

The Pennsylvania State University

The Graduate School

**DIVERSIFIED INTEGRATED PEST MANAGEMENT CROPPING SYSTEMS
INFLUENCE PEST POPULATIONS AND THE POTENTIAL FOR ENVIRONMENTAL
POLLUTION**

A Thesis in

Entomology

by

Sarah McTish

© 2019 Sarah McTish

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science

May 2019

The thesis of Sarah McTish was reviewed and approved* by the following:

John F. Tooker
Associate Professor of Entomology and Extension Specialist
Thesis Advisor

Heather D. Karsten
Associate Professor of Crop Production/Ecology and Extension Specialist

Heather E. Gall
Assistant Professor of Agricultural and Biological Engineering

Mary E. Barbercheck
Professor of Entomology and Extension Specialist

Gary W. Felton
Professor of Entomology
Head of the Department of Entomology

*Signatures are on file in the Graduate School

ABSTRACT

As agricultural intensification continues in the United States there is a need for developing and using sustainable practices that maintain productive yields while also protecting the environment. For my first chapter, I compared two crop rotations, one representative of a diverse dairy rotation typical of the Mid-Atlantic United States. More specifically, over two field seasons, I compared a 2-yr low plant diversity, preemptive pest control rotation to two, 6-yr high plant diversity, integrated pest management (IPM) rotations and how these different rotations influenced pest and predator populations, with a focus on slugs and their ground beetle predators. Overall, I found that in many cases increasing plant diversity within rotations and decreasing insecticide use suppressed pest populations and fostered higher predator populations. In 2017, the maize grown for grain in the high-diversity, IPM rotation produced higher yields than the low-diversity, preemptive pest control rotation. Also, in 2017, soybean yields were equal between these rotations. My results suggest that increasing plant diversity within cropping rotations and using integrated pest management with reduced insecticides can be an effective means for controlling pests to remain below economically damaging levels.

Because of my interest in reducing insecticide use in Chapter One, I wanted to explore possible negative consequences of one of the preventative insecticides used in the low-diversity, high insecticide input rotation: the neonicotinoid insecticides. For Chapter Two, I examined the potential for these insecticides to be transported from agricultural fields to nearby aquatic environments. These highly water-soluble insecticides, used as seed coatings, are translocated throughout growing seedlings, but very little of the active ingredient applied to seeds is actually taken up by the growing seedling. The majority of the active ingredient is then retained in soil, where it is susceptible to transport via surface runoff, leaching to groundwater, biodegradation, sorption to soil, and drift as planting dust. Because neonicotinoids are highly water-soluble, they have a high potential to impact water quality and pose a risk to aquatic ecosystems, making it critical to understand how neonicotinoids leave crop fields in water and at what concentrations. To determine these concentrations of active ingredient applied to seed coatings leaving the

crop field in water, I used field lysimeter plots to collect surface and subsurface water runoff from corn fields planted with seeds coated with the neonicotinoid thiamethoxam. Samples were then analyzed for concentrations of thiamethoxam and its degradant clothianidin using HPLC/MSMS-Orbitrap. These concentrations paired with water flow data provide the mass loss of active ingredient that occurs with each runoff event. After collecting surface and subsurface runoff, I found that 1.3% of thiamethoxam and its metabolite clothianidin are lost to the surrounding aquatic environment where it could have negative unintended consequences.

TABLE OF CONTENTS

LIST OF FIGURES	vii
LIST OF TABLES.....	xi
ACKNOWLEDGEMENTS.....	xii
 Chapter 1 The potential for high diversity integrated pest management cropping systems to reduce slug damage and increase natural enemy populations in no-till maize	1
Introduction.....	1
Materials and Methods.....	5
Study Site Description	5
Early Season Damage Assessments.....	7
Slug Activity-Density	7
Late Season European Corn Borer Assessments	8
Potato Leafhopper Abundance in Alfalfa	8
Predatory Arthropod Activity-Density	8
Seasonal Predation on Sentinel Prey Caterpillars	9
Statistical Analyses	10
Results.....	11
Early Season Damage Assessments.....	11
Slug Activity-Density	12
Seasonal Predation on Sentinel Prey Caterpillars	13
Predatory Arthropod Activity-Density.....	14
Relationships between Slug Activity and their Predators	16
Late Season European Corn Borer Assessments	16
Potato Leafhopper Abundance in Alfalfa	17
Discussion.....	18
Conclusions.....	24
References.....	24
Tables.....	33
Figures	39
 Chapter 2 Neonicotinoid insecticides in the aquatic environment: A step towards solving the mass balance equation.....	55
Introduction.....	55
Materials and Methods.....	57
Collection Site Descriptions	57
Sample Collection and Storage	57
Sample Preparation and Analysis	58
Statistical Analyses	59
Results.....	60
Discussion.....	61

Conclusions.....	64
References.....	64
Tables.....	71
Figures	72
Appendix Supplemental tables describing neonicotinoid insecticide concentrations in Ch. 2.....	75

LIST OF FIGURES

- Figure 1-1.** 2017 cropping schematic showing within rotation comparisons. The Pest and Manure Rotations provide year-round ground cover, while the Control Rotation has no ground cover between maize and soybean crops.....39
- Figure 1-2.** 2018 cropping schematic showing the addition of the BAU Rotation in the pest management comparison.40
- Figure 1-3.** (A) 2017 mean proportion of maize seedlings damaged by slugs (LS-Means \pm SE) based on growth stage and rotation. Values marked with letters are significantly different (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by stage. (B) 2018 mean proportion of maize seedlings damaged by slugs (LS-Means \pm SE) based on growth stage and rotation. There is no significant Stage*Rotation interaction (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by stage.41
- Figure 1-4.** 2017 mean proportion of maize seedlings damaged by slugs (LS-Mean \pm SE) in the Manure Rotation based on the maize entry and stage. Values marked with letters are significantly different (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by stage.....42
- Figure 1-5.** 2018 mean proportion of maize seedlings damaged by slugs (LS-Mean \pm SE) in the Manure Rotation based on the previous cover in the plot (FT: Fall Terminated, ST: Spring Terminated). Values marked with letters are significantly different (PROC MIXED; Covariate Type VC; $P \leq 0.05$) based on Tukey's post-hoc test.43
- Figure 1-6.** 2017 mean slugs per shingle trap (LS-Mean \pm SE) in maize grown for grain plots in the Manure and Control Rotation. Values mark with (*) are statistically different (PROC MIXED with repeated measures; Covariate Type VC; Kenward

- Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by date.....44
- Figure 1-7.** 2018 mean slugs per shingle trap (LS-Mean \pm SE) in maize grown for grain plots in the Manure and Control Rotation and the maize grown for silage following alfalfa with orchardgrass in the Manure Rotation. Values mark with (*) are statistically different (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by date.45
- Figure 1-8.** 2018 mean slugs per shingle trap (LS-Mean \pm SE) in maize entries in the Manure Rotation (AOg2CsO: maize silage following two years of alfalfa with orchardgrass; RCcCgInt: maize grain following either rye silage or crimson clover). Values mark with (*) are statistically different (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by date.46
- Figure 1-9. (A)** 2017 mean proportion of caterpillars surviving (LS-Mean \pm SE) during the day and night across rotation and time. Values marked with letters are statistically significant (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by month. **(B)** 2018 mean proportion of caterpillars surviving (LS-Mean \pm SE) during the day and night across rotation and time. Values marked with letters are statistically significant (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by month.47
- Figure 1-10.** 2018 mean proportion of caterpillars surviving (LS-Mean \pm SE) during the day and night across Manure Rotation previous cover and time. Values marked with letters are statistically significant (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by month.48

Figure 1-11. Regression of mean proportion of sentinel caterpillars surviving during the night and the mean number of slugs found under shingle traps in 2017 and 2018 split by the maize grown for grain in the Manure and Control Rotations.. .49

Figure 1-12. Mean arthropod predators (LS-Mean \pm SE) in pitfall traps per plot in the Manure Rotation maize grown for grain and the Control Rotation maize grown for grain (Formicidae = Ants; Araneae = Spiders; Lycosidae = Wolf Spiders; *Pterostichus melanarius* = a common ground beetle species; Carabidae = Ground Beetles). Values marked with letters are statistically significant (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; Bonferroni Correction $P \leq 0.01$) based on SLICE analysis of simple effects by month.50

Figure 1-13. 2018 Manure Rotation mean arthropod predators (LS-Mean \pm SE) in pitfall traps based on previous cover (FT: Fall Terminated; ST: Spring Terminated) (Araneae = Other Spiders; *Pterostichus melanarius* = a common ground beetle species; Carabidae = Ground Beetles). Values marked with letters are statistically significant (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; Bonferroni Correction $P \leq 0.017$) based on SLICE analysis of simple effects by month.....51

Figure 1-14. Mean slug arthropod predators (LS-Mean \pm SE) in pitfall traps per plot in the Manure Rotation maize grown for grain and the Control Rotation maize grown for grain. Values marked with letters are statistically significant (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.07$)..52

Figure 1-15. 2017 mean potato leafhoppers (PLH) per sweep (LS-Means \pm SE) in first and second year Pest and Manure Rotation treatments (\$: Insecticide spray in all four treatments; \$¹: Insecticide spray in Manure-Resistant treatment; \uparrow : Harvest). Values marked with * are statistically different (PROC MIXED with repeated measures; Covariate Type VC; $P \leq 0.05$) based on SLICE analysis of simple effects by date.53

- Figure 1-16.** 2018 mean potato leafhoppers (PLH) per sweep (LS-Means \pm SE) in first year Pest, Manure and BAU Rotation treatments (\$: Insecticide spray on Manure-Susceptible, Manure-Resistant, and BAU-Susceptible treatments; \$¹: Insecticide spray on BAU-Susceptible treatment; †: Harvest). Values marked with * are statistically different (PROC MIXED with repeated measures; Covariate Type VC; $P \leq 0.05$) based on SLICE analysis of simple effects by date.....54
- Figure 2-1.** Top: Sum surface water flow by date. Bottom: Mean thiamethoxam and clothianidin mass loss (mg/ha) by date (Mean \pm SE) in surface water flow.72
- Figure 2-2.** Top: Sum subsurface water flow by date. Bottom: Mean thiamethoxam and clothianidin mass loss (mg/ha) by date (Mean \pm SE) in subsurface water flow.73
- Figure 2-3.** The proportion of applied neonicotinoid mass loss and proportion of water flow as measured by the data logger in the lysimeter separated by subsurface and surface water runoff. These proportions represent May 2017 through April 2018.74

LIST OF TABLES

Table 1-1. Overview of the four different rotations.....	33
Table 1-2. 2017 and 2018 maize grown for grain management practices.	34
Table 1-3. 2017 and 2018 maize grown for silage management practices.	35
Table 1-4. Top: First year alfalfa management. Bottom: Second year alfalfa management	36
Table 1-5. Total Carabidae caught in 2017 and 2018 pitfall traps.	37
Table 1-6. 2017 and 2018 European corn borer damage by year and rotation (LS-Mean \pm SE). The Manure Rotation is also broken down by maize entry (R/CC,Cg: Maize grain following rye silage and crimson clover cover; AOg2,Cs: Maize silage following second year alfalfa with orchardgrass; Int,Cs: Maize silage following interseeded cover crop). Values marked with letters are significantly different (PROC MIXED; Covariate Type VC; $P \leq 0.05$) based on Tukey's post-hoc test.....	38
Table 2-1. Percent of thiamethoxam and clothianidin applied to maize plots exported from the field via water surface and subsurface flow.	71
Table A-1. Neonicotinoid concentrations (ug/L) in Spring Creek and vernal pool samples. (VP=Vernal Pool; SC=Spring Creek; Numbers indicate locations).....	75
Table A-2. Thiamethoxam concentrations (ug/L) from lysimeter samples by month. (nd= no detection; – = no samples)	76
Table A-3. Clothianidin concentrations (ug/L) from lysimeter by month. (– = no samples)	77

ACKNOWLEDGEMENTS

I would like to thank the wonderful group of people that supported me over the past two years, making this thesis possible. First, thank you my lab mates Kirsten Pearsons, Julie Baniszewski, Elizabeth Rowen, Angela Coco, Julie Golinski, and Eric Yip for their advice, feedback, help during experiments, and friendships. While trying to figure out how to analyze my neonicotinoid samples, I especially appreciate Kirsten for her patience and willingness to help guide me through that process. In addition, a special thanks to Andrew Aschwanden for his helping hand while I learned the ropes of the Northeast SARE project. Over the two years, many undergraduate research assistants helped with field experiments: Dan Wisniewski, Ken Kim, Ken Koepplinger, Garret Reiter, Hayden Bock, Jen Halterman, Kyra Hoerr, Brandon Wilt, Sonia Klein, and Amanda Seow. Finally, I appreciate my advisor, Dr. John Tooker, for welcoming me into his lab five years ago and helping to shape my academic career. I am immensely thankful for his encouragement and guidance in making me a better entomologist.

During my thesis research, I was very fortunate to be a part of the NESARE Dairy Cropping Systems team. Thank you to Dr. Heather Karsten for leading this team with enthusiasm. Apurba Sutradhar and Tom Adams also deserve a thank you for coordinating the many efforts of this project. Scott Harkcom, Kaleb Wolfe, and the rest of the farm crew worked very hard to make field operations possible and I thank them for always trying to work around my field experiments.

My committee members, Drs. Heather Gall, Heather Karsten, and Mary Barbercheck provided valuable support and feedback throughout this process. Through their help, I feel that I have become a stronger scientist and writer. A special thanks to Heather Karsten for taking the time to mentor me through the statistics of the Northeast SARE project. In addition, to my committee, I am extremely grateful to have been a part of the Penn State Entomology Department, where I have made many good friendships and found support from other students and faculty. Outside of the department, I am

thankful for Dr. Kyle Elkin for his help in analyzing my neonicotinoid samples and for Lou Saporito for his help in using the lysimeter.

Thank you to my friends and family for their love and support throughout this process. I want to thank my parents, James and Mary McTish, and my sisters, KC and Liz, for always supporting my entomology endeavors, even if that meant stopping to look at cool insects on every sidewalk. Thank you to Lew for always supporting my dreams and encouraging me to be my best self.

For the funding of my thesis I thank USDA Northeast SARE, Sigma Xi research society, Penn State's Office of Physical Plant, and the Department of Entomology. The USDA National Institute of Food and Agriculture, Northeast Sustainable Agriculture Research and Education funds were for the federally funded project titled, "Advanced Sustainable Cropping Systems for Dairy Farms in the Northeast." The project number is LNE16-354. Findings and conclusions from this study do not necessarily reflect the view of the funding agency.

Chapter 1

The potential for high diversity integrated pest management cropping systems to reduce slug damage and increase natural enemy populations in no-till maize

Introduction

In the United States, agricultural intensification can be seen in monoculture plantings of continuous maize (*Zea mays*) (Plourde 2013), which tend to rely on intensive use of chemical inputs for pest control (Lechenet et al. 2014). Further, as agricultural intensification has increased, biodiversity of plants, animals, vertebrates and invertebrates, and microorganisms has decreased (Emmerson et al. 2016), as have associated environmental or ecosystem services that these communities provide. Simplified, preemptive pest control cropping rotations or schemes, such as continuous maize can have lower yields (Porter et al. 1997; Smith et al. 2008) and natural enemy populations (O'Rourke 2008) than diversified, integrated pest management (IPM) cropping rotations. Overall, high plant diversity, IPM maize cropping rotations have the potential to maintain or increase yield, improve soil health, reduce both insect and weed pest populations, and conserve natural enemy populations (Davis et al. 2012).

Crop rotations have the ability to increase maize yields compared to continuous maize production (Grover et al. 2009). Even a simple maize-soybean rotation can increase maize yields by 10% and soybean (*Glycine max*) yields by 8% compared to either maize or soybean continuous monocultures (Crookston et al. 1991). Additionally, maize yields, as well as environmental benefits, can be conserved or elevated with the introduction of a perennial forage, such as alfalfa (*Medicago sativa*) into the rotation (Porter et al. 1997; Olmstead and Brummer 2008). Increased crop species diversity (Smith et al. 2008) and decreased external inputs (Davis et al. 2012; Lechenet et al. 2014) can either increase or have no impact on maize yields and profitability. In addition to

increased crop diversity within rotations, the addition of cover crops in rotations can increase or maintain yields (Wittwer et al. 2017) while also increasing ecosystem services such as soil fertility, erosion control, weed control, and beneficial-insect populations (Schipanski et al. 2014). Overall, high diversity, IPM cropping systems can compete, both in profitability and in yield, with lower diversity, preemptive pest control cropping systems.

Incorporating cover crops into a rotation is one way of increasing plant diversity within cropping systems to increase ecosystem services, such as pest suppression and beneficial insect conservation. Some cover crops can harbor greater arthropod densities and diversity than others (House and Alzugaray 1989), whether these arthropods are pests or natural enemies. It is important to understand how different cover crops will interact with arthropods in the system; for example, a cereal rye (*Secale cereale*) cover crop before maize can increase damage resulting from true armyworm (*Mythimna unipuncta*) and black cutworm (*Agrotis ipsilon*) populations that share a plant host range with cereal rye (Dunbar et al. 2016). However, increasing crop diversity in rotations, especially with the addition of perennial and cover crop species, and increasing polycultures can decrease the potential for pest outbreaks (Altieri 1999; Staudacher et al. 2013). Therefore, it is critical to efficiently manage cover crops in crop rotations so that they can be added to the rotation profitably and conserve biodiversity (Lundgren et al. 2013).

Having a diverse community of arthropod predators, both generalists and specialists, can be effective in reducing pest populations in agroecosystems (Cardinale et al. 2003). For instance, predation of western corn rootworm (*Diabrotica virgifera virgifera*) was greater in maize following cover crops compared to maize following bare soil due to a wider range of predator species in the maize following cover crops (Lundgren and Fergen 2011). Similar to pest populations, natural-enemy populations can vary based on cover crop management. In a comparison of a mowed cover crop versus an herbicide killed cover crop, the mowed cover crop harbored more carabid beetles (Coleoptera: Carabidae) and wolf spiders (Araneae: Lycosidae), leading to increased predation of armyworms (*Pseudaletia unipuncta*) (Laub and Luna 1992). Further, planting maize into a living mulch of alfalfa and kura clover (*Trifolium ambiguum*)

increased predator abundance and predation rates of sentinel European corn borer (*Ostrinia nubilalis*) pupae (Prasifka et al. 2006). In addition to increased plant diversity and cover crop use, decreased management intensity, such as reduced insecticide use, also encourages arthropod diversity (Adams et al. 2017) and beneficial insect populations (Witmer et al. 2003).

In the Mid-Atlantic region of the United States, the switch to no-till and conservation tillage practices has, in part, been associated with an increase in slug damage, particularly from the grey garden slug (*Deroceras reticulatum*) to field crops in agroecosystems (Barratt et al. 1994; Douglas and Tooker 2012). To combat slug populations in reduced-tillage agroecosystems, some growers are turning towards high diversity, IPM crop rotations, which reduces pesticide applications. Slugs preferentially feed on different plant species to fulfill their nutritional requirements (Cook et al. 2000), which can make intercrops and cover crop residue beneficial for managing slug damage to cash crops by providing an alternative food source for slug populations (Brooks et al. 2006; Le Gall and Tooker 2017). In addition, high diversity, IPM cropping systems can increase carabid beetle activity-density and species richness (O'Rourke et al. 2008). In field crops, such as maize, carabid beetles are important generalist predators (Menalled et al. 1999), particularly *Pterostichus melanarius*, a significant early season slug predator species (Symondson et al. 2002; Wendland et al. 2017). Decreasing insecticide use in these systems is also beneficial because broad spectrum and prophylactic insecticide use can cause secondary pest (e.g. slugs) outbreaks by reducing predatory beetle populations (Hill et al. 2017; Tooming et al. 2017).

For this chapter, I conducted research in collaboration with the Northeast Dairy Cropping Systems project team (Penn State 2019). The overarching goal of this project is to identify sustainable crop management strategies for the average-sized dairy farm in Pennsylvania to produce all of the needed feed, forage, and fuel on farm while minimizing external inputs, such as pesticides and synthetic fertilizers (Penn State 2019). My goal with this project was to compare a simple, low plant diversity, preemptive pest control two-year cropping rotation with transgenic Bt maize, insecticide-coated seed, and prophylactic insecticide use to two, more complex, 6-year crop rotations that included

cover crops and reduced-insecticide-input with non-transgenic seed and insecticide use based on need. In this comparison, I studied the differences in pest and predator populations between these rotations, with a focus on slugs and carabid beetles.

My objectives for this project are as follows: (1) assess the effects of crop diversity and insecticide intensity on pest populations and their associated natural predators in maize; (2) assess the effects of cover crops and cover crop management within the high diversity, IPM rotation on slug and other invertebrate pests in maize in the following year; and (3) compare potato leafhopper (*Empoasca fabae*) management strategies in potato leafhopper-resistant and non-resistant alfalfa varieties.

I hypothesized that greater crop and cover-crop species diversity, and decreased insecticide use in the diversified Manure Rotation would be associated with lower slug abundance and greater abundance of slug predators compared to the Control Rotation. Because no-till and cover-crop use provides diverse habitat for predators and an alternative food source for slugs, and because decreased insecticide usage conserves beneficial insects, I hypothesized that predation would be greater in the diversified Manure Rotation and lower in the Control Rotation. Due to the long-term non-Bt corn seed use in the Manure and Pest Rotations of this project, I hypothesized that European corn borer (*Ostrinia nubilalis*) damage will be greater in these rotations compared to the Control Rotation that does use Bt seed targeted for European corn borer control. Because the trichomes in potato leafhopper resistant alfalfa take about a year to develop, I hypothesized that potato leafhopper populations (*Empoasca fabae*) in alfalfa (*Medicago sativa*) would be equal between resistant and non-resistant alfalfa in the first year. I hypothesized that in the second year, resistant alfalfa would have fewer potato leafhoppers than the non-resistant alfalfa. Even though the “Business-as-Usual” (BAU) non-resistant alfalfa received an insecticidal spray after each cutting while the other treatments only received an insecticidal spray if potato leafhoppers exceeded the economic threshold, I hypothesized that there would be no yield difference between the BAU alfalfa and the resistant and non-resistant alfalfa that was sprayed only when necessary. I hypothesized that natural enemies of potato leafhoppers would control populations in treatments that did not receive an insecticidal spray.

Materials and Methods

Study Site Description

I conducted my field experiments in 2017 and 2018 as a part of the Diversified Dairy Cropping Systems project located at Penn State's Russell E. Larson Agricultural Research Center (Pennsylvania Furnace, PA; 40.721384, -77.919952). This long-term systems project was established in 2010 and compares two, six-year, diversified, IPM cropping rotations (Manure and Pest Rotations) to a two-year corn-soy, preemptive pest management crop rotation (Control Rotation), which is typical of grain crop rotations in Pennsylvania. The experimental design is a nested split-split plot randomized complete block design within each rotation with four blocks, each block containing 14 plots measuring 27.4 m wide and 36.6 m long. Each plot is surrounded by a grass alleyway at least 12 m wide.

In 2017, the Pest Rotation, which compared standard herbicide use to reduced herbicide use, and the Manure Rotation, which compared broadcast manure to injected manure comprised the diversified rotations (Malcolm et al. 2015; Snyder et al. 2016) (Figure 1-1), were planted with perennial and annual crops as well as with winter cover crops between each cash crop. The maize varieties used in these rotations did not have transgenic, insect-resistant traits (e.g. Bt traits) and were not coated with neonicotinoid insecticides (Table 1-2 and 1-3). Moreover, the diversified rotations did not receive a preventative insecticidal spray shortly after planting. The maize-soybean Control Rotation was managed with common preventative insect management tactics that included Bt-traited maize varieties, neonicotinoid seed coatings on both the maize and soybean, and a preventative application of a pyrethroid insecticide on maize shortly after planting (Table 1-2).

In 2018, the Manure and Control Rotations were managed as in 2017. However, the Pest Rotation was split to now include a new "Business-As-Usual" (BAU) Rotation informed by discussion with local dairy farmers (Figure 1-2). The BAU rotation reflects typical local crop and pest management on dairy farms in the region. This rotation is

manure intensive and includes three years of alfalfa followed by three years of maize silage with a winter cover crop (typically cereal rye, *Secale cereal*) between each year of maize silage or oats (*Avena sativa*) after maize and prior to alfalfa establishment. In addition, maize varieties in the BAU rotation, as in the Control Rotation, contained Bt traits, the seed was coated with neonicotinoid insecticides, and the treatments annually received a preventative application of a pyrethroid insecticide shortly after planting. Because of this addition splitting the Pest Rotation in half in space, the Pest Rotation for 2018 only included the reduced herbicide treatment, with the details the same as were used in 2017. In both years, I monitored and sampled all maize entries in all rotations, as well as the first- and second-year stands of alfalfa/orchardgrass (*Dactylis glomerata*) in the Manure and Pest Rotations. A crop entry for this project represents each crop option that would occupy a position in a rotation. For example, in a maize-soybean-wheat rotation each crop is an entry and would be represented every year of the research study. Management practices for maize and alfalfa for both years are summarized in Tables 1-2, 1-3 and 1-4. Throughout this thesis chapter, I mainly focus on the comparison between the maize grown for grain in the Control and Manure Rotations because each of these entries has the same manure management comparison and because the maize grain varieties would be planted and harvested around the same time. Because I also sampled in some of the maize grown for silage entries in the Manure Rotation, I used them in comparisons, but they are not as good of a comparison because maize silage and maize grain would be managed differently with different harvest times.

Both years of my field research experienced very different weather patterns. In 2017, April was unusually warm and then was followed by a rainy, cloudy, cool spring that favored an increase in slug populations. From April through August 2017, this field site received 55.9 cm of rain. In 2018, April was particularly cold and was followed by 79.5 cm of rain between April and August. The greater rainfall in 2018 compared to 2017 made it difficult for the farm crew to get in the fields at normal crop management times; so, yields in 2018 were lower than previous years of the project (NESARE 2018).

Early Season Damage Assessments

At the V2 (2017: starting June 2; 2018: starting June 15) and V5 (2017: starting June 14; 2018: starting June 25) growth stages of maize, I conducted early season damage assessments in all maize entries. In each maize split-split plot, I assessed two 3 m row sections for the number of plants present, presence or absence of slug or insect damage, and a damage rating. The damage rating scale ranged from 0 to 4 depending on proportion of leaf area removed by early season pests (0: no damage, 1: <25%, 2: 25-50%, 3: 50-75%, 4: >75%). Early season maize pests included slugs (*Deroceras reticulatum*; *Deroceras laeve*; *Arion fasciatus*; *Arion subfuscus*), true armyworm (*Pseudaletia unipuncta*), black cutworm (*Agrotis ipsilon*), bill bug (*Sphenophorus aequalis aequalis*), and stinkbug (*Halyomorpha halys*).

Slug Activity-Density

I monitored slug and selected arthropod activity-density throughout the growing season (2017: April 28 – October 26; 2018: May 17 – October 2) using 0.09 m² pieces of white roofing shingles (Owens Corning Rolled Roofing Material, color: Shasta White). By providing shelter for slugs, roofing shingle pieces are a valuable tool for recording slug populations (Schrim and Byers 1980). In maize, I placed two shingles between rows six and seven of each split-split plot, 9.1 and 18.3 m in from the west side of the plot. I pushed aside any residue on the ground before placing the shingles. On a weekly (occasionally every other week) basis, I checked shingles in the morning before 9:00 am, to avoid letting the shingles heat up too much and drive away slugs (Hommay et al. 2003). When checking shingles, I recorded the numbers of *Deroceras reticulatum* (Agriolimacidae) (Gray Garden Slug), *Deroceras laeve* (Agriolimacidae) (Marsh Slug), *Arion fasciatus* (Arionidae) (Banded Slug), millipede (class Diplopoda), ground beetle (Coleoptera: Carabidae), and wolf spider (Araneae: Lycosidae).

Late Season European Corn Borer Assessments

I assessed maize plots for European corn borer damage close to corn harvest (starting August 30 both years) to capture damage from both generations of European corn borer (ECB) (*Ostrinia nubilalis*) (Bohnenblust et al. 2013). I measured ECB damage in rows four and eight of each maize split-split plot. In each of these rows, I sampled two maize plants every 6.1 m and counted the number of entry holes caused by ECB caterpillars per plant. Through this process, I sampled 6 plants per row, or 12 plants per split-split plot. In addition to counting entry holes, I also tallied the number of lodged maize plants in the rows on either side of me as I walked the entire length of the row.

Potato Leafhopper Abundance in Alfalfa

Potato leafhoppers (*Empoasca fabae*) arrive in Pennsylvania on storm fronts in the spring to early summer (Medler 1957). Therefore, I started sampling for potato leafhoppers in alfalfa after the first few big storms in spring (2017: starting June 8; 2018: starting June 6). I sampled potato leafhoppers using a sweep net (15" diameter), taking 10 sweeps (a sweep equals one 180° swing of the net through the upper canopy) two times in each first- and second-year alfalfa split-split plots and measured the height of four random alfalfa plants in each split-split plot. Using the average number of potato leafhoppers per plant, average plant height, cost of insecticide application, and value of the hay I then determined if the potato leafhopper populations exceeded the economic threshold and required an insecticidal spray (Calvin et al. 2003).

Predatory Arthropod Activity-Density

Once per month from June through August (2017: June 30, July 24, August 14; 2018: June 28, July 30), I assessed predatory arthropod activity-density with pitfall traps.

I installed pitfall traps in three entries of maize (maize in the Control Rotation, maize following cereal rye cover in the Pest Rotation, and maize following cereal rye silage and crimson clover (*Trifolium incarnatum*) in the Manure Rotation) in 2017 and four entries of maize (including the same entries as in 2017, and also including maize following alfalfa/orchardgrass in the Manure Rotation) in 2018. Two pitfall traps were placed between rows 6 and 7 of each split-split plot 9.1 and 18.3 m from the west edge of the plot. I used plastic deli cups (16oz, 11.5cm diameter, 8cm tall) for the pitfalls and sunk them into the ground so that the lip of the cup was level with the soil surface. A Styrofoam plate supported by nails (8.5cm long) served as a roof and protected the pitfall trap from rain. When setting the pitfalls, I filled them with 60ml of a 50% propylene glycol/50% water solution as a killing agent and left them open for 72 hours. At the end of the sampling period, I collected pitfall traps and returned them to the laboratory, where I removed the arthropods and transferred them to 80% ethanol. Ground beetles (Family: Carabidae) were identified to species using a key for Northeastern North America (Bousquet 2010).

Seasonal Predation on Sentinel Prey Caterpillars

Once per month from June through August (2017: June 26, July 20, August 10; 2018: June 21, July 26, August 28) I assessed predation by epigeal arthropods using waxworm caterpillars (*Galleria mellonella*) as sentinel prey to represent predation of slugs (Douglas et al. 2015). To exclude vertebrate predators, I placed the sentinel caterpillars in cages consisting of 1.3cm hardware mesh to allow entry of predaceous arthropods but exclude vertebrates, such as mice and birds. The cages were constructed out of a rectangle (9.5 × 11.5 cm) of hardware cloth fastened into a circular cage (11.5 cm diameter); I topped the cage with a deli-cup lid to exclude large predators and rain. I placed four of these cages in between rows 7 and 8 of each split-split at 4.6, 9.1, 13.7, and 18.3 m from the west edge of plot. In 2017, I assessed predation in three maize entries

and in 2018, I assessed predation in four maize entries (the same entries as predatory arthropod activity-density).

To prepare sentinel prey, I pinned individual waxworms to small pieces of clay and stored them at 4 degrees Celsius until deployed in the field the following day. To initiate the predation assay, starting at 8am, I placed one pinned waxworm in the center of each cage, burying the ball of clay so that the caterpillar laid on the soil surface. I checked the wax worms for signs of predation at 3, 12, 15, and 24 h after deployment. At 12 h, I replaced any dead, partial, or missing waxworms to compare day and night predation. During statistical analysis, I excluded any waxworm that was dead, but without any signs of predation.

Statistical Analyses

I used linear mixed models for most statistical analyses (SAS 9.4, SAS Institute Inc. 2016). When comparing the Manure Rotation to the Control Rotation, I excluded the synthetic fertilizer and chisel disc treatments from the Control Rotation maize to make an accurate comparison between the inject manure and broadcast manure treatments.

To compare the effect of rotation and previous cover crop on seasonal predation, predatory arthropod and slug activity-density, and early season damage assessments, I used a repeated measures analysis with variance component (VC) that best fit the covariance structure. My model for effect of rotation included rotation (Control, Pest, Manure, BAU), type of manure management (broadcast vs. injection), previous cover nested in rotation, and sample date as fixed factors as well as the interactions of manure management with rotation and previous cover nested in rotation, and the interactions of sample date with rotation, manure management and previous cover nested in rotation. My model for the effect of previous cover within the Manure Rotation included manure management, previous cover, and date as fixed factors as well as the manure management interaction with previous cover and the date interactions with manure management and previous cover. Block was a random factor for both models and I used a Kenward-Roger

degrees of freedom correction. After analysis, I used a SLICE analysis of simple effects by date for means separation (SAS 9.4, SAS Institute Inc. 2016). When assessing predatory arthropod activity-density, I used a Bonferroni correction, which decreased my p-value from 0.05 to 0.01 when making comparisons across the Manure and Control Rotations and to 0.017 when making comparisons within the Manure Rotation.

To compare the effect of alfalfa treatments on potato leafhopper populations I used a repeated measures analysis with VC best fit covariance structure. My model included rotation, main management nested in rotation, and date. I also included the interactions of rotation and main management nested in rotation with date. Block was a random factor and I used a Kenward-Roger degrees of freedom correction. After analysis, I used a SLICE analysis of simple effects by date for means separation (SAS 9.4, SAS Institute Inc. 2016).

To assess the effect of rotation on European corn borer tunnels per plant and percent of plants lodged, I used linear mixed models (SAS 9.4, SAS Institute Inc. 2016) and Tukey's HSD test for means separation. For my model, I used block as a random factor with rotation and maize entry nested in rotation as fixed factors.

In addition to using linear mixed models, I also used a regression (SAS 9.4, SAS Institute Inc. 2016) to assess the relationship between factors such as night predation rates, slug activity density, carabid beetle activity-density, and early season slug damage.

Results

Early Season Damage Assessments

In 2017, based on the mean proportion of maize seedlings damaged by slugs, I found a significant stage \times rotation interaction between the Control Rotation maize grown for grain and the Manure Rotation maize grown for silage following an interseeded cover crop (Stage*Rotation Interaction: $F_{1,36.6} = 11.9$, $P = 0.001$), and between the Control Rotation maize grown for grain and the Manure Rotation maize

grown for silage following alfalfa and orchardgrass (Stage*Rotation Interaction: $F_{1,30.8} = 16.5$, $P = 0.0003$; Figure 1-3A). Within the Manure Rotation, previous cover did not significantly influence slug damage; however, there was a significant interaction of maize entry and stage (Stage*Maize Entry: $F_{2,74} = 21.4$, $P < 0.0001$) on proportion of maize seedlings damaged by slugs (Figure 1-4). At V2, both of the maize silage entries in the Manure Rotation had the lowest proportion of slug damage compared to the maize grown for grain.

In 2018, the proportion of maize seedlings damaged by slugs did not differ between the Manure and Control Rotations (Figure 1-3B). However, there was a higher proportion of maize seedlings with slug damage at the V5 growth stage compared to the V2 growth stage (Control Maize Grain vs. Manure Maize Grain, V2 vs. V5: $F_{1,20} = 14.7$, $P = 0.001$; Control Maize Grain vs. Manure Maize Silage following Interseeded Cover, V2 vs. V5: $F_{1,30.2} = 21.6$, $P < 0.0001$; Control Corn Grain vs. Manure Corn Silage following Alfalfa and Orchardgrass, V2 vs. V5; $F_{1,20} = 31.2$, $P < 0.0001$). Likewise, within the three maize entries of the Manure Rotation there was a higher proportion of maize seedlings with slug damage at the V5 growth stage compared to the V2 growth stage (V2 vs. V5: $F_{1,48.2} = 39.9$, $P < 0.0001$). Within the Manure Rotation, maize preceded by cereal rye silage and spring-terminated alfalfa with orchardgrass had the lowest proportion of maize seedlings damaged by slugs overall (Previous Cover: $F_{2,23.6} = 7.1$, $P = 0.004$; Figure 1-5).

Slug Activity Density

During the 2017 and 2018 growing seasons, I detected three species of slugs in maize plots: *D. reticulatum*, *D. laeve*, and *A. fasciatus*. For analyses, I pooled the three species together as “total slugs.”

In 2017, there was a significant date \times rotation interaction (Date*Rotation: $F_{17,340} = 6.3$, $P < 0.0001$) between the maize grown for grain in the Manure and Control Rotations. In the beginning of the growing season, there was no difference between the

two rotations and slug populations remained relatively low. For six of the final eight sampling dates at the end of the growing season (end of August through October), the control rotation averaged significantly greater slug activity-density and about twice as many slugs under shingle traps than the Manure Rotation (Figure 1-6), perhaps because the inter-seeded cover crop in the Manure Rotation provided habitat for predatory arthropods that help limit slug populations.

In 2018, I did not detect a significant date \times rotation interaction (Figure 1-6) in the mean number of slugs under shingle traps between the maize grown for grain in the Manure and Control Rotations; however, there was a significant date \times rotation interaction (Date*Rotation: $F_{9,180} = 2.9$, $P = 0.003$) between the Control Rotation maize grown for grain and the Manure Rotation maize grown for silage following two years of alfalfa with orchardgrass. On the first two sampling dates, there were two- to three- times more slugs under shingle traps in the Manure Rotation silage maize compared to the Control Rotation grain maize (Figure 1-6). This could be due to the two years of alfalfa and orchardgrass providing ample food for the slug population.

Within the Manure Rotation in 2018, I found a significant date \times crop entry interaction (Date*Crop Entry: $F_{9,225}=3.9$, $P=0.0001$) in the mean number of slugs under the traps in the maize following alfalfa with orchardgrass and the maize following cereal rye silage and crimson clover in the Manure Rotation. On the first three and final sampling dates, there were on average more slugs under shingles traps in maize following alfalfa with orchardgrass compared to maize following cereal rye silage and crimson clover (Figure 1-8).

Seasonal Predation on Sentinel Prey Caterpillars

In 2017, predation rates on sentinel caterpillars differed significantly during both the day and night in maize grown for grain between the Control and Manure Rotations (Day: $F_{1,15} = 8.4$, $P=0.01$; Night: $F_{1,18}=5.5$, $P=0.03$). In July, there was greater predation during both the day and night in the Manure Rotation compared to the Control Rotation (Figure 1-9A). I also detected significant differences during both the day and night in

predation rates across sampling dates with greater predation at the end of the growing season compared to the beginning of the growing season (Day: $F_{2,40}=5.5$, $P=0.008$; Night: $F_{2,40}=60.3$, $P<0.0001$).

In 2018, during the day, predation rates differed significantly between maize grown for grain between the Control and Manure Rotations ($F_{1,55}=7.8$, $P=0.007$) and across time ($F_{2,55}=22.3$, $P<0.0001$) with greater predation in the Manure Rotation in August compared to the Control Rotation (Figure 1-9B). During evening, predation was greater in the Manure Rotation compared to the Control Rotation in June and August (Month*Rotation Interaction: $F_{2,58}=4.7$, $P=0.01$; Figure 1-9B).

In 2018, within the Manure Rotation maize during the day in July and August, previous cover had an influence on predation rates (Month*Previous Cover: $F_{4,54}=3.6$, $P=0.01$). In July, maize preceded by spring-terminated alfalfa/orchardgrass had about double the predation rate of maize preceded by fall-terminated alfalfa/orchardgrass (Figure 1-10), and in August, maize preceded by crimson clover cover had about double the predation rate of the spring-terminated alfalfa/orchardgrass (Figure 1-10). During evening in August, predation was significantly greater in the maize grown for grain entry following cereal rye silage or crimson clover compared to the maize grown for silage entry following alfalfa/orchardgrass (Month*Maize Entry: $F_{2,54}=5.4$, $P=0.007$).

Predatory Arthropod Activity-Density

Over the 2017 and 2018 growing seasons, I collected 2,137 carabid beetles. The most common carabid was *Pterostichus melanarius*, comprising 65% of beetles collected. The second most common species was *Harpalus pensylvanicus* (13.2%) and the third most common species was *Bembidion quadrimaculatum* (7%). The remaining beetle species made up less than 5% of total beetles collected (Table 1-5).

In June of 2017, ants (Family: Formicidae) and in June of 2018, ants (Family: Formicidae), spiders (Order: Araneae), and wolf spiders (Family: Lycosidae) were significantly more abundant ($P<0.01$, Figure 1-12) in maize in the Manure Rotation than

in maize in the Control Rotation. Wolf spiders were analyzed separately from spiders generally. In July of 2017, there were significantly more wolf spiders ($P < 0.01$, Figure 1-12) in maize grown for grain in the Manure Rotation than in maize grown for grain in the Control Rotation. These trends in predator numbers were also evident when I compared the maize grown for silage following alfalfa/orchardgrass in the Manure Rotation to the maize grown for grain in the Control Rotation in 2018.

In both years, I did not find any significant differences between rotations each month in total carabids or *P. melanarius* captured in pitfalls traps. However, carabids and *P. melanarius* significantly increased over the course of the growing season (2017 Carabidae: $F_{2,51.6} = 11.5$, $P < 0.0001$; 2017 *P. melanarius*: $F_{2,51.4} = 14.9$, $P < 0.0001$; 2018 Carabidae: $F_{1,20} = 8.4$, $P = 0.009$; 2018 *P. melanarius*: $F_{1,20} = 6.8$, $P = 0.02$) with more carabids and *P. melanarius* captured at the end of the season compared to the beginning of the season.

In 2018, within the Manure Rotation, previous cover had a significant effect on the mean number of spiders, Carabidae, and *P. melanarius* captured in pitfall traps in different months (Spiders Month*Previous Cover: $F_{2,39} = 5.6$, $P = 0.007$; Carabidae Month*Previous Cover: $F_{2,27} = 10.1$, $P = 0.0005$; *P. melanarius* Month*Previous Cover: $F_{2,27} = 9.1$, $P = 0.0009$) (Figure 1-13). In June, spiders were more abundant in the maize following spring-terminated alfalfa/orchardgrass and the cereal rye silage previous covers compared to the other previous cover treatments in the Manure Rotation. Maize preceded by a crimson clover cover crop harbored the lowest number of spiders. Also, in July, the greatest numbers of Carabidae and *Pterostichus melanarius* were captured in pitfall traps in maize preceded by crimson clover.

I also compared slug predator activity-density between rotations overall each year. These slug predators included rove beetles (Coleoptera: Staphylinidae), ground beetles (Coleoptera: Carabidae), and harvestmen (Order Opiliones). In 2017, there was a marginally significant difference between rotations with more slug predators in the Manure Rotation compared to the Control Rotation ($F_{1,3.02} = 8.47$, $P = 0.061$) (Figure 1-14). However, in 2018, compared to the Control Rotation there were significantly more slug predators in the Manure Rotation ($F_{1,6.32} = 8.31$, $P = 0.026$) (Figure 1-14).

Relationships Between Slug Activity and their Predators

In both years of the Manure and Control rotations, I also assessed the relationship between factors such as night predation rates, slug activity density, carabid beetle activity-density, and early season slug damage. There was no significant relationship between carabid beetle activity-density and slug activity-density, nor between carabid beetle activity-density and the proportion of maize seedlings with slug damage at both the V2 and V5 maize growth stages. In the Control Rotation in 2017, there was no relationship between predation rates and slug activity density. However, in the Manure Rotation in 2017, as predation increased, the average number of slugs under shingles also increased ($P = 0.027$, R-Square = 0.10; Figure 1-11). A different effect was seen in both rotations in 2018. As predation rates decreased, the average number of slugs under shingles increased (2018 Control: $P = 0.0004$, R-Square = 0.45; 2018 Manure: $P = 0.032$, R-Square = 0.096) (Figure 1-11). While there was not a significant relationship in 2018 between night predation and carabid beetle activity-density, in 2017 as night predation increased so did carabid beetle activity-density ($P = 0.0003$, R-Square = 0.18).

Late Season European Corn Borer Assessments

Damage by the European corn borer (*Ostrinia nubilalis*) was low and well below economic threshold of a 2.5% yields loss per tunnel per plant in both 2017 and 2018 (Bode and Calvin 1990). In 2017, the mean number of tunnels per plant and in 2017 and 2018, the percentage of plants lodged were not different between rotations. However, in 2018, there was a significant difference between rotations ($F_{3,9}=10$, $P=0.003$; Table 1-6) in the mean number of tunnels per plant. While still low, the mean number of tunnels per plant were greatest in the Manure Rotation, which was likely driven by the corn grain following rye silage and crimson clover cover.

Potato Leafhopper Abundance in Alfalfa

In 2017, the interaction of date and treatment significantly affected the mean number of potato leafhoppers (PLH) per sweep in both the first and second year alfalfa and orchardgrass mixture (Year One Date*Trt: $F_{8,57}=9.4$, $P<0.0001$; Year Two Date*Trt: $F_{8,48}=1.8$, $P=0.09$). On July 25, 2017 in the first year stands of alfalfa/orchard grass, numbers of PLH per sweep in the PLH-susceptible variety grown in the Manure Rotation were greater than those in the PLH-resistant variety grown in the Pest Rotation treatment (Figure 1-15). This sampling date in the first year stands of alfalfa/orchardgrass also appeared to have the highest number of PLH per sweep overall, likely due to the insecticide spray on June 15, 2017 knocking down populations until the population could increase again in July. On June 21, 2017, in the second year stands of alfalfa/orchardgrass, the resistant Manure Rotation treatment has the least PLH per sweep, again, likely due to the insecticide spray on June 15, 2017 on that treatment knocking down PLH populations.

In 2018, the first-year alfalfa/orchardgrass mixture mean number of PLH per sweep were significantly affected by the date \times treatment interaction (Year One Date*Trt: $F_{3,41.6}=10.6$, $P<0.0001$). On June 6, 2018 and June 25, 2018, the numbers of PLH per sweep were lowest in the PLH-resistant variety in the Pest Rotation (Figure 1-16), which could be explained by either the development of the trichomes making this alfalfa variety PLH resistant or by the oats that were planted with this treatment as a nurse crop or both. In 2018, the weather promoted the growth of the oats over the growth of the alfalfa, which could lead to fewer PLH in that treatment. On July 28, 2018, the PLH-resistant variety in the Pest Rotation and PLH-susceptible variety in the BAU Rotation had the fewest PLH per sweep (Figure 1-16). The low PLH per sweep in the susceptible BAU Rotation treatment could be due to the insecticide spray on that treatment on July 20, 2018.

Discussion

As a part of the Northeast Dairy Cropping Systems team, I studied the effect on insect pest and predator populations in agroecosystems of increased rotational plant diversity, through use of annual and perennial crops as well as the use of continuous year-round ground cover, and decreased insecticide use. I focused on slugs as pests of maize and their ground beetle (Coleoptera: Carabidae) predators. In agroecosystems, increased plant species diversity, particularly the presence of continuous year-round ground coverage by cover crops and winter cash crops, can provide soil health benefits (Tiemann 2015; McDaniel and Grandy 2016) and suppress insect pests (Koch 2015; Lundgren and Fergen 2011). In addition, the use of integrated pest management (IPM), in which pesticide use is reduced, in these cropping systems can promote and maintain pest suppression by natural enemies (Stern et al. 1959). Plant diversity contributes to greater numbers of natural enemies, which are then conserved by the reduced pesticide usage of IPM (Lundgren and Fergen 2011; Brust and King 1994).

Widespread adoption of conservation tillage in the Mid-Atlantic United States has been associated with development of slugs as a major pest in maize agroecosystems (Barratt et al. 1994; Douglas and Tooker 2012). Slug populations and behavior vary greatly in response to maize growth, weather, and other environmental factors, making it difficult to set an economic injury level for slug damage (Byers and Calvin 1994). Economic injury levels have ranged from 2% to 59% defoliation of maize plants by slugs (Byers and Calvin 1994). Additionally, slug control in maize is difficult as many baits and molluscicides are expensive, toxic to non-target species, and can be ineffective in controlling slugs (Douglas and Tooker 2012).

In 2017 and 2018, I found that the proportion of maize seedlings damaged by slugs was equal between the maize grown for grain in the Control Rotation and the maize grown for grain in the Manure Rotation. This similarity was also reflected in slug densities samples using shingles. At the beginning of each growing season (2017, 2018), numbers of slugs under shingles were similar in the two rotations. The lack of differences in slug activity-density and slug damage in the two rotations could be due to unique

environmental and management factors associated with each rotation. Maize in the Control Rotation was planted with neonicotinoid insecticide coated seeds and received a preventative pyrethroid insecticidal spray shortly after planting, both of which have been shown to reduce natural enemy populations (Douglas and Tooker 2016). Reduction in natural enemy numbers in turn results in reduced predation on slugs (Douglas et al. 2015). Slugs feeding on crop seedlings established with neonicotinoid-coated seeds can pass the insecticide, which has no effect on the slug, into their ground beetle predators, impairing or killing greater than 60% of the ground beetles feeding on those slugs (Douglas et al. 2015, Douglas 2016). These treatments also did use a winter cover crop, which can increase or decrease slug populations in the following crop depending on the winter cover crop used (Vernava et al. 2004). In contrast, maize grown for grain in the Manure Rotation was planted after winter cover crops of either cereal rye grown for silage or crimson clover. In addition, maize seed in the Manure Rotation was not coated with neonicotinoid insecticides and did not receive a preventative pyrethroid insecticidal spray. Reduced pesticide use and increased crop diversity within a rotation promotes populations of ground-dwelling predators, such as carabid beetles (Witmer et al. 2003; Nash et al. 2008). A winter cover crop compared to bare soil preceding maize can also promote greater slug populations (Hammond and Stinner 1987). However, the presence of ground cover or plant residue during the time that maize seedlings are vulnerable to slug damage can provide an alternative, sometimes nutritionally better, food source for slug populations than maize alone in a field (Cook et al. 2000, Le Gall and Tooker 2017).

In 2018, half of the mixture of alfalfa/orchardgrass preceding maize in the Manure Rotation was terminated in the fall of 2017, and half was terminated in the spring of 2018. Spring-terminated alfalfa/orchardgrass was associated with greater slug damage compared to slug damage in the fall-terminated alfalfa/orchardgrass. Recently terminated alfalfa/orchardgrass residue in the spring could have provided habitat for slugs (Glen 2000). However, this residue should also have provided favorable habitat for predator populations. This is supported by my observation in July 2018 in which daytime predation of sentinel prey was greater in maize preceded by spring-terminated alfalfa/orchardgrass compared to maize preceded by fall-terminated alfalfa/orchardgrass.

Pterostichus melanarius populations in crop fields tend to peak around mid-July and into August (Thomas et al. 1998); so, perhaps predator populations and the control they can provide were not sufficient to overcome the slug population encouraged by the spring-killed ground cover in June when there was greater slug damage in the maize preceded by spring-terminated alfalfa.

Within the Manure Rotation in 2018, I found significantly more slug damage in the maize grown for silage when it followed an inter-seeded mixture of annual rye (*Lolium multiflorum*) and red clover (*Trifolium pratense*) than maize that followed cereal rye harvested for silage. In recent years, many no-till farmers in Mid-Atlantic region have begun planting maize or soybeans into a standing green cover crop, usually cereal rye. This practice is commonly known as “planting green.” The cover crop is typically terminated using glyphosate up to a week after planting the cash crop. When established in this sequence, some evidence suggests that slugs will feed preferentially on the dying cover crop rather than the newly emerging maize seedlings (Le Gall and Tooker 2017). My results reveal a reduction in slug damage to maize seedlings following cereal rye grown for silage compared to an inter-seeded cover crop. While the cereal rye was removed as a silage instead of being left in the field for planting green, the cereal rye stubble remaining in the maize plot may have provided an alternative food source for slugs, reducing damage to maize seedlings. Additionally, the lack of decomposing plant residue on the soil surface could have allowed the soils to warm up and dry out enough to make the maize preceded by cereal rye silage treatment less hospitable to slugs than the nearby maize preceded by a crimson clover cover crop that did have decomposing cover crop residue.

In 2017 and 2018, whenever there were significant differences (Figures 1-9A and 1-9B) between rotations, predation rates were greater in the Manure Rotation than in the Control Rotation. Following sentinel prey assays in July 2017, wolf spider abundance was greater in pitfall traps in the Manure Rotation than the Control Rotation. Throughout the 2018 growing season, I consistently found greater abundance of ants and wolf spiders, and other spiders in pitfall traps in the Manure Rotation. However, I found that there was no significant difference between rotations in total activity-density of ground

beetles or *Pterostichus melanarius*. This result could be due in part to the small plot size of this experiment and the ability of ground beetles to colonize small plots quickly from the plot borders (Kromp 1999). Even though there were no significant differences between rotations in carabid beetle abundances, in both years, when slug predators were pooled together there were more in the Manure Rotation compared to the Control Rotation (Figure 1-14). Up until year four of this project, the slug predator activity-density in the Control and Manure Rotations was equal (Busch et al. submitted). Starting in year four and continuing through my part in the project, slug predator activity-density has been greater in the Manure Rotation compared to the Control Rotation, indicating that farmers installing these types of diversified, integrated pest management rotations will need to be patient until they see the full benefit of this system (Busch et al. submitted).

During the sentinel prey assays in both years, I observed a wide range of predatory arthropods known to be slug predators (Barker 2004), such as ground beetles, ants, wolf spiders, harvestmen, and centipedes, actively consuming sentinel waxworms. During certain times of the growing season, slug predators were more abundant and predation was greater in the Manure Rotation, which had greater plant species diversity within the rotation and eliminated unnecessary insecticide use compared to the Control Rotation. Greater abundance and diversity of predators, both generalists and specialists, can effectively reduce pest populations compared to lower diversity systems (Cardinale et al. 2003), as supported by higher levels of predation in the Manure rotation. When I used slug activity-density to predict night predation (Figure 1-11), I found inconsistent results. In 2017 in the Manure Rotation, I discovered that nighttime predation increased with higher activity-densities of slugs, a density-dependent response, which could be explained by predators responding to higher densities of slugs. In 2018 in both rotations, however, I found that increases in slug activity-density were associated with decreases in nighttime predation rates (Figure 1-11), suggesting that slug predation was negatively associated with slug population density. One possible explanation for this response is that the increased levels of rainfall in 2018 favored the activity of slugs over their predators. Unfortunately, these inconsistent results make it difficult for me to draw any conclusions on the relationship between slug populations and predation. Nevertheless, even without

this detail clarified, my results suggest that a combination of no-till, greater rotational crop diversity with continuous ground cover, and reduced insecticide use can foster populations of natural enemies (Witmer et al. 2003) that can provide effective control of invertebrate pests in agroecosystems.

In both years, damage from European corn borers (ECB) was well below the threshold of one tunnel per plant, which can result in an expected yield loss of about 2.5% (Bode and Calvin 1990). In 2017, there were no differences in damage from ECB among the three rotations. In 2018, the number of tunnels per plant was greatest in the Manure Rotation with on average 0.17 tunnels per plant. The greater number of tunnels per plant in the Manure Rotation was likely driven by the maize grain preceded by cereal rye or crimson clover. Due to weather delaying field activities, I was unable to do ECB damage assessments in that treatment until October 1, 2018, which could have allowed for more ECB damage than the treatments I had assessed in August and early September. The transgenic Bt maize varieties in the Control Rotation, and BAU Rotation in 2018 (Table 1-2), prevented ECB damage. Maize in the Pest and Manure Rotations was not transgenic. These results are comparable to those observed in previous years of this project and raise concern about whether or not the added cost of Bt-traited seed is always necessary for control of ECB. In a study of 29 sites over three years in the Pennsylvania, use of Bt maize for ECB control did not consistently improve yields, and because of the higher cost of transgenic seed, did not reliably improve profits (Bohnenblust et al. 2014). This could be due to a “halo effect,” by which large areas planted to transgenic Bt-maize results in reduced ECB populations within the field and neighboring fields. In this way, high adoption rates of Bt-maize in a region can suppress ECB populations over a large agricultural area (Hutchison et al. 2010). High adoption of Bt-maize can directly benefit growers who do not plant transgenic-Bt varieties by eliminating the cost of Bt-traited varieties to growers of non-Bt-traited varieties of maize (Hutchison et al. 2010). This “halo effect” has also been observed in China where the use of Bt cotton varieties has reduced pink bollworm damage for growers of non-Bt cotton varieties (Wan et al. 2012).

Similar to my comparison of the effects of transgenic Bt maize with non-traited varieties, I compared potato leafhopper (PLH) abundances in resistant and susceptible

alfalfa varieties planted in a mixture with orchardgrass. In 2017, in first-year alfalfa in the Pest and Manure Rotations, PLH populations were lower in plots of PLH-resistant alfalfa compared with non-resistant alfalfa on one sampling date. Likewise, in the second-year alfalfa stands in the Manure Rotation, populations of PLH were lower in resistant compared with non-resistant alfalfa on one sample date. With the addition of the BAU Rotation in 2018, I still compared PLH populations in resistant and susceptible alfalfa varieties using sweep net scouting to determine if control measures were warranted. In addition, I was then also able to compare those treatments in the Pest and Manure Rotations to the BAU PLH-susceptible alfalfa that received an insecticide spray for PLH between each cutting regardless of scouting efforts. On two dates in 2018, PLH populations were lowest in the first-year stands of alfalfa and orchardgrass with resistant alfalfa in the Pest Rotations. Unfortunately, the effect of PLH-resistant varieties on PLH populations was inconsistent. PLH populations on one sampling date were low in both the PLH-resistant alfalfa in the Pest Rotation and the PLH-susceptible alfalfa in the BAU Rotation. Even though PLH-resistant alfalfa varieties can reduce PLH populations, we did not find any overall seasonal yield differences between treatments (NESARE 2018), indicating that the added cost of PLH resistant alfalfa varieties may not be worth the added expense to farmers. In addition, reducing insecticide use by scouting to determine economic thresholds could conserve natural enemies, reduce environmental pollution, and reduce risk to the applicator. While I was unable to test the effect of intercropping orchardgrass with alfalfa on PLH populations, previous work has shown that addition of orchardgrass causes PLH to move between plants more often, increasing predation rates of PLH (Straub et al. 2013). In addition, over a three-year study there were no differences in dry matter yields, crude protein concentration, and acid detergent fiber concentrations between PLH-resistant and nonresistant alfalfa varieties under high and low PLH populations in plots without insecticide treatments (Dellinger et al. 2006).

Conclusions

Over two years, I compared high-diversity, IPM cropping systems to low-diversity, preemptive pest control cropping systems on arthropod- and mollusk-pest and arthropod-predator populations. In many instances, the more diverse Manure Rotation provided equal or better predator activity-density and pest suppression than did the less-diverse Control Rotation. Maize grown for grain in the Manure Rotation yielded better than maize grown for grain in the Control Rotation in 2017, while yields in these two rotations were similar in 2018 (NESARE 2018). My results suggest that, in comparison to the use of preventative tactics for management of invertebrate pests, combination of plant diversity within cropping rotations and using IPM for pest control can be effective means of maintaining pest populations below economically damaging levels. This thesis provides a part of the larger picture of this NESARE Dairy Cropping Systems Project. My data, combined with data generated in the previous seven years (Busch et al. submitted), should provide new insights on the benefits of diversified, low-insecticide input cropping systems over time.

References

- Adams, P., Orr, D., Arellano, C., & Cardoza, Y. (2017). Soil and foliar arthropod abundance and diversity in five cropping systems in the coastal plains of North Carolina. *Environmental Entomology*, 46(4), 771-783. doi:10.1093/ee/nvx081
- Altieri, M. A. (1999). The ecological role of biodiversity in agroecosystems. *Agriculture, Ecosystems and Environment*, 74(1), 19-31. doi:10.1016/S0167-8809(99)00028-6
- Barker, G.. Natural Enemies of Terrestrial Molluscs, CABI, 2004. ProQuest Ebook Central, <https://ebookcentral.proquest.com/lib/pensu/detail.action?docID=301669>.
- Barratt, B. I. P., Byers, R. A., & Bierlein, D. L. (1994). Conservation tillage crop yields in relation to grey garden slug [*Deroceras reticulatum* (Müller)] (Mollusca: Agriolimacidae) density during establishment. *Crop Protection*, 13(1), 49-52. doi:10.1016/0261-2194(94)90136-8

- Bode, W. M., & Calvin, D. D. (1990). Yield-loss relationships and economic injury levels for European corn borer (Lepidoptera: Pyralidae) populations infesting Pennsylvania field corn. *Journal of Economic Entomology*, 83(4), 1595-1603. doi:10.1093/jee/83.4.1595
- Bohnenblust, E. W., Breining, J. A., Shaffer, J. A., Fleischer, S. J., Roth, G. W., & Tooker, J. F. (2014). Current European corn borer, *Ostrinia nubilalis*, injury levels in the northeastern United States and the value of Bt field corn. *Pest Management Science*, 70(11), 1711-1719. doi:10.1002/ps.3712
- Bousquet, Y. 2010. Illustrated Identification Guide to Adults and Larvae of Northeastern North American Ground Beetles (Coleoptera: Carabidae). Pensoft Publishers, Sofia-Moscow, Bulgaria.
- Brooks, A. S., Wilcox, A., Cook, R. T., James, K. L., & Crook, M. J. (2006). The use of an alternative food source (red clover) as a means of reducing slug pest damage to winter wheat: Towards field implementation. *Pest Management Science*, 62(3), 252-262. doi:10.1002/ps.1155
- Brust, G. E., & King, L. R. (1994). Effects of crop rotation and reduced chemical inputs on pests and predators in maize agroecosystems. *Agriculture, Ecosystems and Environment*, 48(1), 77-89. doi:10.1016/0167-8809(94)90077-9
- Busch, A. K., Douglas, M. R., Malcolm, G. M., Karsten, H. D., & Tooker, J. F. A high-diversity/IPM cropping system fosters beneficial arthropod populations, limits invertebrate pests, and produced competitive corn yields. (Submitted to *Agriculture, Ecosystems, and Environment*, April 2019).
- Byers, R. A., & Calvin, D. D. (1994). Economic injury levels to field corn from slug (Stylommatophora: Agrolimacidae) feeding. *Journal of Economic Entomology*, 87(5), 1345-1350. doi:10.1093/jee/87.5.1345
- Calvin, D., Hower, A., & Tooker, J. (2003). Potato Leafhopper on Alfalfa. Retrieved February 7, 2019, from <https://ento.psu.edu/extension/factsheets/potato-leafhopper-alfalfa>
- Cardinale, B. J., Harvey, C. T., Gross, K. and Ives, A. R. (2003), Biodiversity and biocontrol: emergent impacts of a multi-enemy assemblage on pest suppression

and crop yield in an agroecosystem. *Ecology Letters*, 6: 857-865.

doi:10.1046/j.1461-0248.2003.00508.x

Cook, R. T., Bailey, S. E. R., McCrohan, C. R., Nash, B., & Woodhouse, R. M. (2000).

The influence of nutritional status on the feeding behaviour of the field slug,

Deroceras reticulatum (Müller). *Animal Behaviour*, 59(1), 167-176.

doi:10.1006/anbe.1999.1275

Crookston, R., Kurle, J., Copeland, P., Ford, J., & Lueschen, W. (1991). Rotational

cropping sequence affects yield of corn and soybean. *Agronomy Journal*, 83(1),

108-113. doi:10.2134/agronj1991.00021962008300010026x

Davis, A. S., Hill, J. D., Chase, C. A., Johanns, A. M., & Liebman, M. (2012). Increasing

cropping system diversity balances productivity, profitability and environmental

health. *PLoS One*, 7(10), e47149. doi:10.1371/journal.pone.0047149

Dellinger, T. A., Youngman, R. R., Laub, C. A., Brewster, C. C., & Kuhar, T. P. (2006).

Yield and forage quality of glandular-haired alfalfa under alfalfa weevil

(Coleoptera: Curculionidae) and potato leafhopper (Hemiptera: Cicadellidae) pest pressure in Virginia. *Journal of Economic Entomology*, 99(4), 1235-1244.

doi:10.1603/0022-0493(2006)99[1235:YAFQOG]2.0.CO;2;10.1603/0022-0493-99.4.1235;

Douglas, M. R., & Tooker, J. F. (2012). Slug (Mollusca: Agriolimacidae, Arionidae)

ecology and management in no-till field crops, with an emphasis on the Mid-

Atlantic region. *Journal of Integrated Pest Management*, 3(1), C1-C9.

doi:10.1603/IPM11023

Douglas, M., Rohr, J., & Tooker, J. (2015). Neonicotinoid insecticide travels through a

soil food chain, disrupting biological control of non-target pests and decreasing

soya bean yield. *Journal of Applied Ecology*, 52(1), 250-260. doi:10.1111/1365-

2664.12372

Douglas, M. R. 2016. *Ecological trade-offs associated with insecticide use, from*

Pennsylvania to Bangladesh. (Doctoral Dissertation, Pennsylvania State

University)

- Douglas, M., & Tooker, J. (2016). Meta-analysis reveals that seed-applied neonicotinoids and pyrethroids have similar negative effects on abundance of arthropod natural enemies. *PeerJ*, 4, e2776. doi:10.7717/peerj.2776
- Dunbar, M. W., O'Neal, M. E., & Gassmann, A. J. (2016). Increased risk of insect injury to corn following rye cover crop. *Journal of Economic Entomology*, 109(4), 1691-1697. doi:10.1093/jee/tow101
- Emmerson, M., Morales, M. B., Oñate, J. J., Batáry, P., Berendse, F., Liira, J., ... (2016). How agricultural intensification affects biodiversity and ecosystem services. *Advances in Ecological Research*, 55, 43-97.
- Glen, D. M. (2000). The effects of cultural measures on cereal pests and their role in integrated pest management. *Integrated Pest Management Reviews*, 5(1), 25-40. doi:10.1023/A:1009609504464
- Grover, K. K., Karsten, H. D., & Roth, G. W. (2009). Corn grain yields and yield stability in four long-term cropping systems. *Agronomy Journal*, 101(4), 940-946. Retrieved from <http://ezaccess.libraries.psu.edu/login?url=https://search-proquest-com.ezaccess.libraries.psu.edu/docview/346933856?accountid=13158>
- Hammond, R. B., & Stinner, B. R. (1987). Seedcorn maggots (Diptera: Anthomyiidae) and slugs in conservation tillage systems in Ohio. *Journal of Economic Entomology*, 80(3), 680-684. doi:10.1093/jee/80.3.680
- Hill, M., Macfadyen, S., & Nash, M. (2017). Broad spectrum pesticide application alters natural enemy communities and may facilitate secondary pest outbreaks. *PeerJ*, 5, e4179. doi:10.7717/peerj.4179
- Hommay, G., Kienlen, J.C, Jacky, F., & Gertz, C. (2003). Daily variation in the number of slugs under refuge traps. *Annals of Applied Biology*, 142(3), 333-339. doi:10.1111/j.1744-7348.2003.tb00258.x
- House, G. J., & Alzugaray, M. D. R. (1989). Influence of cover cropping and no-tillage practices on community composition of soil arthropods in a North Carolina agroecosystem. *Environmental Entomology*, 18(2), 302-307. doi:10.1093/ee/18.2.302

- Hutchison, W., Burkness, E., Mitchell, P., Moon, R., Leslie, T., Fleischer, S., . . . Raun, E. (2010). Areawide suppression of European corn borer with Bt maize reaps savings to non-Bt maize growers. *Science*, 330(6001), new series, 222-225. Retrieved from <http://www.jstor.org.ezaccess.libraries.psu.edu/stable/40931819>
- Koch, R. L., Sezen, Z., Porter, P. M., Ragsdale, D. W., Wyckhuys, K. A. G., & Heimpel, G. E. (2015). On-farm evaluation of a fall-seeded rye cover crop for suppression of soybean aphid (Hemiptera: Aphididae) on soybean: Suppression of soybean aphid with rye cover crop. *Agricultural and Forest Entomology*, 17(3), 239-246. doi:10.1111/afe.12099
- Kromp, B. (1999). Carabid beetles in sustainable agriculture: A review on pest control efficacy, cultivation impacts and enhancement. *Agriculture, Ecosystems and Environment*, 74(1), 187-228. doi:10.1016/S0167-8809(99)00037-7
- Laub, C. A. & Luna, J. M. (1992). Winter cover crop suppression practices and natural enemies of armyworm (Lepidoptera: Noctuidae) in no-till corn, *Environmental Entomology*, Volume 21, Issue 1, Pages 41–49, <https://doi-org.ezaccess.libraries.psu.edu/10.1093/ee/21.1.41>
- Lechenet, M., Bretagnolle, V., Bockstaller, C., Boissinot, F., Petit, M., Petit, S., & Munier-Jolain, N. (2014). Reconciling pesticide reduction with economic and environmental sustainability in arable farming. *Plos One*, 9(6), e97922. doi:10.1371/journal.pone.0097922
- Le Gall, M., & Tooker, J. (2017). Developing ecologically based pest management programs for terrestrial molluscs in field and forage crops. *Journal of Pest Science*, 90(3), 825-838. doi:10.1007/s10340-017-0858-8
- Lundgren, J. G., Hesler, L. S., Clay, S. A., & Fausti, S. F. (2013). Insect communities in soybeans of eastern South Dakota: The effects of vegetation management and pesticides on soybean aphids, bean leaf beetles, and their natural enemies. *Crop Protection*, 43, 104-118. doi:10.1016/j.cropro.2012.08.005

- Lundgren, J. G., & Fergen, J. K. (2011). Enhancing predation of a subterranean insect pest: A conservation benefit of winter vegetation in agroecosystems. *Applied Soil Ecology*, *51*, 9-16. doi:10.1016/j.apsoil.2011.08.005
- Malcolm, G. M., Camargo, G. G. T., Ishler, V. A., Richard, T. L., & Karsten, H. D. (2015). Energy and greenhouse gas analysis of northeast U.S. dairy cropping systems. *Agriculture, Ecosystems and Environment*, *199*, 407-417. doi:10.1016/j.agee.2014.10.007
- McDaniel, M. D., & Grandy, A. S. (2016). Soil microbial biomass and function are altered by 12 years of crop rotation. *Soil*, *2*(4), 583-599. doi:10.5194/soil-2-583-2016
- Medler, J. T. (1957). Migration of the potato leafhopper—A report on a cooperative Study. *Journal of Economic Entomology*, *50*(4), 493-497. doi:10.1093/jee/50.4.493
- Menalled, F. D., Lee, J. C., & Landis, D. A. (1999). Manipulating carabid beetle abundance alters prey removal rates in corn fields. *Biocontrol*, *43*(4), 441-456. doi:10.1023/A:1009946004251
- Nash, M. A., Thomson, L. J., & Hoffmann, A. A. (2008). Effect of remnant vegetation, pesticides, and farm management on abundance of the beneficial predator *Notonomus gravis* (Chaudoir) (Coleoptera: Carabidae). *Biological Control*, *46*(2), 83-93. doi:10.1016/j.biocontrol.2008.03.018
- NESARE (2018). Advanced sustainable cropping systems for dairy farms in the Northeast. Retrieved March 27, 2019, from <https://projects.sare.org/project-reports/lne16-354r/>
- Olmstead, J., & Brummer, E. C. (2008). Benefits and barriers to perennial forage crops in Iowa corn and soybean rotations. *Renewable Agriculture and Food Systems*, *23*(2), 97-107. doi:10.1017/S1742170507001937
- O'Rourke, M. E., Liebman, M., & Rice, M. E. (2008). Ground beetle (Coleoptera: Carabidae) assemblages in conventional and diversified crop rotation

systems. *Environmental Entomology*, 37(1), 121-130. doi:10.1603/0046-225X(2008)37[121:GBCCAI]2.0.CO;2

The Pennsylvania State University. 2019. Sustainable Dairy Cropping Systems. Accessed 2/12/2019: <https://plantscience.psu.edu/research/areas/crop-ecology-and-management/cropping-systems>

Plourde, J. D., Pijanowski, B. C., & Pekin, B. K. (2013). Evidence for increased monoculture cropping in the central united states. *Agriculture, Ecosystems and Environment*, 165, 50-59. doi:10.1016/j.agee.2012.11.011

Porter, P. M., Crookston, R. K., Ford, J. H., Huggins, D. R., & Lueschen, W. E. (1997). Interrupting yield depression in monoculture corn: Comparative effectiveness of grasses and dicots. *Agronomy Journal*, 89(2), 247. doi:10.2134/agronj1997.00021962008900020015x

Prasifka, J., Schmidt, N., Kohler, K., O'Neal, M., Hellmich, R., & Singer, J. (2006). Effects of living mulches on predator abundance and sentinel prey in a corn-soybean-forage rotation. *Environmental Entomology*, 35(5), 1423-1431. doi:10.1093/ee/35.5.1423

SAS Institute Inc. 2016. SAS/STAT® 9.4 User's Guide. Cary, NC: SAS Institute Inc.

Schipanski, M. E., Barbercheck, M., Douglas, M. R., Finney, D. M., Haider, K., Kaye, J. P., . . . White, C. (2014). A framework for evaluating ecosystem services provided by cover crops in agroecosystems. *Agricultural Systems*, 125, 12-22. doi:10.1016/j.agsy.2013.11.004

Schrim, M., & Byers, R. A. (1980). A method for sampling three slug species attacking sod-seeded legumes [*Derocerus reticulatum*, *Derocerus laeve*]. *Melsheimer Entomological Series*.

Smith, R. G., Gross, K. L., & Robertson, G. P. (2008). Effects of crop diversity on agroecosystem function: Crop yield response. *Ecosystems*, 11(3), 355-366. doi:10.1007/s10021-008-9124-5

- Snyder, E. M., Curran, W. S., Karsten, H. D., Malcolm, G. M., Duiker, S. W., & Hyde, J. A. (2016). Assessment of an integrated weed management system in no-till soybean and corn. *Weed Science*, *64*(4), 712-726.
doi:<http://dx.doi.org.ezaccess.libraries.psu.edu/10.1614/WS-D-16-00021.1>
- Staudacher, K., Schallhart, N., Thalinger, B., Wallinger, C., Juen, A., & Traugott, M. (2013). Plant diversity affects behavior of generalist root herbivores, reduces crop damage, and enhances crop yield. *Ecological Applications*, *23*(5), 1135-1145.
doi:10.1890/13-0018.1
- Stern VMRF, Smith R, Van den Bosch R, Hagen K. 1959. The integration of chemical and biological control of the spotted alfalfa aphid: the integrated control concept. *Hilgardia* 29:81–101
- Straub, C. S., Simasek, N. P., Gapinski, M. R., Dohm, R., Aikens, E. O., & Muscella, S. (2013). Influence of nonhost plant diversity and natural enemies on the potato leafhopper, *Empoasca fabae*, and pea aphid, *Acyrtosiphon pisum*, in alfalfa. *Journal of Pest Science*, *86*(2), 235-244. doi:10.1007/s10340-012-0465-7
- Symondson, W. O. C., Glen, D. M., Ives, A. R., Langdon, C. J., & Wiltshire, C. W. (2002). Dynamics of the relationship between a generalist predator and slugs over five years. *Ecology*, *83*(1), 137-147. doi:10.1890/0012-9658(2002)083[0137:DOTRBA]2.0.CO;2
- Thomas, C. F. G., L. Parkinson, and E. J. P. Marshall. 1998. Isolating the components of activity density for the carabid beetle *Pterostichus melanarius* in farmland. *Oecologia* 116: 103–112.
- Tiemann, L. K., Grandy, A. S., Atkinson, E. E., Marin-Spiotta, E., McDaniel, M. D., & Hooper, D. (2015). Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecology Letters*, *18*(8), 761-771.
doi:10.1111/ele.12453
- Tooming, E., Merivee, E., Must, A., Merivee, M., Sibul, I., Nurme, K., & Williams, I. H. (2017). Behavioural effects of the neonicotinoid insecticide thiamethoxam on the

predatory insect *Platynus assimilis*. *Ecotoxicology*, 26(7), 902-913.
doi:10.1007/s10646-017-1820-5

Vernava, M. N., Phillips-Aalten, P. M., Hughes, L. A., Rowcliffe, H., Wiltshire, C. W., & Glen, D. M. (2004). Influences of preceding cover crops on slug damage and biological control using *Phasmarhabditis hermaphrodita*. *Annals of Applied Biology*, 145(3), 279-284. doi:10.1111/j.1744-7348.2004.tb00384.x

Wan P, Huang Y, Tabashnik BE, Huang M, Wu K (2012) The halo effect: Suppression of pink bollworm on non-Bt cotton by Bt cotton in China. *PLoS ONE* 7(7): e42004. <https://doi.org/10.1371/journal.pone.0042004>

Wendland, S., Baudy, P., Fusser, M. S., Entling, M. H., & Schirmel, J. (2017). Contrasting predation pressure of different ground beetles on slugs and their eggs. *Biocontrol*, 62(6), 749-756. doi:10.1007/s10526-017-9845-5

Witmer, J. E., Hough-Goldstein, J. A., & Pesek, J. D. (2003). Ground-dwelling and foliar arthropods in four cropping systems. *Environmental Entomology*, 32(2), 366-376. doi:10.1603/0046-225X-32.2.366;10.1603/0046-225X(2003)032[0366:GAFAIF]2.0.CO;2;

Wittwer, R. A., Dorn, B., Jossi, W., & van der Heijden, Marcel G A. (2017). Cover crops support ecological intensification of arable cropping systems. *Scientific Reports*, 7(1), 41911. doi:10.1038/srep41911

Tables

Table 1-1. Overview of the four different rotations.

	Control Rotation	Manure Rotation	Pest Rotation	BAU Rotation
Comparison	Broadcast Manure vs. Inject Manure	Broadcast manure vs Inject Manure	Standard Herbicide vs. Reduced Herbicide	Standard Herbicide
Crop Rotation	2yr with Annuals	6yr with Annuals and Perennials	6yr with Annuals and Perennials	6yr with Annuals and Perennials
Winter Cover	No	Yes	Yes	Yes
Pest Management	Preventative: Bt, Pyrethroids and Neonicotinoids	Integrated Pest Management	Integrated Pest Management	Preventative: Bt, Pyrethroids and Neonicotinoids

Table 1-2. 2017 and 2018 maize grown for grain management practices.

Year	Management Practice	Control Rotation	Manure Rotation
2017	Maize Variety	TA550-20ND	TA550-18ND
	Seed Treatment	Neonic	—
	Transgenic Traits	GT, CB, LL, Bt	GT
	Soil Insecticide/Rate	Warrior/3.5oz.	—
	Application Date	5/24/2017	—
	Previous Crop	Soybean	Sorghum Sudangrass
	Previous Cover Crop	—	Rye or CC
	Planting Date	5/23/2017	5/24/2017
	Interseed Date	—	7/7/2017
2018	Maize Variety	MC 5663 300GT	MC 5661
	Seed Treatment	THX	—
	Transgenic Traits	GT, CB, LL, Bt	GT
	Soil Insecticide/Rate	Warrior/3.5oz.	—
	Application Date	6/15/2018	—
	Previous Crop	Soybean	Sorghum Sudangrass
	Previous Cover Crop	—	Rye or CC
	Planting Date	5/30/2018	6/5/2018
	Interseed Date	—	7/10/2018

Key GT: Glyphosate Tolerant
 LL: Liberty Link
 CB: Corn Borer
 Bt: *Bacillus thuringiensis*
 Neonic: Neonicotinoid Insecticide
 THX: Thiamethoxam
 CC: Crimson Clover

Table 1-3. 2017 and 2018 maize grown for silage management practices.

Year	Management Practice	Pest Rotation	Manure Rotation	
			After Int	After AOg2
2017	Maize Variety	TA290-18	TA445-18	TA445-18
	Planting Date	5/10/2017	6/1/2017	5/18/2017
	Transgenic Traits	GT	GT	GT
	Previous Crop	Soybean	Maize Grain	AOg2
	Previous Cover Crop	Rye	Int	—
2018	Maize Variety	TA477-18	TA477-18	TA447-18
	Planting Date	5/31/2018	5/30/2018	6/1/2018
	Transgenic Traits	GT	GT	GT
	Previous Crop	Soybean	Maize Grain	AOg2
	Previous Cover Crop	Rye	Int	—

Key GT: Glyphosate Tolerant

Int: Interseeded Cover Crop (annual rye and clover mix)

AOg2: Second Year Alfalfa and Orchard Grass

Table 1-4. Top: First year alfalfa management. Bottom: Second year alfalfa management.

Year	Management Practice	Pest Rotation		Manure Rotation	
		Resistant	Susceptible	Resistant	Susceptible
2017	Variety	Nexgrow6422Q		Nexgrow6422Q	
	Planting Date	4/17/2017	4/17/2017	4/17/2017	4/17/2017
	Insecticide/Rate	Warrior/3oz.		Warrior/3oz.	
	Application Dates	6/15		6/15	
	Harvest Dates	6/29; 8/1; 9/19		6/29; 8/1; 9/19	
2018	Variety	FSG42OLH	Nexgrow6422Q	FSG42OLH	Nexgrow6422Q
	Planting Date	4/30/2018	4/30/2018	4/30/2018	4/30/2018
	Insecticide/Rate	Warrior/3oz.		Warrior/3oz.	
	Application Dates	—	6/8	—	6/8
	Harvest Dates	7/2; 8/9; 9/19		7/2; 8/9; 9/19	

Year	Management Practice	Pest Rotation		Manure Rotation	
		Resistant	Susceptible	Resistant	Susceptible
2017	Variety	SW420LH		42OLG	Nexgrow6422Q
	Planting Date	4/19/2016	4/19/2016	4/19/2016	4/19/2016
	Insecticide/Rate	—		Warrior/3oz.	
	Application Dates	—		6/15	—
	Harvest Dates	5/22; 6/26; 7/25; 9/8		5/22; 6/29; 7/25; 9/8	
2018	Variety	FSG42OLH	Nexgrow6422Q	FSG42OLH	Nexgrow6422Q
	Planting Date	4/17/2017	4/17/2017	4/17/17	4/17/17
	Insecticide/Rate	Warrior/3oz.		—	
	Application Dates	6/15		—	
	Harvest Dates	5/29; 7/2; 8/7; 9/19		5/30; 7/2; 8/7; 9/19	

Table 1-5. Total Carabidae caught in 2017 and 2018 pitfall traps.

Species	2017		2018		Combined	
	Total	%	Total	%	Total	%
<i>Pterostichus melanarius</i>	1010	66.4	378	61.3	1388	65
<i>Pterostichus mutus</i>	33	2.2	37	6	70	3.3
<i>Bembidion quadrimaculatum</i>	84	5.5	66	10.7	150	7
<i>Chlaenius tricolor</i>	65	4.3	21	3.4	86	4
<i>Harpalus pensylvanicus</i>	273	18	10	1.6	283	13.2
<i>Harpalus affinis</i>	4	0.3	2	0.3	6	0.3
<i>Harpalus sp.</i>	6	0.4	6	1	12	0.6
<i>Amara sp.</i>	7	0.5	37	6	44	2.1
<i>Bembidion sp.</i>	1	0.07	16	2.6	17	0.8
<i>Poecilus lucublandus</i>	8	0.5	2	0.3	10	0.5
<i>Poecilus chalcites</i>	2	0.1	2	0.3	4	0.2
<i>Bradycellus rupestris</i>	7	0.5	–	–	7	0.3
<i>Dyschirius sp.</i>	2	0.1	13	2.1	15	0.7
<i>Scarites quadriceps</i>	2	0.1	–	–	2	0.1
<i>Scarites subterraneus</i>	1	0.07	3	0.5	4	0.2
<i>Diplochelia obtusa</i>	3	0.2	–	–	3	0.1
<i>Agonum sp.</i>	11	0.7	17	2.8	28	1.3
<i>Colliuris pensylvanica</i>	1	0.07	1	0.2	2	0.1
<i>Badister sp.</i>	–	–	3	0.5	3	0.1
<i>Stenolophus sp.</i>	–	–	1	0.2	1	0.05
<i>Notiophilus sp.</i>	–	–	1	0.2	1	0.05
<i>Cicindela sp.</i>	–	–	1	0.2	1	0.05
Total	1520	100	617	100	2137	100

Table 1-6. 2017 and 2018 European corn borer damage by year and rotation (LS-Mean \pm SE). The Manure Rotation is also broken down by maize entry (R/CC,Cg: Maize grain following rye silage and crimson clover cover; AOg2,Cs: Maize silage following second year alfalfa with orchardgrass; Int,Cs: Maize silage following interseeded cover crop). Values marked with letters are significantly different (PROC MIXED; Covariate Type VC; $P \leq 0.05$) based on Tukey's post-hoc test.

Year	Rotation	Tunnels/Plant	Percent Plants Lodged
2017	Control	0.07 \pm 0.027	0.84 \pm 1.121
	Manure	0.06 \pm 0.016	2.19 \pm 0.879
	R/CC,Cg	0.02 \pm 0.027	0.57 \pm 1.121
	AOg2,Cs	0.10 \pm 0.027	2.66 \pm 1.121
	Int,Cs	0.06 \pm 0.027	3.35 \pm 1.121
	Pest	0.07 \pm 0.027	1.41 \pm 1.121
	2018	Control	0 \pm 0.035
BAU		0 \pm 0.027 	0.01 \pm 0.198
Manure		0.17 \pm 0.027 a	0.67 \pm 0.198
R/CC,Cg		0.36 \pm 0.035	1.56 \pm 0.214
AOg2,Cs		0.08 \pm 0.035	0.12 \pm 0.214
Int,Cs		0.08 \pm 0.035	0.32 \pm 0.214
Pest		0.04 \pm 0.035 b	0.30 \pm 0.214

Figures

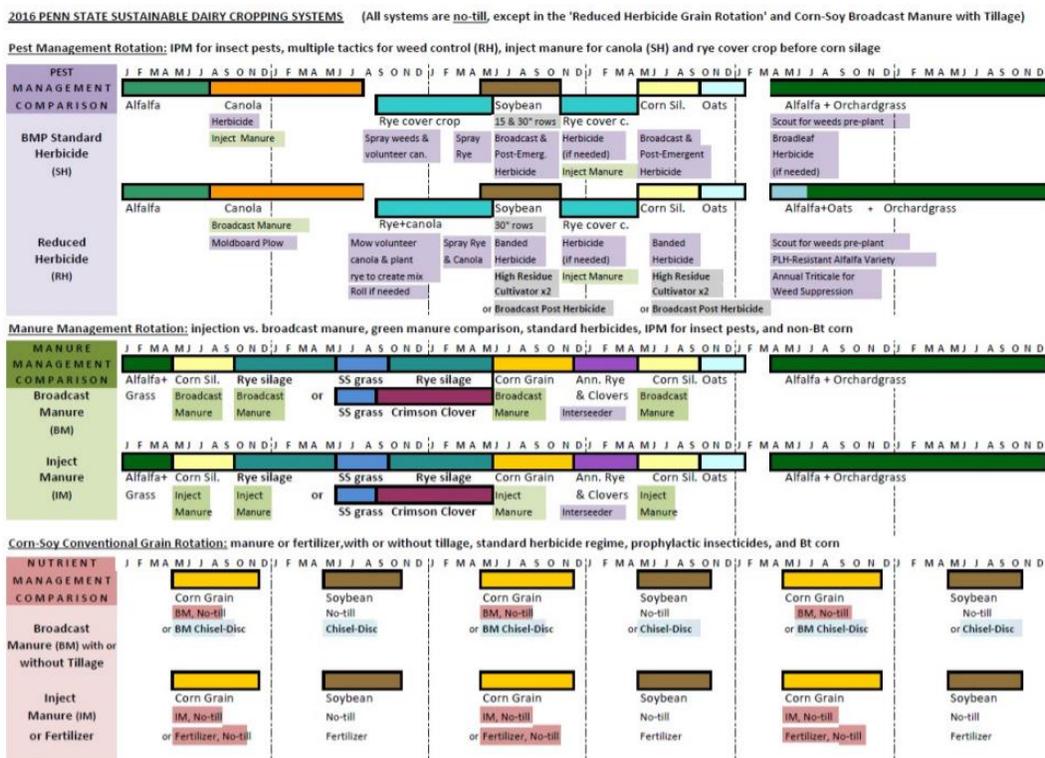


Figure 1-1. 2017 cropping schematic showing within rotation comparisons. The Pest and Manure Rotations provide year-round ground cover, while the Control Rotation has no ground cover between maize and soybean crops.

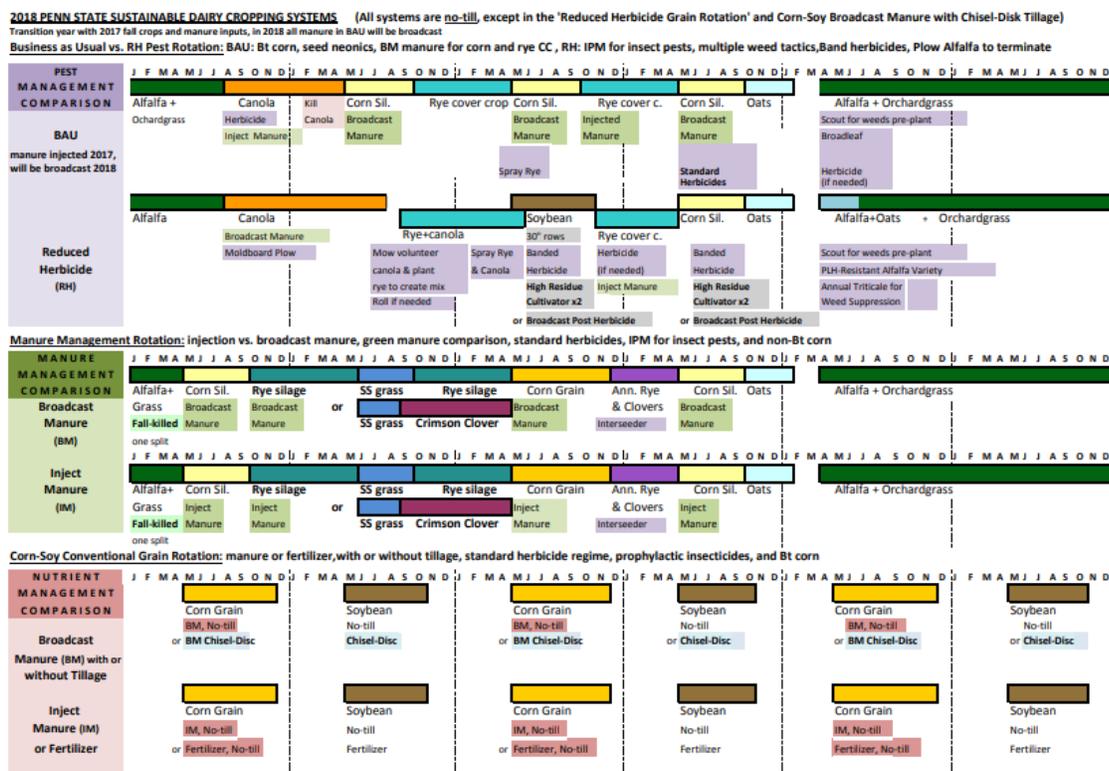


Figure 1-2. 2018 cropping schematic showing the addition of the BAU Rotation in the pest management comparison.

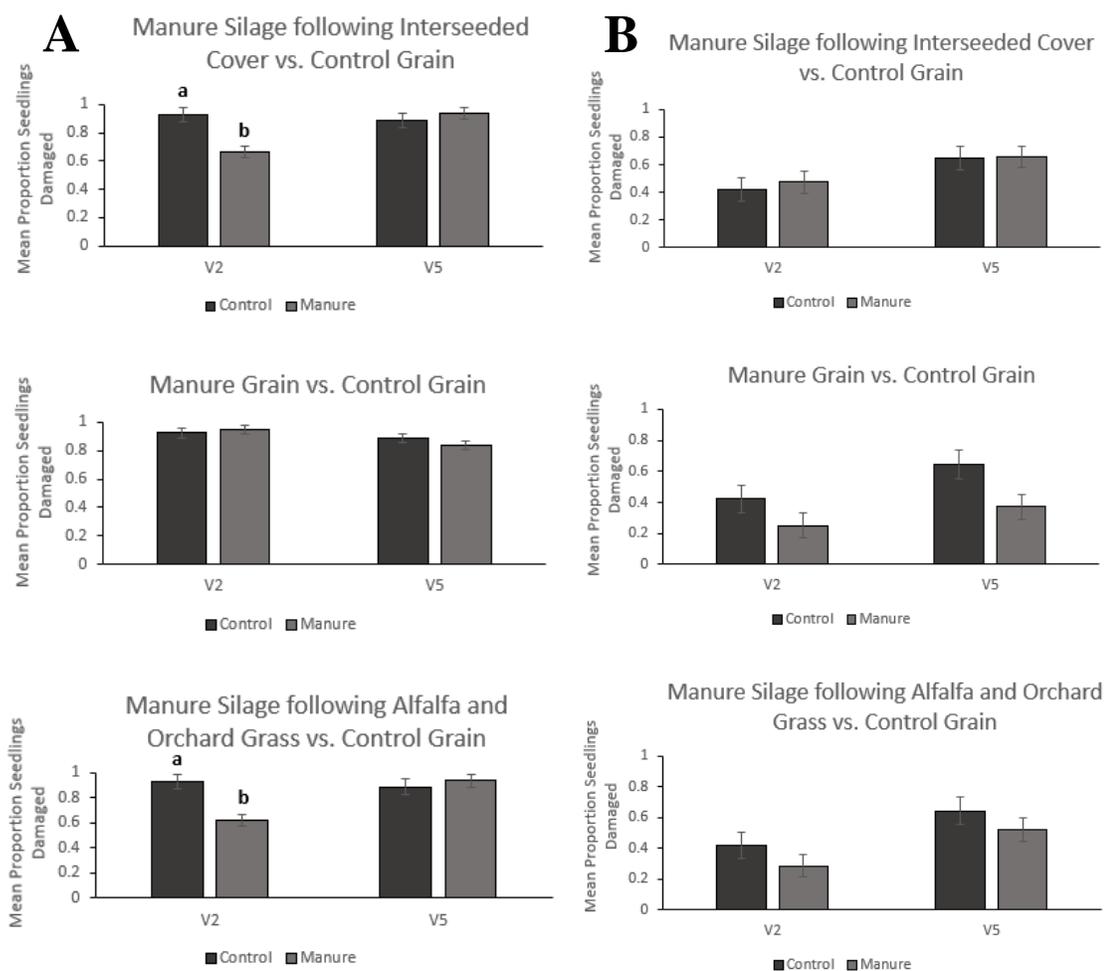


Figure 1-3. (A) 2017 mean proportion of maize seedlings damaged by slugs (LS-Means \pm SE) based on growth stage and rotation. Values marked with letters are significantly different (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by stage. (B) 2018 mean proportion of maize seedlings damaged by slugs (LS-Means \pm SE) based on growth stage and rotation. There is no significant Stage*Rotation interaction (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by stage.

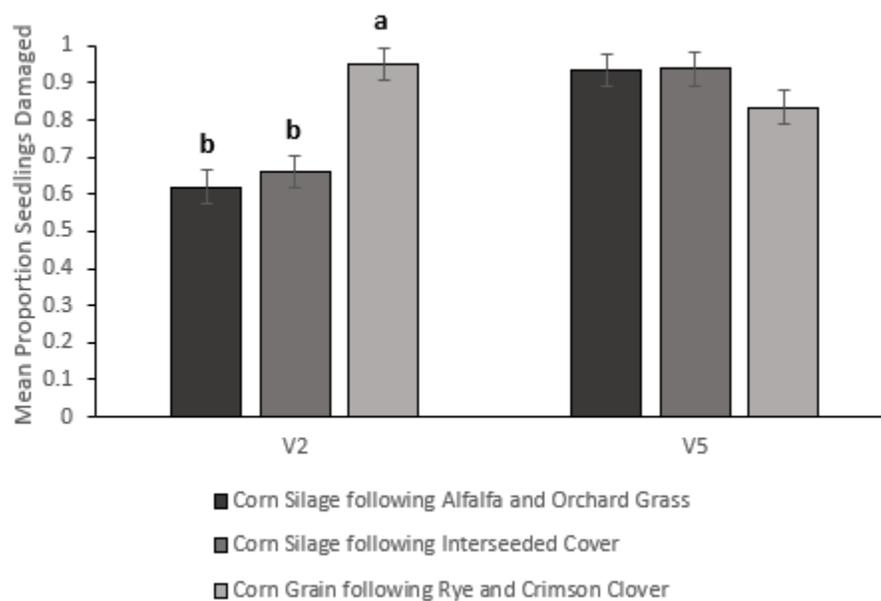


Figure 1-4. 2017 mean proportion of maize seedlings damaged by slugs (LS-Mean \pm SE) in the Manure Rotation based on the maize entry and stage. Values marked with letters are significantly different (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by stage.

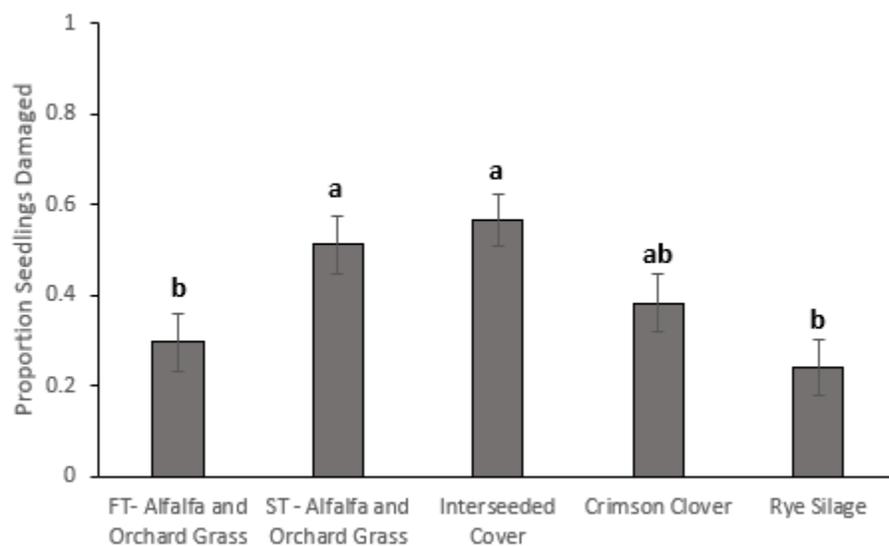


Figure 1-5. 2018 mean proportion of maize seedlings damaged by slugs (LS-Mean \pm SE) in the Manure Rotation based on the previous cover in the plot (FT: Fall Terminated, ST: Spring Terminated). Values marked with letters are significantly different (PROC MIXED; Covariate Type VC; $P \leq 0.05$) based on Tukey's post-hoc test.

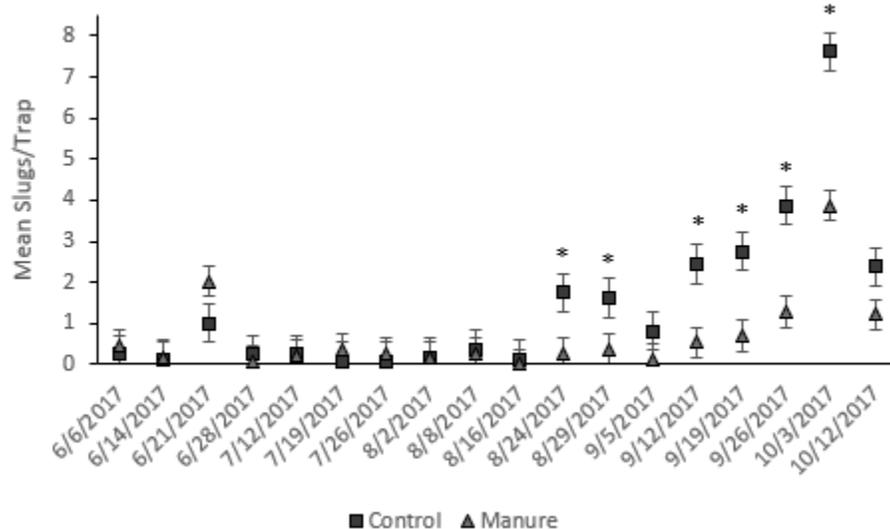


Figure 1-6. 2017 mean slugs per shingle trap (LS-Mean \pm SE) in maize grown for grain plots in the Manure and Control Rotation. Values mark with (*) are statistically different (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by date.

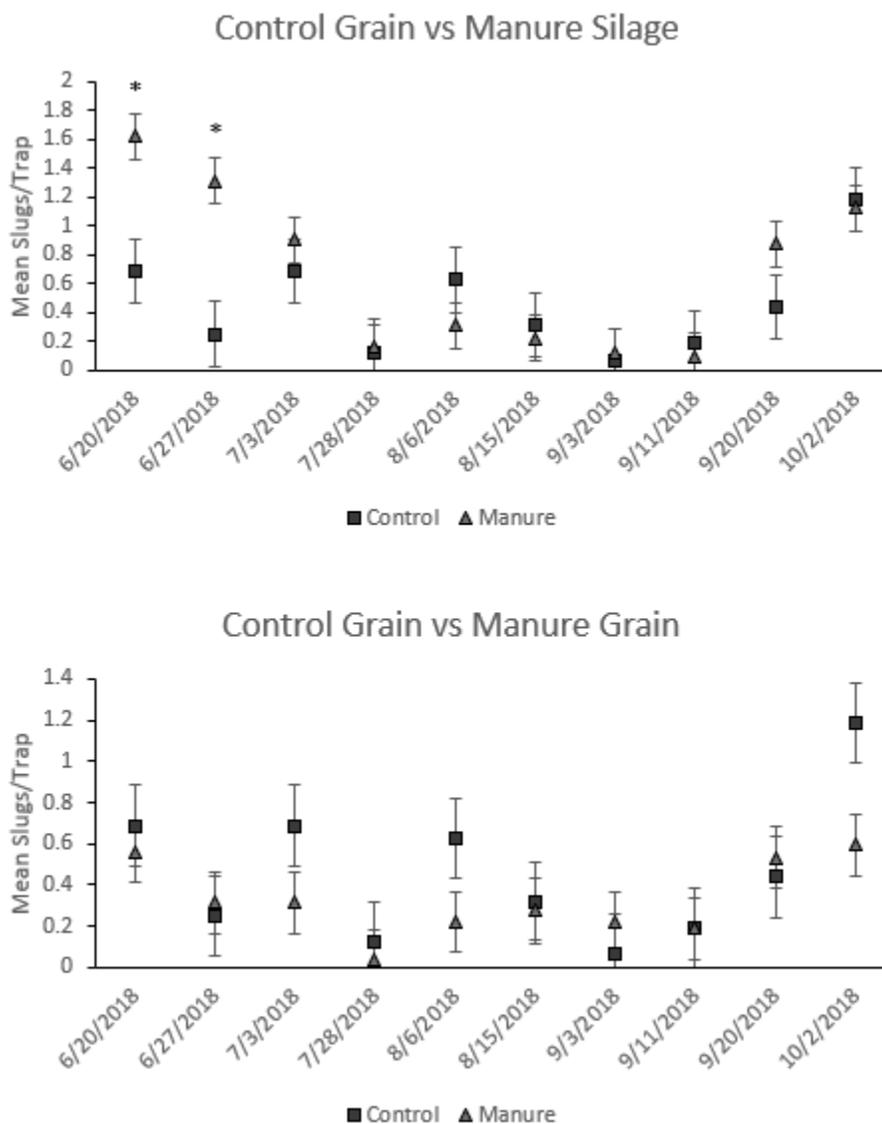


Figure 1-7. 2018 mean slugs per shingle trap (LS-Mean \pm SE) in maize grown for grain plots in the Manure and Control Rotation and the maize grown for silage following alfalfa with orchardgrass in the Manure Rotation. Values mark with (*) are statistically different (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by date.

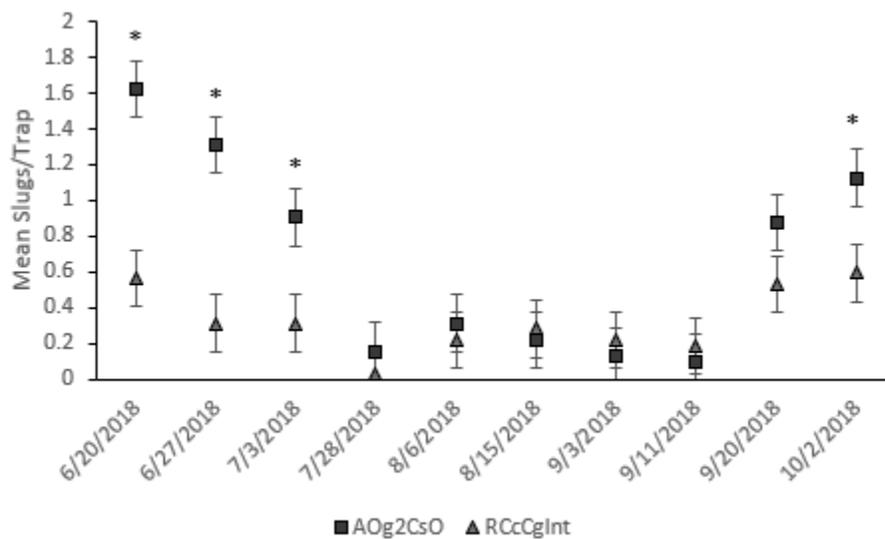


Figure 1-8. 2018 mean slugs per shingle trap (LS-Mean \pm SE) in maize entries in the Manure Rotation (AOg2CsO: maize silage following two years of alfalfa with orchardgrass; RCcCgInt: maize grain following either rye silage or crimson clover). Values mark with (*) are statistically different (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by date.

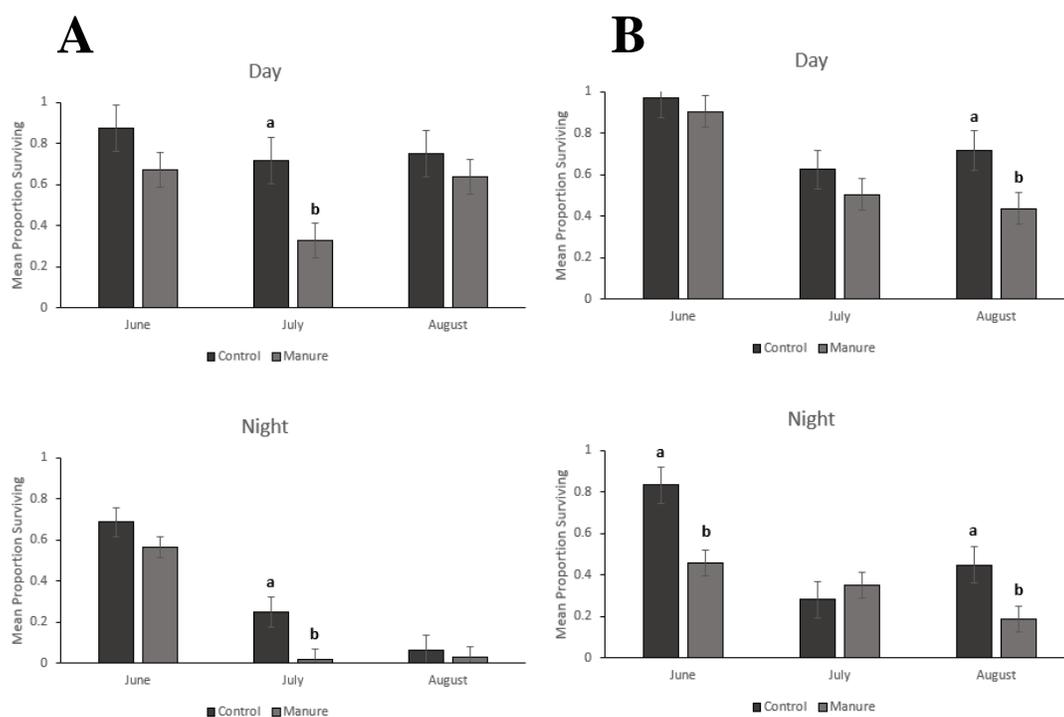


Figure 1-9. (A) 2017 mean proportion of caterpillars surviving (LS-Mean \pm SE) during the day and night across rotation and time. Values marked with letters are statistically significant (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by month. (B) 2018 mean proportion of caterpillars surviving (LS-Mean \pm SE) during the day and night across rotation and time. Values marked with letters are statistically significant (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by month.

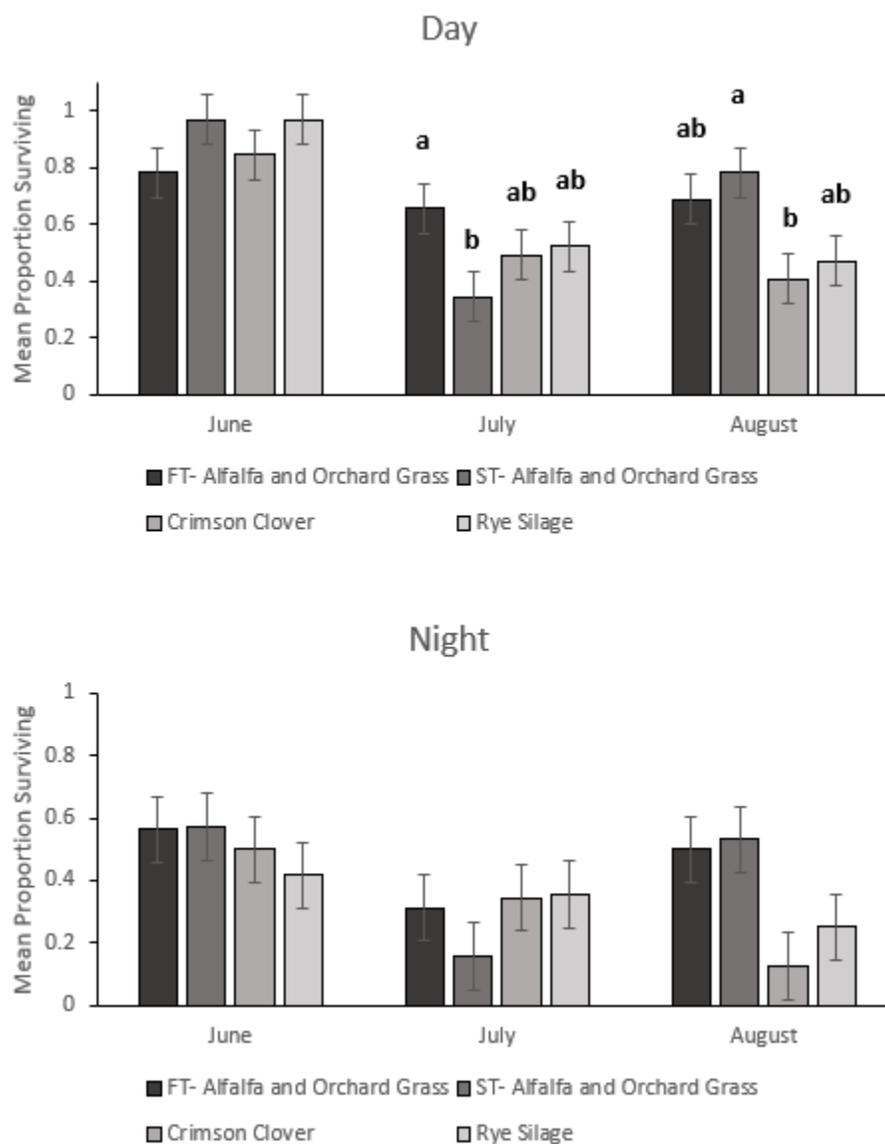


Figure 1-10. 2018 mean proportion of caterpillars surviving (LS-Mean \pm SE) during the day and night across Manure Rotation previous cover and time. Values marked with letters are statistically significant (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.05$) based on SLICE analysis of simple effects by month.

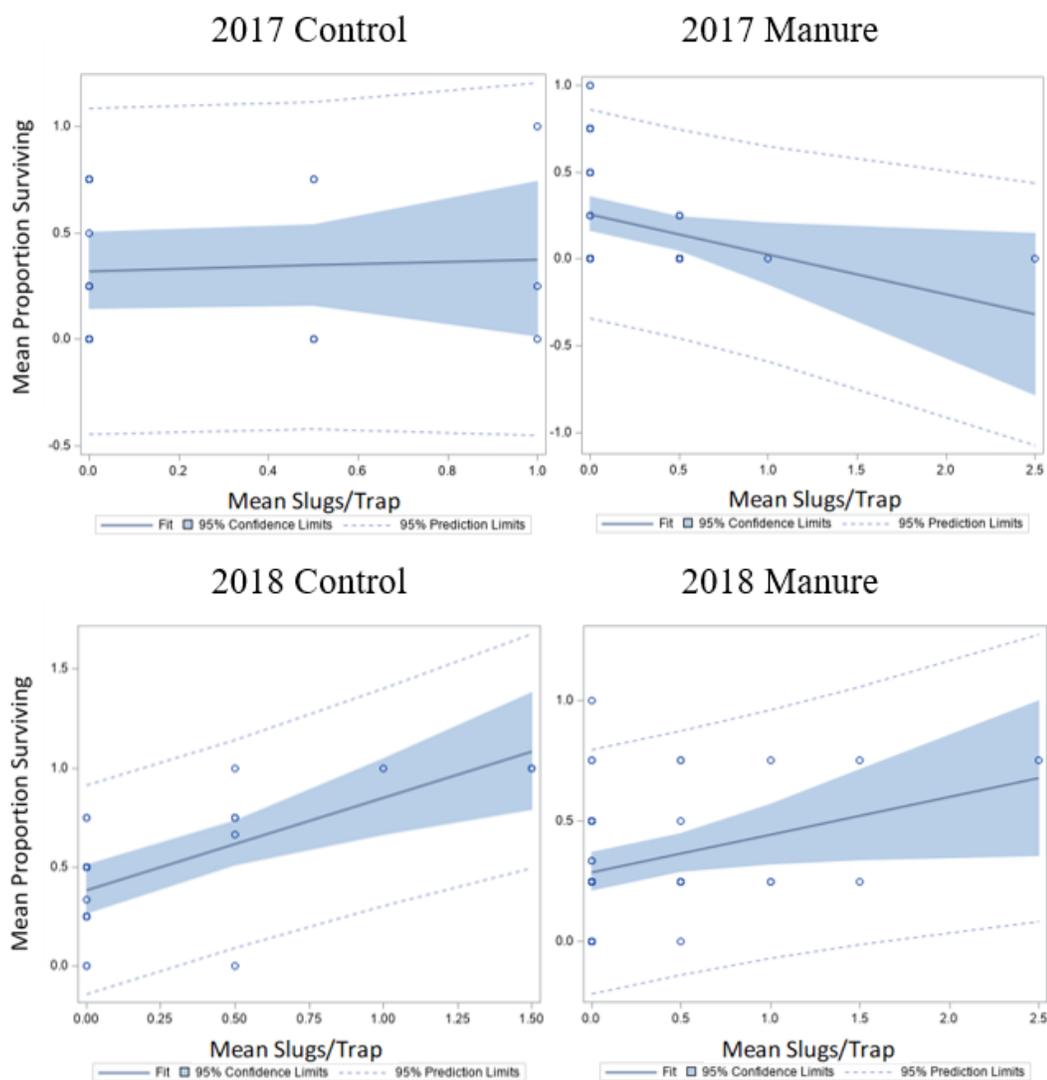


Figure 1-11. Regression of mean proportion of sentinel caterpillars surviving during the night and the mean number of slugs found under shingle traps in 2017 and 2018 split by the maize grown for grain in the Manure and Control Rotations.

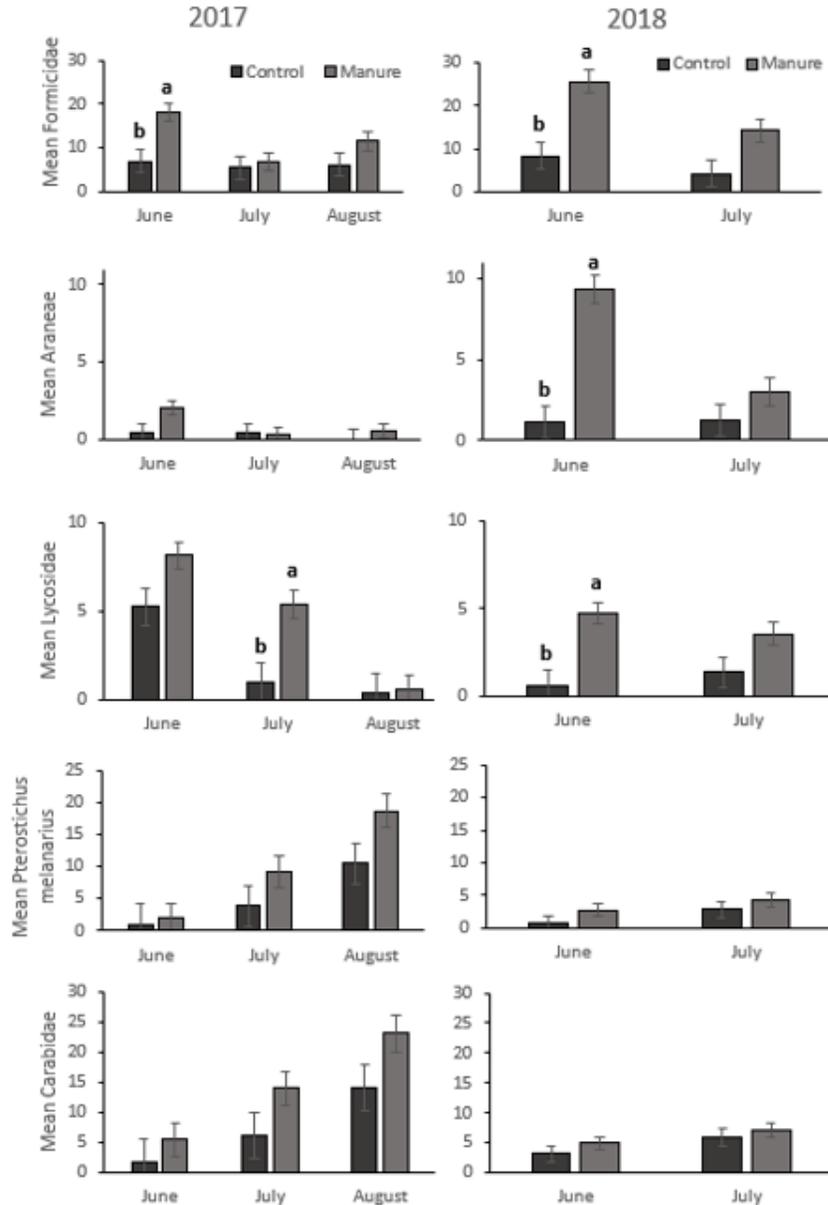


Figure 1-12. Mean arthropod predators (LS-Mean ± SE) in pitfall traps per plot in the Manure Rotation maize grown for grain and the Control Rotation maize grown for grain (Formicidae = Ants; Araneae = Spiders; Lycosidae = Wolf Spiders; *Pterostichus melanarius* = a common ground beetle species; Carabidae = Ground Beetles). Values marked with letters are statistically significant (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; Bonferroni Correction $P \leq 0.01$) based on SLICE analysis of simple effects by month.

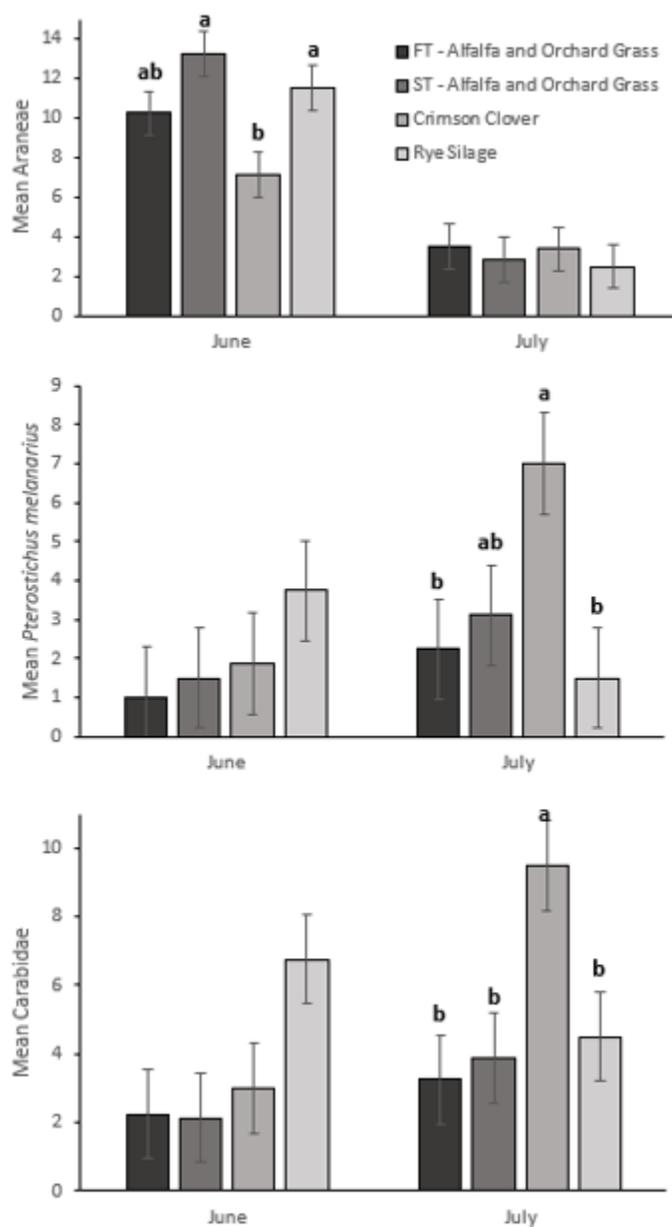


Figure 1-13. 2018 Manure Rotation mean arthropod predators (LS-Mean \pm SE) in pitfall traps based on previous cover (FT: Fall Terminated; ST: Spring Terminated) (Araneae = Other Spiders; *Pterostichus melanarius* = a common ground beetle species; Carabidae = Ground Beetles). Values marked with letters are statistically significant (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; Bonferroni Correction $P \leq 0.017$) based on SLICE analysis of simple effects by month.

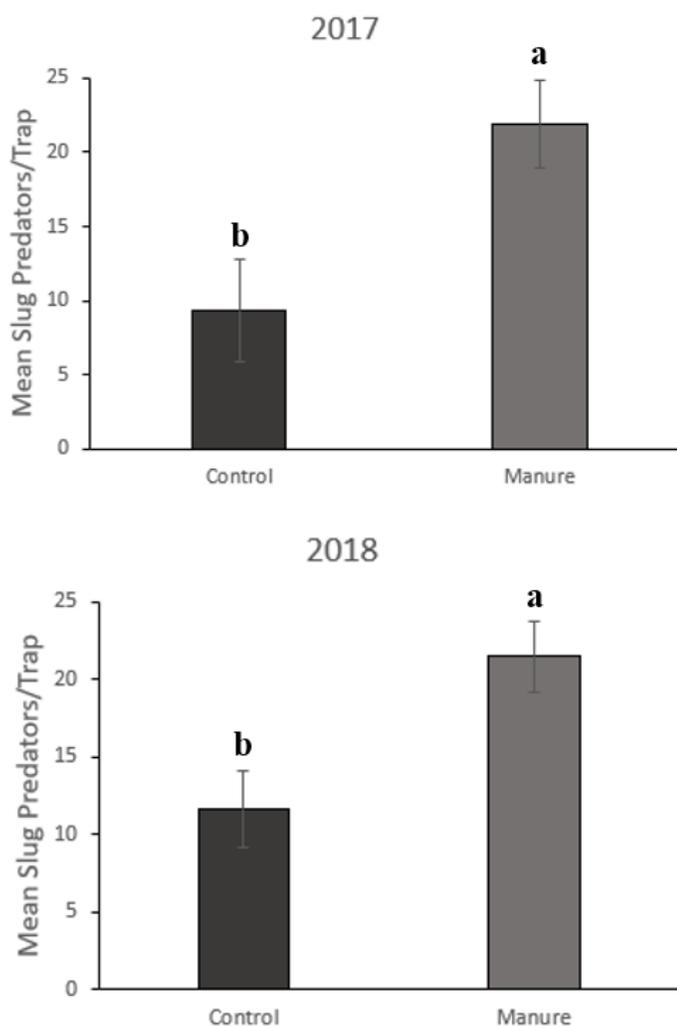


Figure 1-14. Mean slug arthropod predators (LS-Mean \pm SE) in pitfall traps per plot in the Manure Rotation maize grown for grain and the Control Rotation maize grown for grain. Values marked with letters are statistically significant (PROC MIXED with repeated measures; Covariate Type VC; Kenward Roger approximation; $P \leq 0.07$).

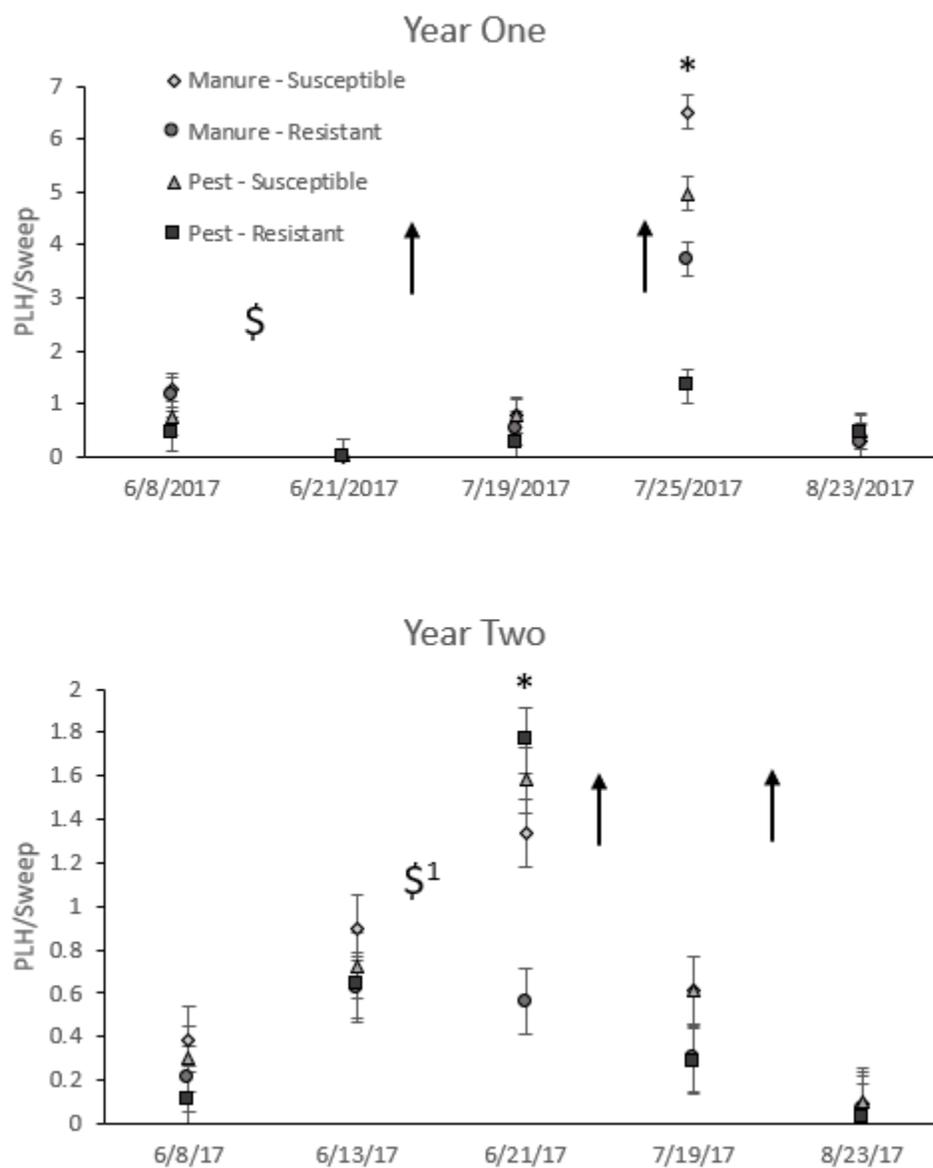


Figure 1-15. 2017 mean potato leafhoppers (PLH) per sweep (LS-Means \pm SE) in first and second year Pest and Manure Rotation treatments (\$: Insecticide spray in all four treatments; \$¹: Insecticide spray in Manure-Resistant treatment; ↑: Harvest). Values marked with * are statistically different (PROC MIXED with repeated measures; Covariate Type VC; $P \leq 0.05$) based on SLICE analysis of simple effects by date.

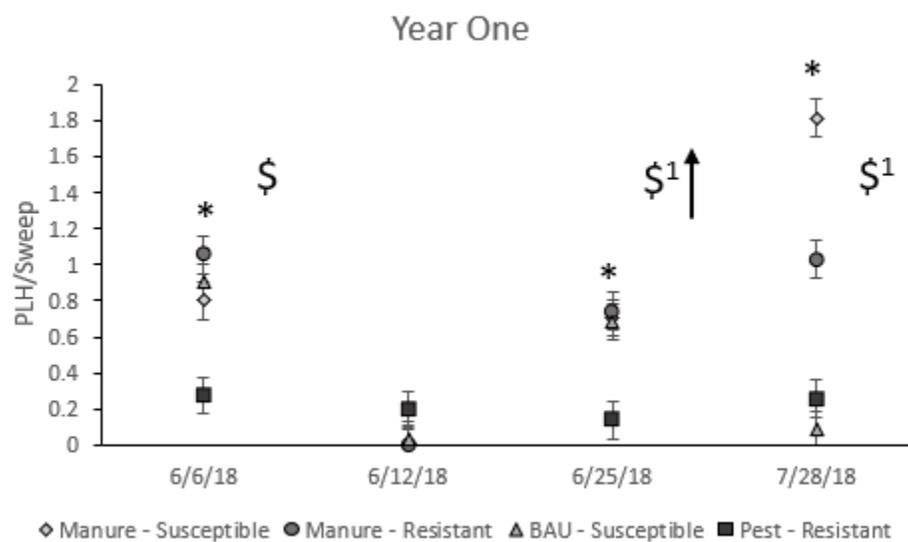


Figure 1-16. 2018 mean potato leafhoppers (PLH) per sweep (LS-Means \pm SE) in first year Pest, Manure and BAU Rotation treatments (\$: Insecticide spray on Manure-Susceptible, Manure-Resistant, and BAU-Susceptible treatments; \$¹: Insecticide spray on BAU-Susceptible treatment; ↑: Harvest). Values marked with * are statistically different (PROC MIXED with repeated measures; Covariate Type VC; $P \leq 0.05$) based on SLICE analysis of simple effects by date.

Chapter 2

Neonicotinoid insecticides in the aquatic environment: A step towards solving the mass balance equation

Introduction

Neonicotinoid insecticides are currently the most commonly used insecticides in the world (Jeschke et al. 2011), accounting for about one third of the global insecticide market (Simon-Delso et al. 2015). Within the United States, the majority of these insecticides are used in maize, soybean, and cotton production with 2011 data showing an area about the size of California planted with neonicotinoid coated seeds in just these three crops (Douglas and Tooker 2015). Between 2011 and 2014, the amount of these insecticides applied to maize alone doubled, even though maize acreage remained roughly the same (Tooker et al. 2017).

Neonicotinoid insecticides are typically used in feed-grain and forage crops as prophylactic seed coatings (Douglas and Tooker 2015; Hladik et al. 2018b). At 20 degrees Celsius, the solubility of the neonicotinoids clothianidin and thiamethoxam in water are 0.327 g/L and 4.1 g/L, respectively (EPA 2003; Banerjee et al. 2008). Because these insecticides are so water soluble, plant roots can take up the insecticide from the seed coat and translocate the active ingredient systemically (Alford and Krupke 2017; Bonmatin 2005), making the plant toxic to feeding insects. While these insecticides can be effective at controlling pest populations, high concentrations of active ingredient within plants do not necessarily correspond to pest phenology, rendering the insecticide ineffective (Alford and Krupke 2017; Krupke et al. 2017a).

Previous studies have described the inconsistent effectiveness of these insecticides at reducing pest populations and saving crop yield, while suggesting that use of neonicotinoid seed coatings should be better targeted against known pest populations (Douglas et al. 2015; Krupke et al. 2017b; Milosavljevic 2019). Notably, only about 2 to 20% of the active ingredient applied to seeds is actually absorbed by target maize plants

(Alford and Krupke 2017; Sur and Stork 2003), leaving the remaining 80 to 98% of the active ingredient susceptible to loss to the surrounding environment, particularly in surface runoff from crop fields. Following a nationwide study of neonicotinoid concentrations in streams, 53% of collected samples across 24 states yielded at least one chemical in this class of insecticides (Hladik and Kolpin 2016).

Due to the widespread occurrence of neonicotinoid seed coatings and the corresponding abundance of these insecticides in the aquatic environment (Hladik et al. 2014), they pose serious concern for aquatic ecosystem function. In the Netherlands, concentrations of these insecticides in water have been linked to declines in populations of insectivorous birds (Hallmann et al. 2014) that rely on aquatic insects for food; these declines are likely due to decreased aquatic insect populations associated with increased neonicotinoid insecticides concentrations (Mohr et al. 2012; Morrissey et al. 2015; Van Dijk et al. 2013). Additionally, these insecticides in water negatively influence trophic relationships between aquatic species (Miles et al. 2017) and establishment of aquatic invertebrates in different habitats (Basley and Goulson 2018).

For this study, I used field lysimeters to investigate the loss via water of the neonicotinoid insecticide seed coating thiamethoxam from maize plots. My objectives for this project were: (1) track over time neonicotinoid mass loss from treated maize seed in runoff, (2) compare neonicotinoid mass loss via surface and subsurface water runoff, and (3) determine what percent of applied thiamethoxam, and its metabolite clothianidin, is lost in water. This research provides an important step towards discovering the behavior of neonicotinoid insecticides in the environment and the potential negative unintended consequences of their use in agriculture.

Methods

Site Description

For this project, I conducted research within the lysimeter plots at Penn State's Russell E. Larsen Agricultural Research farm in Rock Springs, PA. The lysimeters were installed in 12 0.1-acre maize plots (27-m long, 15-m wide) and collect the surface and subsurface runoff flow from each of the plots. The plots were installed in a sloped field and designed to be hydrologically separate from each other with a berm installed at the bottom of each plot and 30-meter grass alleyways between each plot. Pipes carried water to two collection houses, and a datalogger tracked water flow data from each collection point. On 19 and 22 May 2017, the plots were planted with maize seeds treated with 0.25 mg of the neonicotinoid thiamethoxam per seed (CruiserMaxx Corn 250 Seed Treatment; Syngenta US). It had been at least two years since neonicotinoid-coated seeds were planted in these plots, meaning that any detected thiamethoxam or clothianidin, a breakdown product of thiamethoxam (Nauen 2003), likely came from the maize seeds we planted in 2017. In 2018, untreated corn seed (i.e., seeds not coated with insecticides) was planted in these same plots. Based on historical water flow data, these plots were grouped into an unconventional randomized complete block design with six blocks, so that plots in each block have similar water flow patterns (Duncan and Beegle 2016).

Beyond collecting water from these lysimeter plots, I also collected weekly water samples from Spring Creek in Bellefonte, Pennsylvania, and from vernal pools in an agricultural area of Penn State's Living Filter; however, due to technical issues during analysis, I only have a few of those results. Concentrations from these samples can be found in Appendix Table A-1.

Water Sample Collection and Storage

Within 24 hours of a significant rainfall, I checked each of the 24 collection points in the lysimeter collection houses for potential runoff. Generally speaking, the

plots will reliably produce runoff after about 2.54 cm of rainfall (Duncan and Beegle 2016). I collected samples into 500 ml polypropylene bottles (SKS Science, Saratoga Springs, NY) and shielded them from direct sunlight to prevent UV light degradation of the neonicotinoids in the samples (Gupta et al. 2008; Englert et al. 2018; Acera et al. 2019). I returned samples to the laboratory and froze the samples (-18° C) until I prepared them for analysis.

Sample Preparation and Analysis

To extract neonicotinoid insecticides from the water samples, I followed an established protocol (Hladik and Calhoun 2012). After allowing samples to thaw overnight in a dark drawer, I filtered the water samples through glass microfiber filter paper (Grade 8, 90-mm diameter; Fisher Scientific). I then spiked filtered water samples with an internal standard of deuterated thiamethoxam and clothianidin to adjust final neonicotinoid concentrations based on the percent recovery of the internal standard. I precleaned Waters Oasis HLB SPE cartridges (6mL, 500 mg; Waters; Milford, MA) by placing them on a vacuum manifold and pumping through the cartridges 6 mL of dichloromethane followed by 6 mL acetone and 6 mL deionized water. After cleaning the cartridges, I used the vacuum manifold to pull the water samples through the cartridge at 10 mL per minute. Once the entire sample was pulled through the cartridge, I allowed the cartridge to dry under vacuum for 1 h. I stored these cartridges frozen (-18° C) until analysis.

Prior to analysis, I allowed frozen cartridges to warm up to room temperature. Placing the room temperature cartridges inside a 28.5mL disposable borosilicate glass test tube (18mm diameter, 150mm length; Fisher Scientific), I removed the neonicotinoid insecticides and internal standard from the cartridge using 10mL of a 1:1 dichloromethane:acetone solution. Depending on the expected concentration of neonicotinoids (based on how soon after maize planting the sample was taken), I either did not subsample, subsampled 1 mL, or subsampled 0.5 mL from the 10 mL eluent. Using a vacuum, I evaporated this sample to 0.5 mL and then added 0.5 mL of

acetonitrile, which I evaporated to about 200 μL . I reconstituted this 200 μL sample into either 400 or 800 μL of acetonitrile depending on the original subsample amount. Prior to analysis, I stored these samples at 4° C in a labelled amber GC-MS vial (pre-cleaned, 2mL; Sigma-Aldrich: St. Louis, MO).

Prior to analyzing samples, I made a set of samples to prepare a standard curve using laboratory standards of imidacloprid, clothianidin, and thiamethoxam. This standard curve allowed me to detect concentrations between 0.1 ng/mL and 500 ng/mL. I then analyzed samples using HPLC/MS/MS-Orbitrap. After analysis, I used the percent recovery of the internal standards to correct and back calculated the concentrations of thiamethoxam and clothianidin. I excluded any samples with less than a 20% recovery of internal standard or had no detection. If a concentration was below the limit of detection of 0.1 ng/mL, I changed this concentration to zero. Additionally, if a sample exceeded the upper limit of quantitation, which was 500ng/mL, I adjusted the reported concentration to the maximum allowable concentration based on the standard curve. 6.4% and 11.2% of thiamethoxam and clothianidin concentrations, respectively, were under the limit of detection and 8% of thiamethoxam concentrations exceeded the upper limit of quantitation. To calculate loads, I multiplied the corrected concentrations, based on the percent recover of internal standard, by the water flow in the lysimeters recorded by the datalogger.

Statistical Analyses

I analyzed thiamethoxam and clothianidin mass loss over time with analysis of variance (PROC GLM; SAS 9.4, SAS Institute Inc. 2016) and used least significant differences to separate means.

Results

I started collecting water samples from the lysimeters about one week following maize planting. Overall, I collected and analyzed 187 samples from 27 runoff events in 10 months between May 2017 and April 2018. The lysimeters did not produce any runoff in the months of September and December 2017. Of the 187 samples, 126 were from subsurface flow and 61 were from surface flow. In addition, 95 samples had no detection for thiamethoxam and 2 samples had no detection for clothianidin.

When I compared mean thiamethoxam and clothianidin mass loss in surface flow by sampling date, I found no significant differences between dates even though there appeared to be peaks in the sum of surface water flow over my year of sampling (Figure 2-1). In contrast, I did find that mean thiamethoxam and clothianidin mass loss in subsurface flow did significantly differ by sampling date ($F=2.25$, $P=0.002$) with greater mass loss shortly after planting compared to a year after planting (Figure 2-2). Specific concentrations of thiamethoxam and clothianidin by month are provided below (Tables A-2 and A-3).

From May 2017 through April 2018, the majority of thiamethoxam and clothianidin mass loss from experimental plots was in the subsurface water flow compared to the surface water flow (Figure 2-3). This is not surprising because the majority of water loss from experimental plots was also in the subsurface flow compared to the surface flow (Figure 2-3).

For this experiment, I planted maize seed treated with 0.25 mg of thiamethoxam per seed at a density of 30,000 seeds per acre. Therefore 7,500 mg of thiamethoxam was deployed per acre. Between May 2017 and April 2018, my measurements accounted for 97.5 mg per acre, meaning that on average, 1.3% of applied active ingredient left the field site via water surface and subsurface flow. Based on individual plots, I found that this percent ranged from 0.157% to 4.446%, indicating the flow differences of each lysimeter plot (Table 2-1).

Discussion

My goals for this project were to determine the seasonality of neonicotinoid loss in water from agricultural fields, whether that loss was in surface or subsurface flow, and ultimately to determine what percentage of applied neonicotinoid insecticides were lost from the application site via water runoff. The neonicotinoid mass loss I tracked, in both surface and subsurface flow, continued until about a year post-planting when I stopped collecting runoff samples. Year-round detections of neonicotinoid insecticides in aquatic ecosystems are common throughout the United States (Hladik et al. 2018a) and Canada (Main et al. 2014). I found that for subsurface flow the greatest mass loss of thiamethoxam and clothianidin from maize plots was about one- to two-weeks after planting. Within agricultural areas with large amounts of row-crop production, greater concentrations of neonicotinoid insecticides have been detected in nearby streams (Hladik et al. 2014), wetlands (Williams and Sweetman 2018) and watersheds (Struger et al. 2017) in the spring following planting of neonicotinoid-coated seeds. For example, in streams and watersheds in agricultural areas, the neonicotinoid clothianidin had a max concentration detection in water of about 0.25 µg/L shortly after crop planting and a max concentration detection in water of about 0.025 µg/L later in the growing season around August (Hladik et al. 2014; Struger et al. 2017). Even in a singular storm event, there is an initial flush of neonicotinoids, with greater concentrations at the beginning of a storm event compared to the rest of the storm (Batikian et al. 2019). Concentrations of neonicotinoid residues in puddles of water in maize fields were six times greater for five weeks following planting compared to pre-plant concentrations (Schaafsma et al. 2015). Overall, the concentrations I detected (Tables A-1, A-2, and A-3) are consistent with detections from previous studies (Chrétien et al. 2017; Bradford et al. 2018; Hladik et al. 2014, 2017, 2018a; Klarich et al. 2017).

Comparing surface and subsurface flow, I found that there was greater neonicotinoid mass loss and water flow in the subsurface flow of the lysimeter compared to the surface flow. Transport of neonicotinoids by subsurface flow is key to understand because much of this flow could end up in nearby streams, but some of it is likely to end

up in groundwater reserves. While the mean mass loss via subsurface flow between dates in January and February 2018 were not significantly different, I found a trend towards increased mass loss with increased subsurface flow in February 2018, likely due to the snowmelt (Figure 2-2). In Canada, snowmelt has been shown to transport neonicotinoids from agricultural areas to local wetlands and surface water (Main et al. 2016). During the growing season, the presence of living plants can even increase the vertical transport of thiamethoxam to greater depths in the soil profile along soil macropores formed by roots compared to the absence of living plants (Radolinski et al. 2018, 2019). While living plants within the field can increase transport of neonicotinoids vertically through the soil profile, adding in-field non-crop vegetation can also reduce the transport of neonicotinoids to the surrounding environment (Hladik et al. 2017).

Because of the potential for subsurface water to transport neonicotinoids towards groundwater, there is concern for the potential impact these insecticides could have on human health. In a study of agricultural areas in central Wisconsin, 78% of tested wells had detections of thiamethoxam (Bradford et al. 2018). Water treatment methods and facilities in the mid-west United States and Canada are unable to remove all neonicotinoids from groundwater, allowing them to be present in many samples of drinking water (Klarich et al. 2017; Sultana et al. 2018).

Overall, over the course of a year I found that an average of 1.3% of applied thiamethoxam left the lysimeter plots in water as thiamethoxam or its metabolite clothianidin. In a tile drain cropping system, greater than 0.3% of applied thiamethoxam was exported by surface runoff and tile drain subsurface flow (Chrétien et al. 2017). Similarly, there was a 1.2% mass recovery of thiamethoxam in a sugar beet tile drain system (Wettstein et al. 2016). My results are consistent with these previous efforts and demonstrate that less than 2% of seed applied neonicotinoid insecticide active ingredient appears to leave fields in water, heading for streams, rivers, lakes, and aquifers, among other surface and subsurface elements.

While a 1.3% mass loss to water may not seem large, this has the potential to cause major pollution over large areas. It is estimated that in 2011, in the U.S., 79-100% of maize seed was treated with neonicotinoid insecticides (Douglas and Tooker 2015). In

Pennsylvania, in 2018, there were 1,350,000 acres planted to maize (NASS 2018). If 79% of this maize was planted with neonicotinoid insecticide coated seed that would mean that about 1,066,500 acres of maize have the potential to pollute nearby waterways with these insecticides. Assuming a 1.3% mass loss of these insecticides to water and a range neonicotinoid insecticides seed application rates of 0.25 mg/seed to 0.50 mg/seed, in Pennsylvania alone there is the potential for over 200 to 400 pounds of neonicotinoids, which are extremely toxic to insects, to enter aquatic environments. Extrapolated over the entire United States, this large amount could lead to long-term environmental, and possibly human, health problems (Goulson 2014; Cimino et al. 2017).

Following a review of neonicotinoid insecticide concentrations in surface waters worldwide and the potential for these concentrations to influence aquatic invertebrates, researchers have determined that on average a chronic neonicotinoid concentration of 0.035 $\mu\text{g/L}$ could have effect on aquatic invertebrate communities (Morrissey et al. 2015). Additionally, while the association of imidacloprid with other pesticides found in water was not considered (Vijver and van den Brink 2014), researchers in the Netherlands found a correlation of decreased aquatic invertebrate abundances with imidacloprid concentrations between 0.013 and 0.067 $\mu\text{g/L}$ (Van Dijk et al. 2013). Similarly, a wetland limnocorral study found low concentrations of imidacloprid (0.045 $\mu\text{g/L}$) and clothianidin (0.038 $\mu\text{g/L}$) can have chronic effects on aquatic community assemblages (Cavallaro et al. 2018). In a laboratory study, thiacloprid concentrations in the range from 0.5 to 1.0 $\mu\text{g/L}$ increased amphipod predation of mayfly nymphs and decreased leaf consumption, indicating that low concentrations of these insecticides can alter trophic interactions in an ecosystem (Englert et al. 2012). I found concentrations of thiamethoxam and clothianidin in the ranges of 0 to 6.0 $\mu\text{g/L}$ and 0 to 2.2 $\mu\text{g/L}$, respectively, directly leaving maize plots. This could lead to chronic pulses of these insecticides into nearby surface waters, which appears to be consistent in the literature, that could cause long-term negative side effects to the aquatic invertebrate communities (Morrissey et al. 2015).

Conclusions

My results demonstrate that neonicotinoid mass loss from agricultural fields planted with insecticide-coated maize seeds is greatest at the beginning of the growing season, but continues for the rest of the year. Because the half-life of neonicotinoids in soil can range from 7 to nearly 7000 days (Wood and Goulson 2017), planting neonicotinoid-coated seeds annually may result in concerning levels of these insecticides building up in the environment, resulting in acute and chronic exposure to non-target species. As agricultural intensification continues, there is an increasing need to ensure that potential agricultural pollutants remain in place, rather than runoff into nearby waterways. My results reveal an important view on how pesticide-tainted water leaves crop fields, providing information vital for mitigation efforts.

References

- Aceró, J. L., Real, F. J., Javier Benitez, F., & Matamoros, E. (2019). Degradation of neonicotinoids by UV irradiation: Kinetics and effect of real water constituents. *Separation and Purification Technology*, 211, 218-226. doi:10.1016/j.seppur.2018.09.076
- Alford, A. & Krupke, C. H. (2017) Translocation of the neonicotinoid seed treatment clothianidin in maize. *PLoS ONE* 12(3):e0173836.doi:10.1371/journal.pone.0173836
- Banerjee, K., Patil, S. H., Dasgupta, S., Oulkar, D. P., & Adsule, P. G. (2008). Sorption of thiamethoxam in three Indian soils. *Journal of Environmental Science and Health, Part B*, 43(2), 151-156. doi:10.1080/03601230701795130
- Basley, K., & Goulson, D. (2018). Neonicotinoids thiamethoxam and clothianidin adversely affect the colonisation of invertebrate populations in aquatic microcosms. *Environmental Science and Pollution Research*, 25(10), 9593-9599. doi:10.1007/s11356-017-1125-5

- Batikian, C. M., Lu, A., Watanabe, K., Pitt, J., & Gersberg, R. M. (2019). Temporal pattern in levels of the neonicotinoid insecticide, imidacloprid, in an urban stream. *Chemosphere*, 223, 83-90. doi:10.1016/j.chemosphere.2019.01.165
- Bonmatin, J., Marchand, P., Charvet, R., Moineau, I., Bengsch, E., & Colin, M. (2005). Quantification of imidacloprid uptake in maize crops. *Journal of Agricultural and Food Chemistry*, 53(13), 5336-5341. doi:10.1021/jf0479362
- Bradford, B., Huseh, A., & Groves, R. (2018). Widespread detections of neonicotinoid contaminants in central Wisconsin groundwater. *PloS One*, 13(10), e0201753. doi:10.1371/journal.pone.0201753
- Cavallaro, M. C., Liber, K., Headley, J. V., Peru, K. M., & Morrissey, C. A. (2018). Community-level and phenological responses of emerging aquatic insects exposed to 3 neonicotinoid insecticides: An in situ wetland limnocorral approach: Chronic neonicotinoid toxicity and wetland limnocorrals. *Environmental Toxicology and Chemistry*, 37(9), 2401-2412. doi:10.1002/etc.4187
- Chrétien, F., Giroux, I., Thériault, G., Gagnon, P., & Corriveau, J. (2017). Surface runoff and subsurface tile drain losses of neonicotinoids and companion herbicides at edge-of-field. *Environmental Pollution*, 224, 255-264. doi:10.1016/j.envpol.2017.02.002
- Cimino, A., Boyles, A., Thayer, K., & Perry, M. (2017). Effects of neonicotinoid pesticide exposure on human health: A systematic review. *Environmental Health Perspectives*, 125(2), 155-162. doi:10.1289/EHP515
- Douglas, M., Rohr, J., & Tooker, J. (2015). Neonicotinoid insecticide travels through a soil food chain, disrupting biological control of non-target pests and decreasing soya bean yield. *Journal of Applied Ecology*, 52(1), 250-260. doi:10.1111/1365-2664.12372
- Douglas, M., & Tooker, J. (2015). Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. *Environmental Science & Technology*, 49(8), 5088-5097. doi:10.1021/es506141g

- Duncan, E., & Beegle, D. B. (2016). *Nitrogen cycling and trade-offs with alternative manure application* (Doctoral Dissertation, The Pennsylvania State University)
- Englert, D., Bundschuh, M., & Schulz, R. (2012). Thiacloprid affects trophic interaction between gammarids and mayflies. *Environmental Pollution*, 167, 41-46.
doi:10.1016/j.envpol.2012.03.024
- Englert, D., Zubrod, J. P., Neubauer, C., Schulz, R., & Bundschuh, M. (2018). UV-irradiation and leaching in water reduce the toxicity of imidacloprid-contaminated leaves to the aquatic leaf-shredding amphipod *Gammarus fossarum*. *Environmental Pollution*, 236, 119-125. doi:10.1016/j.envpol.2018.01.050
- EPA. US EPA, Pesticide fact sheet for clothianidin. Office of Prevention, Pesticides and Toxic Substances. 2003.
- Goulson, D. (2014). Pesticides linked to bird declines. *Nature*, 511(7509), 295.
- Gupta, S., Gajbhiye, V. T., & Gupta, R. K. (2008). Effect of light on the degradation of two neonicotinoids viz acetamiprid and thiacloprid in soil. *Bulletin of Environmental Contamination and Toxicology*, 81(2), 185-189.
doi:10.1007/s00128-008-9405-x
- Hallmann, C. A., Foppen, R. P. B., Turnhout, C. A. M. v., Kroon, H. d., & Jongejans, E. (2014). Declines in insectivorous birds are associated with high neonicotinoid concentrations. *Nature*, 511(7509), 341-+. doi:10.1038/nature13531
- Hladik, M. L., & Calhoun, D. L. (2012). Analysis of the herbicide diuron, three diuron degradates, and six neonicotinoid insecticides in water-method details and application to two Georgia streams. *Scientific Investigations Report*. United States Geological Survey
- Hladik, M. L., Kolpin, D. W., & Kuivila, K. M. (2014). Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. *Environmental Pollution*, 193, 189-196.
doi:10.1016/j.envpol.2014.06.033
- Hladik, M., & Kolpin, D. (2016). First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA. *Environmental Chemistry*, 13(1), 12-20.
doi:10.1071/EN15061

- Hladik, M. L., Bradbury, S., Schulte, L. A., Helmers, M., Witte, C., Kolpin, D. W., . . . Harris, M. (2017). Neonicotinoid insecticide removal by prairie strips in row-cropped watersheds with historical seed coating use. *Agriculture, Ecosystems and Environment*, 241, 160-167. doi:10.1016/j.agee.2017.03.015
- Hladik, M. L., Corsi, S. R., Kolpin, D. W., Baldwin, A. K., Blackwell, B. R., & Cavallin, J. E. (2018a). Year-round presence of neonicotinoid insecticides in tributaries to the great lakes, USA. *Environmental Pollution*, 235, 1022-1029.
- Hladik, M., Main, A., & Goulson, D. (2018b). Environmental risks and challenges associated with neonicotinoid insecticides. *Environmental Science & Technology*, 52(6), 3329-3335. doi:10.1021/acs.est.7b06388
- Jeschke, P., Nauen, R., Schindler, M., & Elbert, A. (2011). Overview of the status and global strategy for neonicotinoids. *Journal of Agricultural and Food Chemistry*, 59(7), 2897-2908. 10.1021/jf101303g
- Klarich, K., Pflug, N., DeWald, E., Hladik, M., Kolpin, D., Cwiertny, D., & LeFevre, G. (2017). Occurrence of neonicotinoid insecticides in finished drinking water and fate during drinking water treatment. *Environmental Science & Technology Letters*, 4(5), 168-173. doi:10.1021/acs.estlett.7b00081
- Krupke, C. H., Alford, A. M., Cullen, E. M., Hodgson, E. W., Knodel, J. J., McCornack, B., . . . Welch, K. (2017a). Assessing the value and pest management window provided by neonicotinoid seed treatments for management of soybean aphid (*Aphis glycines matsumura*) in the upper midwestern United States: Value of neonicotinoid seed treatments for soybean aphid management. *Pest Management Science*, 73(10), 2184-2193. doi:10.1002/ps.4602
- Krupke, C. H., Holland, J. D., Long, E. Y., Eitzer, B. D., & Diamond, S. (2017b). Planting of neonicotinoid-treated maize poses risks for honey bees and other non-target organisms over a wide area without consistent crop yield benefit. *Journal of Applied Ecology*, 54(5), 1449-1458. doi:10.1111/1365-2664.12924
- Main, A. R., Headley, J. V., Peru, K. M., Michel, N. L., Cessna, A. J., & Morrissey, C. A. (2014). Widespread use and frequent detection of neonicotinoid insecticides in

- wetlands of Canada's prairie pothole region. *PLoS One*, 9(3)
doi:<http://dx.doi.org.ezaccess.libraries.psu.edu/10.1371/journal.pone.0092821>
- Main, A. R., Michel, N. L., Cavallaro, M. C., Headley, J. V., Peru, K. M., & Morrissey, C. A. (2016). Snowmelt transport of neonicotinoid insecticides to Canadian prairie wetlands. *Agriculture, Ecosystems and Environment*, 215, 76-84.
doi:10.1016/j.agee.2015.09.011
- Miles, J., Hua, J., Sepulveda, M., Krupke, C., & Hoverman, J. (2017). Effects of clothianidin on aquatic communities: Evaluating the impacts of lethal and sublethal exposure to neonicotinoids. *PloS One*, 12(3), e0174171.
doi:10.1371/journal.pone.0174171
- Milosavljević, I., Esser, A. D., Murphy, K. M., & Crowder, D. W. (2019). Effects of imidacloprid seed treatments on crop yields and economic returns of cereal crops. *Crop Protection*, 119, 166-171. doi:10.1016/j.cropro.2019.01.027
- Mohr, S., Berghahn, R., Schmiediche, R., Hübner, V., Loth, S., Feibicke, M., . . . Wogram, J. (2012). Macroinvertebrate community response to repeated short-term pulses of the insecticide imidacloprid. *Aquatic Toxicology*, 110-111, 25-36.
doi:10.1016/j.aquatox.2011.11.016
- Morrissey, C. A., Mineau, P., Devries, J. H., Sanchez-Bayo, F., Liess, M., Cavallaro, M. C., & Liber, K. (2015). Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. *Environment International*, 74, 291-303. doi:10.1016/j.envint.2014.10.024
- National Agricultural Statistics Service. 2018. Quick Stats database. United States Department of Agriculture. Accessed 2/9/19: <https://quickstats.nass.usda.gov/>
- Nauen, R., Ebbinghaus-Kintscher, U., Salgado, V. L., & Kausmann, M. (2003). Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. *Pesticide Biochemistry and Physiology*, 76(2), 55-69.
doi:10.1016/S0048-3575(03)00065-8
- Radolinski, J., Wu, J., Xia, K., & Stewart, R. (2018). Transport of a neonicotinoid pesticide, thiamethoxam, from artificial seed coatings. *Science of the Total Environment*, 618, 561-568. doi:10.1016/j.scitotenv.2017.11.031

- Radolinski, J., Wu, J., Xia, K., Hession, W. C., & Stewart, R. D. (2019). Plants mediate precipitation-driven transport of a neonicotinoid pesticide. *Chemosphere*, 222, 445-452. doi:10.1016/j.chemosphere.2019.01.150
- SAS Institute Inc. 2016. SAS/STAT® 9.4 User's Guide. Cary, NC: SAS Institute Inc.
- Schaafsma, A., Limay-Rios, V., Baute, T., Smith, J., & Xue, Y. (2015). Neonicotinoid insecticide residues in surface water and soil associated with commercial maize (corn) fields in southwestern Ontario. *PLoS One*, 10(2) doi:http://dx.doi.org.ezaccess.libraries.psu.edu/10.1371/journal.pone.0118139
- Simon-Delso, N., Amaral-Rogers, V., Belzunces, L. P., Bonmatin, J. M., Chagnon, M., Downs, C., . . . Wiemers, M. (2015). Systemic insecticides (neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. *Environmental Science and Pollution Research*, 22(1), 5-34. doi:10.1007/s11356-014-3470-y
- Struger, J., Grabuski, J., Cagampan, S., Sverko, E., McGoldrick, D., & Marvin, C. H. (2017). Factors influencing the occurrence and distribution of neonicotinoid insecticides in surface waters of southern Ontario, Canada. *Chemosphere*, 169, 516-523. doi:10.1016/j.chemosphere.2016.11.036
- Sultana, T., Murray, C., Kleywegt, S., & Metcalfe, C. D. (2018). Neonicotinoid pesticides in drinking water in agricultural regions of southern Ontario, Canada. *Chemosphere*, 202, 506-513. doi:10.1016/j.chemosphere.2018.02.108
- Sur, R., & Stork, A. (2003). Uptake, translocation and metabolism of imidacloprid in plants. *Bulletin of Insectology*, 56(1), 35-40.
- Tooker, J. F., Douglas, M. R., & Krupke, C. H. (2017). Neonicotinoid seed treatments: Limitations and compatibility with integrated pest management. *Ael*, 2(1) doi:10.2134/ael2017.08.0026
- Van Dijk, T., Van Staalduinen, M., & Van der Sluijs, J. (2013). Macro-invertebrate decline in surface water polluted with imidacloprid. *PloS One*, 8(5), e62374. doi:10.1371/journal.pone.0062374
- Vijver, M., & van den Brink, P. (2014). Macro-invertebrate decline in surface water polluted with imidacloprid: A rebuttal and some new analyses. *PloS One*, 9(2), e89837. doi:10.1371/journal.pone.0089837

- Wettstein, F., Kasteel, R., Delgado, M., Hanke, I., Huntscha, S., Balmer, M., . . . Bucheli, T. (2016). Leaching of the neonicotinoids thiamethoxam and imidacloprid from sugar beet seed dressings to subsurface tile drains. *Journal of Agricultural and Food Chemistry*, *64*(33), 6407-6415. doi:10.1021/acs.jafc.6b02619
- Williams, N., & Sweetman, J. (2018). Distribution and concentration of neonicotinoid insecticides on waterfowl production areas in west central Minnesota. *Wetlands*, doi:10.1007/s13157-018-1090-x
- Wood, T. J., & Goulson, D. (2017). The environmental risks of neonicotinoid pesticides: A review of the evidence post 2013. *Environmental Science and Pollution Research*, *24*(21), 17285-17325. doi:10.1007/s11356-017-9240-x

Tables**Table 2-1.** Percent of thiamethoxam and clothianidin applied to maize plots exported from the field via water surface and subsurface flow.

Plot	Number of Samples	Percent Exported
1	6	0.206
2	21	1.310
3	17	0.954
4	19	2.941
5	19	1.454
6	11	0.418
7	20	1.305
8	11	0.849
9	25	4.446
10	11	0.222
11	9	1.055
12	14	0.157
Average		1.28

Figures

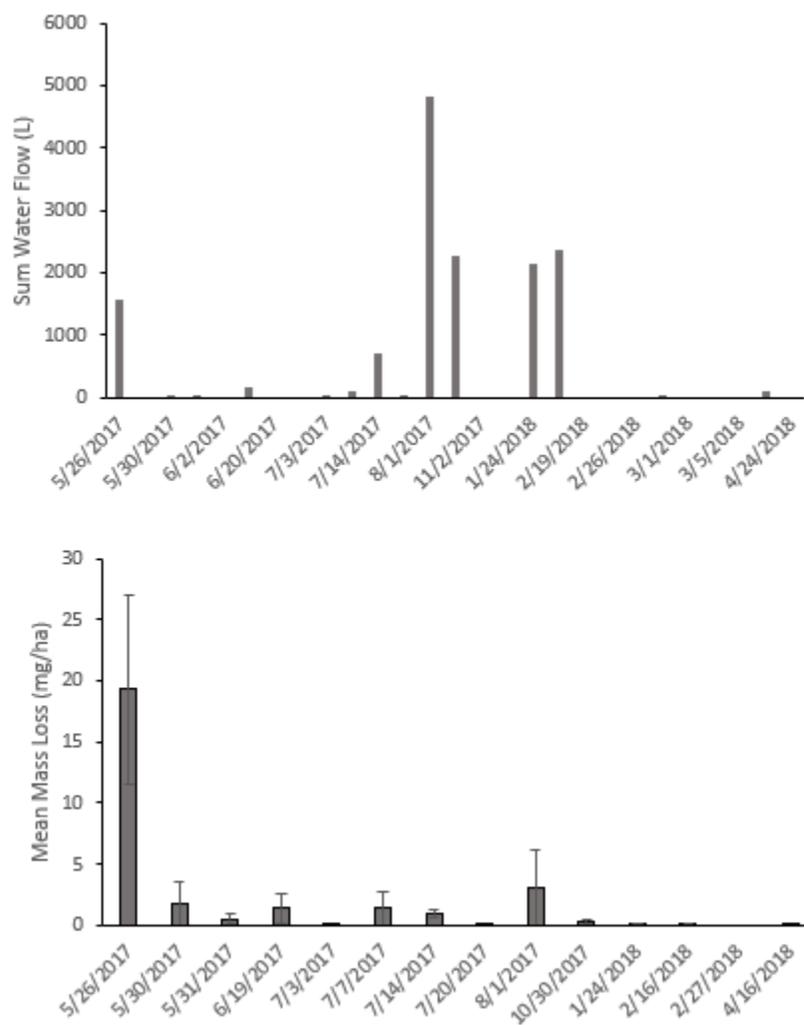


Figure 2-1. Top: Sum surface water flow by date. Bottom: Mean thiamethoxam and clothianidin mass loss (mg/ha) by date (Mean \pm SE) in surface water flow.

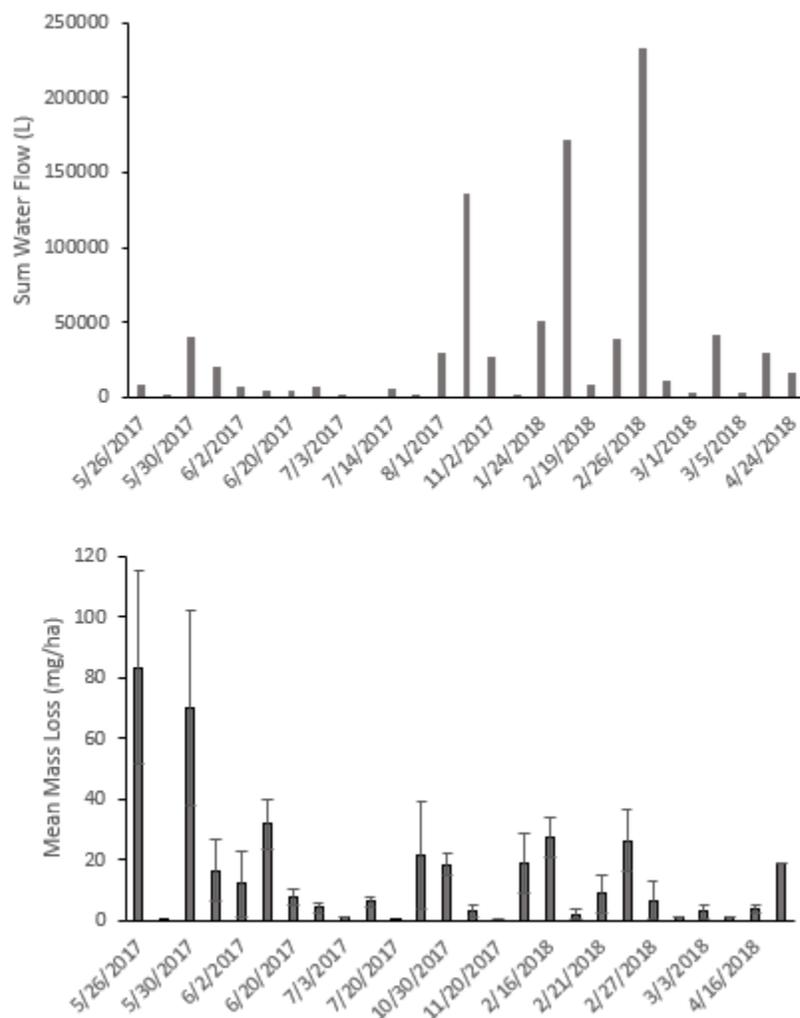


Figure 2-2. Top: Sum subsurface water flow by date. Bottom: Mean thiamethoxam and clothianidin mass loss (mg/ha) by date (Mean \pm SE) in subsurface water flow.

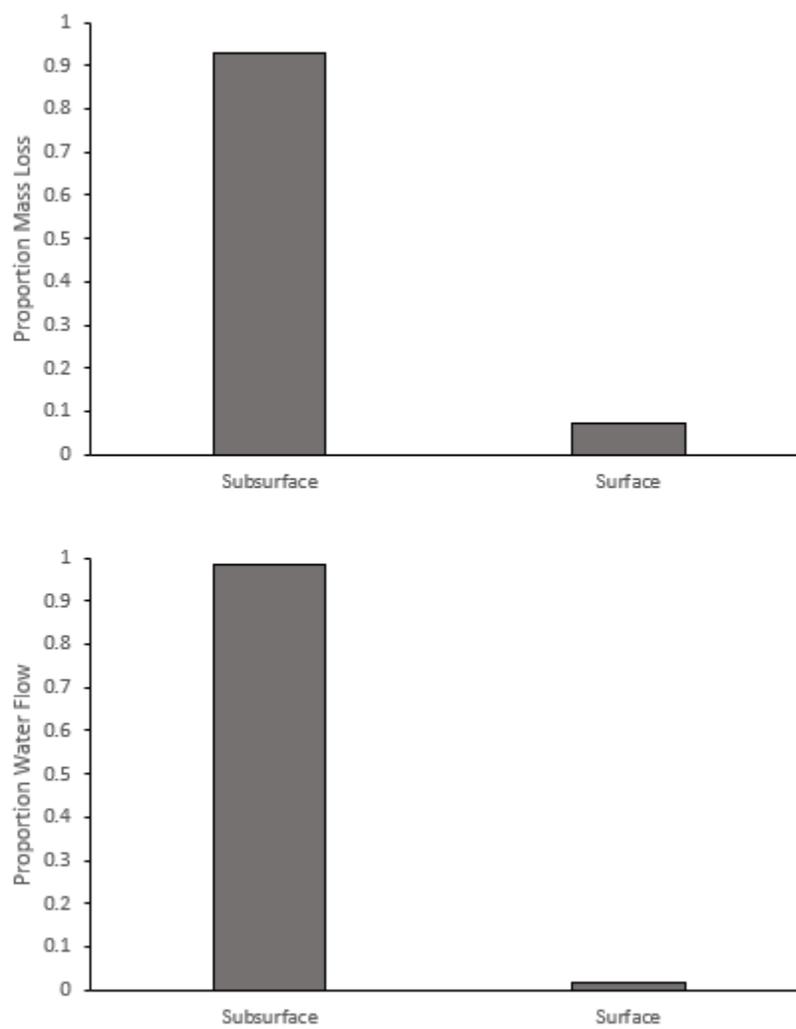


Figure 2-3. The proportion of applied neonicotinoid mass loss and proportion of water flow as measured by the data logger in the lysimeter separated by subsurface and surface water runoff. These proportions represent May 2017 through April 2018.

Appendix

Supplemental tables describing neonicotinoid insecticide concentrations in Ch. 2

Table A-1. Neonicotinoid concentrations (ug/L) in Spring Creek and vernal pool samples. (VP=Vernal Pool; SC=Spring Creek; Numbers indicate locations)

Date	ID	Thiamethoxam	Clothianidin	Imidacloprid
5/19/17	SC2	0.00007	0.00004	0.0008
5/19/17	SC3	0.0002	0.0006	0.003
6/1/17	SC1	0.0005	0.001	0.01
6/29/17	SC3	0.0003	0.0005	0.003
7/13/17	SC1	0.0002	0.0004	0.01
7/13/17	SC3	0.0002	0.0003	0.004
5/22/17	VP3	0.002	0.001	0.004
5/22/17	VP4	0.001	0.0007	0.001
6/15/17	VP9	0.003	0.002	0.005
6/22/17	VP1	0.0002	0.001	0.007
7/16/17	VP1	0.0007	0.0005	0.004
6/22/18	VP2	0.001	0.0007	0.003

Table A-2. Thiamethoxam concentrations (ug/L) from lysimeter samples by month. (nd= no detection; – = no samples)

Month	Surface Flow				Subsurface Flow			
	N	Mean	Median	Range	N	Mean	Median	Range
May 2017	14	3.03	4.68	0.12-5.98	22	1.74	1.14	0.11-4.68
June 2017	4	1.28	1.06	0.07-2.96	11	0.32	0.30	0.03-0.69
July 2017	9	0.37	0.04	0.02-1.54	7	0.07	0.06	0.02-0.19
Aug. 2017	1	0.01	0.01	0.01	2	0.08	0.08	0.01-0.14
Oct. 2017	2	0.08	0.08	0.01-0.15	4	0	0	0
Nov. 2017	–	–	–	–	4	0	0	0-0.0001
Jan. 2018	1	0	0	0	nd	nd	nd	nd
Feb. 2018	2	0	0	0	4	0.01	0	0-0.04
Mar. 2018	–	–	–	–	3	0	0	0
Apr. 2018	nd	nd	nd	nd	1	0	0	0

Table A-3. Clothianidin concentrations (ug/L) from lysimeter by month. (– = no samples)

Month	Surface Flow				Subsurface Flow			
	N	Mean	Median	Range	N	Mean	Median	Range
May 2017	14	0.18	0.03	0.01-2.19	23	0.04	0.03	0.01-0.07
June 2017	3	0.04	0.02	0.02-0.07	12	0.10	0.09	0.03-0.17
July 2017	8	0.13	0.02	0.01-0.87	7	0.12	0.11	0.07-0.19
Aug. 2017	3	0.04	0.04	0.04-0.05	3	0.07	0.06	0.05-0.08
Oct. 2017	5	0.02	0.02	0.008-0.03	9	0.05	0.04	0.03-0.09
Nov. 2017	–	–	–	–	11	0.04	0.03	0.02-0.08
Jan. 2018	8	0.02	0	0-0.05	4	0.05	0.05	0.04-0.06
Feb. 2018	12	0.02	0	0-0.1	38	0.05	0.05	0-0.19
Mar. 2018	–	–	–	–	9	0.02	0.02	0.01-0.03
Apr. 2018	6	0.02	0.02	0-0.04	10	0.04	0.04	0.03-0.07