavailability of fluoranthene in fresh-water sediment toxicity tests. *Environ Toxicol Chem,* 12:155-165.

28. Pennak, R. W. 1989. Fresh-water invertebrates of the United States : protozoa to mollusca. Wiley: New York, USA.

29. OECD, guidelines for the testing of chemicals No. 233. 2010. Sediment-water chironomid life-cycle toxicity test using spiked water or spiked sediment.

APPENDICES

Table 1: Total fungicide application data for three row-crops from approximately 10 yr. ago to the most recent data available (National Agricultural Statistics Service; http://quickstats.nass.usda.gov/). Percent increase provided based on the mass applied per area planted (kg/ha) for the given time interval.

Crop	Fungicide Data Approximately 10 yr. Ago			Most Recent Fungicide Data			% Increase
	Year	Area Planted (ha)	Mass Applied (kg)	Year	Area Planted (ha)	Mass Applied (kg)	
Corn	2003	31,800,000	105,000	2010	35,700,000	337,000	286
Soybean	2002	29,900,000	49,000	2006	30,600,000	212,000	424
Winter Wheat	2000	17.500,000	37,000	2009	17,500,000	132,000	355

Table 2: LC_{50} and LC_{10} values for *Hyalella azteca* comparing toxicity of active strobilurin ingredients and formulations for water-only exposures with 95% confidence intervals shown. Because most of the toxicity occurred within 96 h, values given based on 96 h mortality assessment of the 10 d.

Calculation Method	Endpoint	Pyraclostrobin	Headline®	Trifloxystrobin	Stratego®
Concentrations Based on Total Added to the System	LC_{50} (µg/L)	22.0 (17.2-28.2)	17.0 (14.2-20.4)	29.9 (21.0-43.8)	25.8 (22.7-29.3)
	LC_{10} (µg/L)	9.6 (6.1-12.5)	7.4 (5.2-9.4)	15.0 (6.7-21.34	15.6 (12.2-18.2)
96 h Average Water Concentrations	LC_{50} (µg/L)	25.1 (21.3-29.7)	21.0 (18.0-24.8)	24.7 (17.9-37.9)	20.4 (18.6-22.5)
	LC_{10} (µg/L)	12.1 (9.4-14.7)	9.8 (7.4-12.0)	12.9 (5.5-17.8)	14.2 (11.3-16.0)

Table 3: LC_{50} and LC_{10} for *Hyalella azteca* values in sediment/water microcosms comparing water versus sediment applied formulations following a 168 h exposure with 95% confidence intervals shown. Headline® sediment treated microcosms did not have significant mortality to determine LC values.

Calculation Method	Endpoint	Headline® Water Overspray	Headline® Sediment Overspray	Stratego® Water Overspray	Stratego® Sediment Overspray
Concentrations Based on Total Added to the System	LC_{50} (µg/L)	85.5 (58.7-134.1)	> 284	43.1 (36.0-51.8)	284 (166-756)
	LC_{10} (µg/L)	20.0 (9.5-31.3)	> 284	13.8 (10.1-17.4)	32.4 (15.1-56.4)
96 h Average water Concentrations	LC_{50} (µg/L)	18.3 (11.0-35.4)	> 8.7	16.3 (10.2-25.7)	17.8 (11.7-32.7)
	LC_{10} (µg/L)	3.9 (1.2-7.0)	> 8.7	4.0 (1.4-7.0)	2.6 (1.5-3.8)

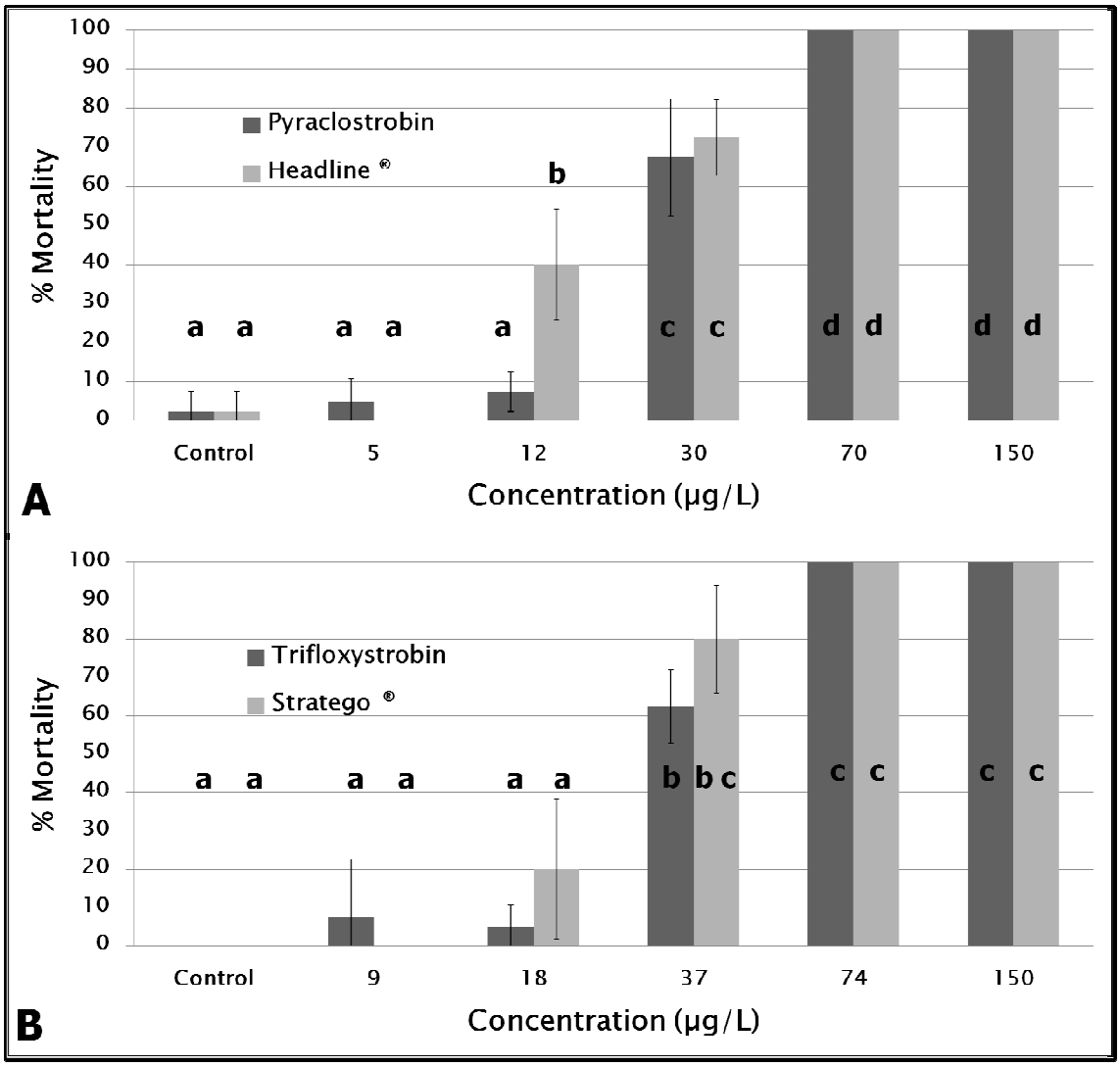

Figure 1: Mean (± standard error) percent mortality of *Hyalella azteca* for water-only exposures for **A**) Headline® or pyraclostrobin and **B**) Stratego® or trifloxystrobin for five concentrations and a control. Provided mortalities correspond to the 96 h assessment which represents the time point when the majority of toxicity had taken place. Each treatment consisted of four replicates (n=4). Categorical letters represent statistical differences between concentrations and/or treatments ($p < 0.05$).

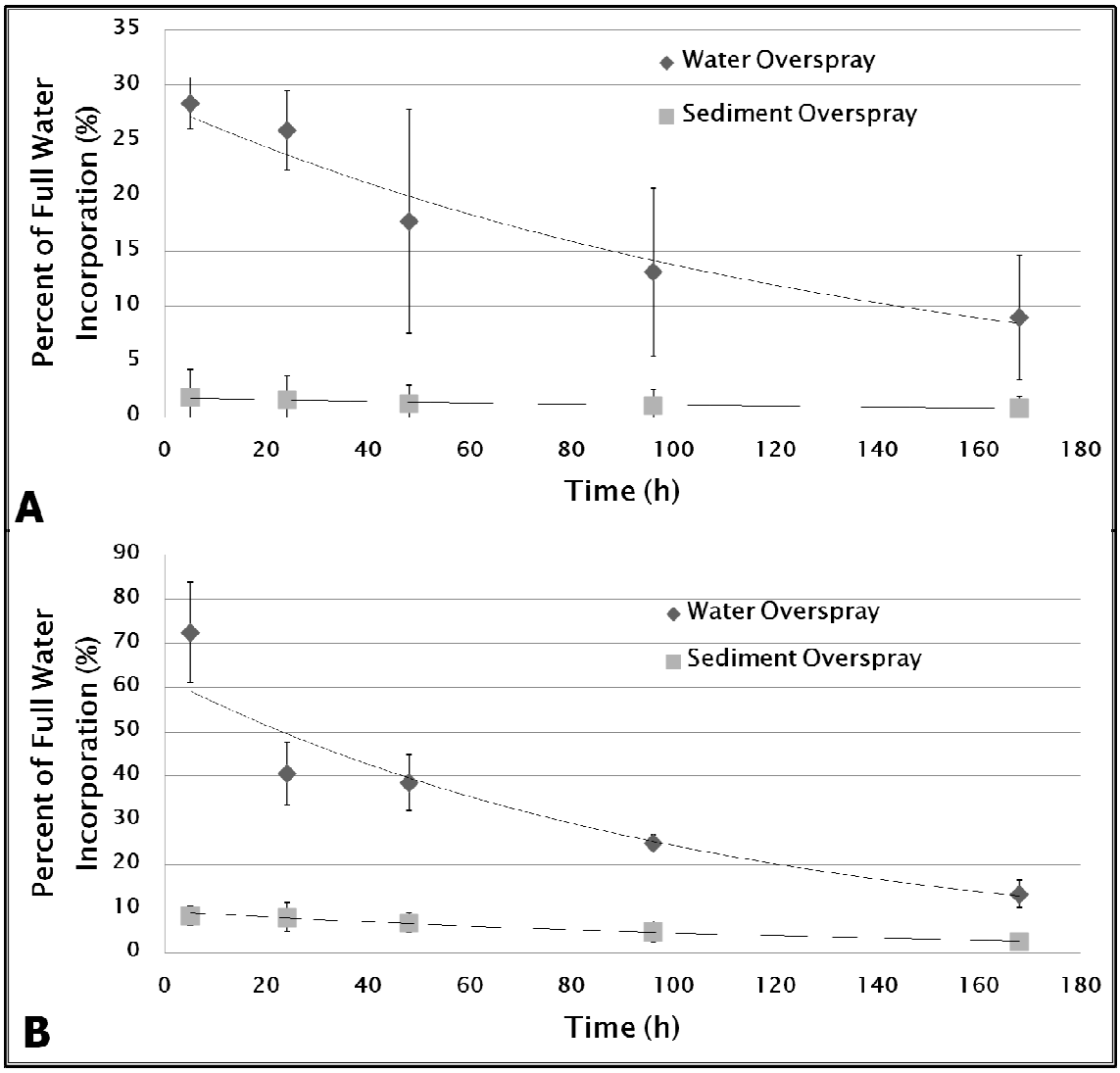

Figure 2: Mean (± standard error) shown for water concentrations of the active strobilurin ingredients across all five treatment concentrations in sediment/water microcosm toxicity tests for **A**) pyraclostrobin and **B**) trifloxystrobin following the application of Headline® and Stratego®, respectively to either overlying water or sediment. Water concentrations are expressed as percent of full water incorporation, assuming complete water partitioning of the total amount of fungicide applied to the system. First order exponential decay curves are fitted through the data to provide a visualization of how water concentrations varied during microcosm toxicity tests.

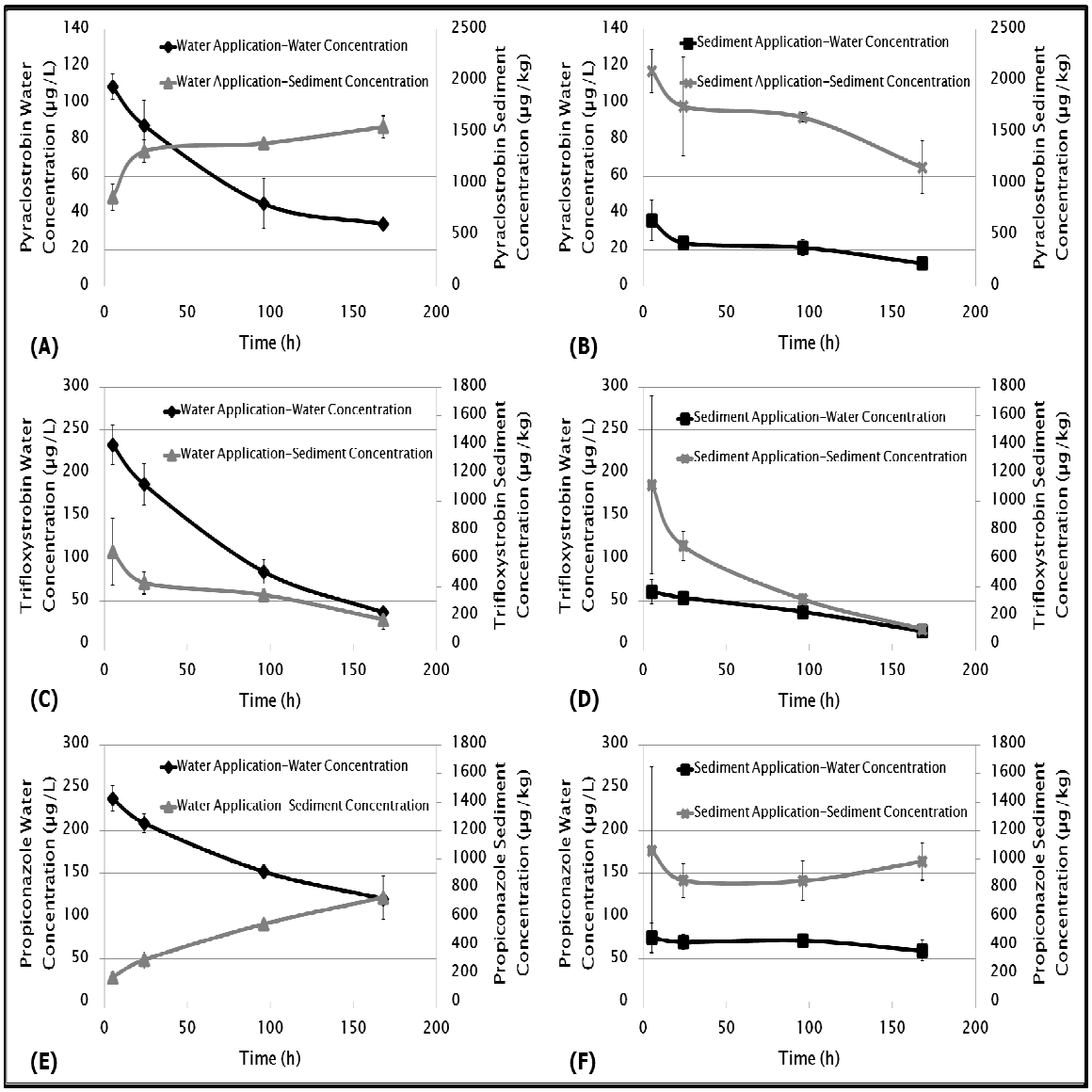

Figure 3: Mean (± standard error) measured water (left axis) and sediment (right axis) concentrations of pyraclostrobin (**A** and **B**), trifloxystrobin (**C** and **D**), and propiconazole (**E** and **F**) in sediment/water microcosms during a parallel study to investigate the fate of active fungicide ingredients following water or sediment applications of Headline® or Stratego®; respectively. Plots **A**, **C**, and **E** represent the dissipation of fungicides following the application of formulations to overlying water. Plots **B**, **D**, and **F** represent the dissipation of fungicides following the application of formulations to sediment 24 h prior to the addition of water. The same application rate was used for both Headline® and Stratego® which corresponded to concentrations of active ingredient concentrations of 300 µg/L in the water phase (assuming complete water partitioning) or; conversely, a sediment concentration of 2300 µg/kg (assuming complete sediment adsorption). Water and sediment concentrations were determined at each time point by destructively sampling three replicates (n=3) and a control.

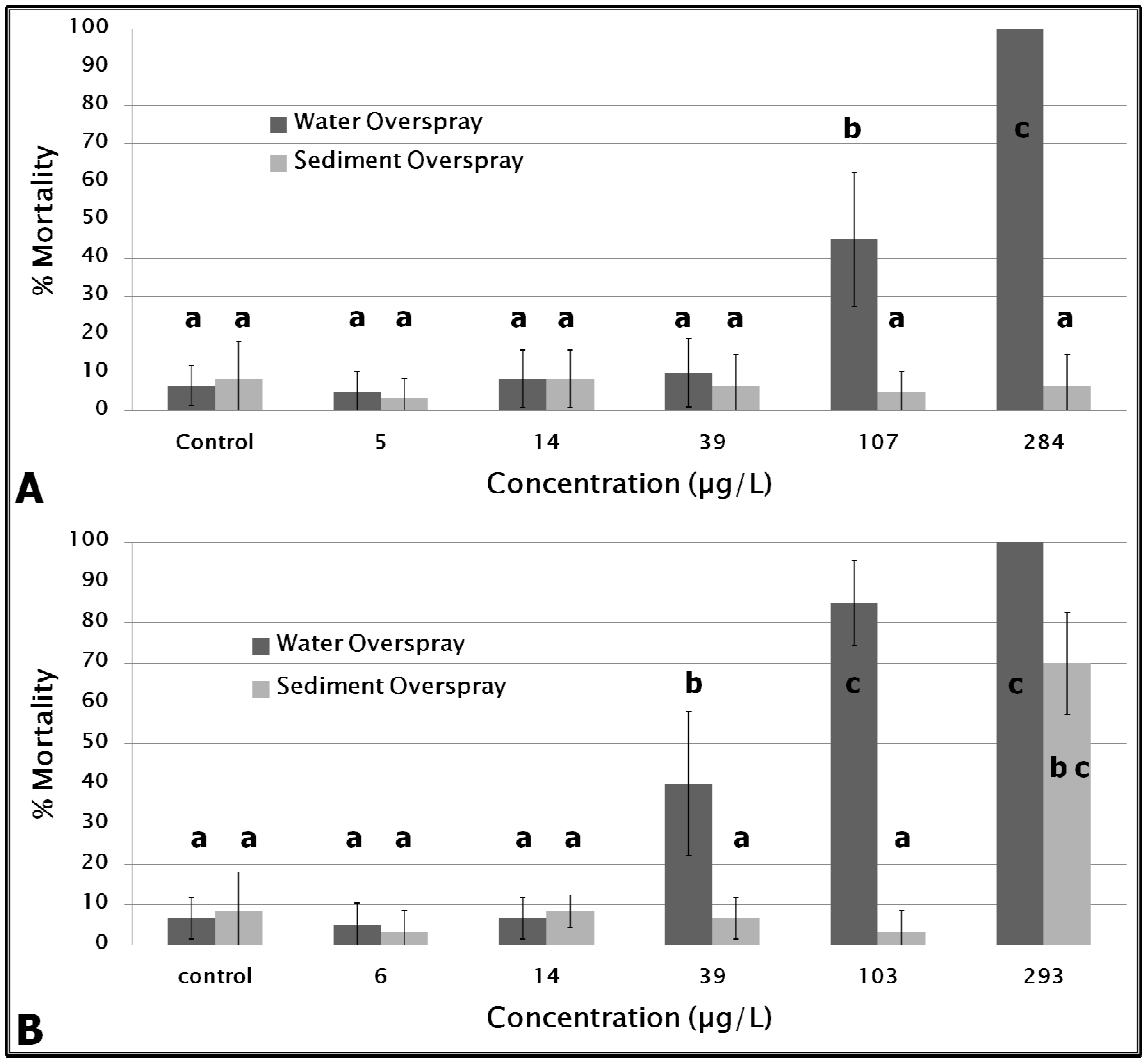

Figure 4: Mean (± standard error) percent mortality of *Hyalella azteca* for sediment/water microcosm exposures comparing toxicity between sediment and water applications of **A**) Headline® and **B**) Stratego® at five concentrations and a control. Formulations were applied to sediment treated microcosms 24 h prior to water addition. Formulations were applied to water treated microcosms following the addition of *H. azteca*. Each treatment consisted of six replicates (n=6). Categorical letters represent statistical differences between concentrations and/or treatments ($p < 0.05$).

VITA

Shane Arthur Morrison

Candidate for the Degree of

Master of Science

Thesis: TOXICITY OF STROBILURIN FUNGICIDES TO *HYALELLA AZTECA*

Major Field: Zoology

Biographical:

Education:

Graduated from John Glenn High School, Bay City, Michigan in May 2005; received Bachelor of Science in Chemistry at Saginaw Valley State University, University Center, Michigan in May 2010

Completed the requirements for the Master of Science in Zoology at Oklahoma State University, Stillwater, Oklahoma in December, 2012.

Experience:

Graduate Research Assistant, Oklahoma State University, 2012. Graduate Teaching Assistant, Oklahoma State University, 2011: Introductory Biology. Regional Student Representative to the Society of Environmental Toxicology and Chemistry Council, Ozark Prairie Chapter, 2011. Independent Undergraduate Researcher, Saginaw Valley State University, 2008-2010. Co-op Laboratory Technician, The Dow Chemical Company, 2006-2010.

Professional Memberships:

Society of Environmental Toxicology and Chemistry