ARTHROPOD RESPONSE TO COVER CROP-BASED REDUCED-TILLAGE ORGANIC CROPPING SYSTEMS

A Dissertation in
Entomology

by

Karly H. Regan

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The dissertation of Karly H. Regan was reviewed and approved* by the following:

Mary Barbercheck
Professor of Entomology
Dissertation Adviser
Chair of Committee

John Tooker
Professor of Entomology

William Curran
Emeritus Professor of Weed Science

Armen Kemanian
Associate Professor of Production Systems and Modeling

Kelli Hoover
Professor of Entomology

Gary Felton
Professor and Department Head of Entomology

*Signatures are on file in the Graduate School.
Abstract

Although all farmers face potential trade-offs between management tactics such as chemical usage, tillage, crop rotation, and other aspects of their production system, these trade-offs can be especially challenging to balance for organic producers. Conservation of natural enemies can be one of the most important tactics for managing invertebrate pests that is available to organic producers (NOP 2005). Through this dissertation, I investigated the response of arthropod pests and predators to organic cropping systems incorporating cover crops and reducing tillage, as well as reviewing broader effects of tillage on pests and predators.

My dissertation addresses multiple objectives through five chapters. Chapter 1 is a general introduction that reviews organic field crop production, tillage as a soil management practice, the use of winter cover crops, and how these agricultural practices influence arthropod pest and predator populations. Chapter 2 assesses the effects of tillage on arthropod populations, particularly predator populations, through a review and meta-analysis conducted in collaboration with Elizabeth Rowen. In addition to comparing the effects of conventionally managed systems with frequent and/or intensive tillage to no-till systems, the meta-analysis examines the effects of reduced or conservation tillage practices on arthropod communities in these systems.

Chapter 3 investigates the effects of winter cover crop species, tillage, and interseeding of cover crops into standing corn on the soil-dwelling arthropod community, with a focus on arthropod predators, in an organic agronomic crop rotation. Characterizing the soil-associated invertebrate community allows us to understand the effects of these practices on arthropod abundance, diversity, and community composition, and the roles that invertebrates play in these systems. I examine the relationship between different predator groups and the response of the predator community to four organic cropping systems that vary in the frequency, intensity, and
timing of disturbance. I also quantify predation exerted by the predator community in the same four cover crop-based, reduced-tillage cropping systems using sentinel prey assays to evaluate the effects of these cropping systems and their associated management practices on the conservation of beneficial predators and enhancement of predation.

Chapter 4 investigates the effects of cropping systems that vary in disturbance on damage from common early and late season invertebrate pests of corn in the Mid-Atlantic US. Through assessment of pest damage, I evaluate risks that producers face when implementing cover crops or reducing tillage in an organic cropping system. Chapter 5 examines the effects of tillage and cover crop management on a pest of large-seeded crops, Delia platura (Diptera: Anthomyiidae), through 6 years of management in an organic cropping system.

Lastly, Chapter 6 serves as a conclusion, in which I summarize the results presented throughout the dissertation and their relevance to agronomic production in Pennsylvania, the surrounding region, and in organic systems more broadly. I also propose suggestions for future research directions.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>List of Tables</th>
<th>viii</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Figures</td>
<td>ix</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>x</td>
</tr>
</tbody>
</table>

### Chapter 1. Introduction to Cover-Crop Based Reduced-Tillage Organic Agriculture
- **Abstract**: 1
- **Introduction to Organic Agriculture**: 1
- **Tillage as a Management Tool**: 4
- **Using Cover Crops to Facilitate Reductions in Tillage**: 6
- **Arthropod Diversity and Biological Control**: 8
- **Conclusions**: 10
- **References**: 11

### Chapter 2. Is Tillage Beneficial or Detrimental for Invertebrate Pest Management? A Meta-Analysis
- **Abstract**: 22
- **Introduction**: 23
- **Materials and Methods**
  - **Data Selection**: 27
  - **Database**: 28
  - **Data Analysis**: 29
  - **Diversity Indices**: 30
- **Results**
  - **Pest Herbivores**: 32
  - **Predators**: 34
  - **Diversity Indices**: 36
- **Discussion**: 40
- **Conclusions**: 46
- **References**: 47

### Chapter 3. Arthropod Predators and Predation in Response to a Cover Crop-Based Reduced-Tillage Organic Feed Grain System
- **Abstract**: 63
- **Introduction**: 64
- **Methods**
  - **Site Description**: 67
  - **Experimental Design**: 67
  - **Arthropod Sampling**: 70
  - **Environmental Conditions**: 72
  - **Data Analysis**: 75
- **Results**
  - **Activity-Density of Macroarthropods**: 79
  - **Diversity of Predatory Macroarthropods**: 80
  - **Community Composition of Predatory Arthropods**: 81
Chapter 4. Prevalence of Early and Late Season Pest Damage in Cover Crop-Based Reduced-Tillage Organic Corn Systems

Abstract ...................................................................................................... 112
Introduction .................................................................................................. 113
Methods
   Site Description ......................................................................................... 117
   Experimental Design ............................................................................... 117
   Early Season Damage to Corn .................................................................. 120
   Late Season Damage to Corn .................................................................. 121
   Environmental Conditions ....................................................................... 121
   Data Analysis ............................................................................................ 124
Results
   Damage Prevalence and Severity in Corn ................................................ 128
   Environmental Conditions Contributing to Damage ............................... 129
   Impacts of Pest Damage on Corn Yield .................................................. 130
   Predators, Sentinel Predation Rate, and Corn Damage .......................... 131
Discussion
   Early Season Damage Prevalence in Corn ............................................. 139
   Late Season Damage Prevalence in Corn ............................................... 143
   Effects of Pest Damage on Yield ............................................................ 145
   Conclusions .............................................................................................. 147
References ..................................................................................................... 149

Chapter 5. Seedcorn Maggot Response to Planting Date, Cover Crops, and Tillage in Organic Cropping Systems

Abstract ...................................................................................................... 162
Introduction .................................................................................................. 163
Methods
   Site Description ......................................................................................... 167
   Experimental Design and Field Operations ............................................. 167
   Sampling Methods .................................................................................. 171
   Environmental Conditions ....................................................................... 171
   Data Analysis ............................................................................................ 174
Results
   Effects of Planting Date and Cash Crop .................................................. 177
   Effects of Cover Crops and Tillage .......................................................... 178
   Natural Enemy Response to Crop Management ...................................... 179
Discussion ..................................................................................................... 187
References ..................................................................................................... 192
Chapter 6. Conclusions .................................................................200
References ..................................................................................206

Appendices
Appendix A: Supplemental Information for Included Chapters
Appendix A3.1: Arthropod Community Overlap Between Four Reduced-Tillage Cropping Systems in ROSE 2.0 .................................................................210

Appendix B: Additional Projects and Protocols
Appendix B-1: Project Proposal for Investigating Contribution of Common Generalist Predators to Predation in Organic Reduced-Tillage Systems
   Abstract .................................................................................211
   Introduction ...........................................................................212
   Methods
      Field Experimental Design .................................................213
      Sentinel Predation Assays .................................................216
      Video Surveillance of Predation .......................................216
      Mark-Recapture Based Gut Content Analysis .................217
   Current Status and Proposed Data Analysis
      Video Surveillance ..........................................................220
      Mark-Recapture Based Gut Content Analysis ..................220

Appendix B-2: Protocol for Using Video Surveillance to Study Predation in Agroecosystems .................................................................222
Appendix B-3: Protocol for Using ELISA to Detect Egg and Chicken Proteins in a Predation Experiment .........................................................230
LIST OF TABLES

Table 3.1 Field operations and sampling dates for each experimental system in each year
........................................................................................................................................78
Table 3.2 Summary statistics for forward-selection RDA models of predator taxa and significant environmental variables..........................................................................................91
Table 4.1 Field operations and damage assessments for each experimental system in each year
........................................................................................................................................127
Table 4.2 Regression models showing significant association of environmental characteristics on prevalence of early season damage categories ........................................136
Table 4.3 Regression models showing significant association of environmental characteristics on prevalence from late season lepidopteran pests ..................................................137
Table 4.4 Relationship between yield of corn harvested as either grain or silage, damage by pests, weed biomass, and soil fertility .....................................................138
Table 4.5 Regression models showing significant association of predator activity-density and predation rate on prevalence of early-season pest damage categories ..................138
Table 5.1. Cash crop planting and emergence trapping dates for ROS E 1.0 and 2.0, growing degree days, and an estimation of growth stage for Delia platura .................................176
Table 5.2 Relationship between Delia platura, Hymenoptera, and Staphylinidae with environmental variables in corn in ROSE 1.0 ........................................................................185
Table 5.3 Relationship between Delia platura, Hymenoptera, and Staphylinidae with environmental variables in corn in ROSE 2.0 ........................................................................186
Table 5.4: Relationship between Delia platura, Hymenoptera, and Staphylinidae with environmental variables in corn in both ROSE 1.0 and ROSE 2.0 ............................186
LIST OF FIGURES

Figure 2.1. Disturbance continuum of tillage practices included in this meta-analysis, based on USDA-NCRS soil disturbance ratings .................................................................................. 31
Figure 2.2. PRISM Diagram of our literature search workflow ............................................. 32
Figure 2.3. The relationship between soil disturbance rating and pest abundance is shown for all pests (A), soil-associated pests (C), and foliar pests (E) in both medium-disturbance and low-disturbance systems .................................................................................................... 37
Figure 2.4. Funnel plots of pest (A) and predator (B) data ....................................................... 38
Figure 2.5. The relationship between soil disturbance rating and predator abundance is shown for all predators (A), soil-associated predators (C), and foliar predators (E) in both medium- Figure 2.6. Differences in predator and pest diversity as a result of soil disturbance 39
Figure 2.6. Differences in predator and pest diversity as a result of soil disturbance .......... 40
Figure 3.1. Activity-density and diversity metrics of arthropod predator community caught prior to cover crop termination ........................................................................................................ 85
Figure 3.2. The activity density per plot of the four most common generalist predator taxa .... 86
Figure 3.3. Multivariate relationships between taxa and environmental variables prior to cover crop termination ......................................................................................................... 88
Figure 3.4. Multivariate relationships between taxa and environmental variables after cover crops are terminated and corn has emerged ................................................................. 89
Figure 3.5. Multivariate relationships between taxa and environmental variables after cover crop interseeding has occurred .............................................................................. 90
Figure 3.6. Principal response curve of predator activity-density in Systems 2-4, relative to System 1 ................................................................................................................................. 92
Figure 3.7. The proportion of waxworms eaten at each sampling date ..................................... 93
Figure 3.8. Linear relationship between sentinel predation rate and spring cover crop biomass prior to cover crop termination .......................................................................................... 94
Figure 3.9. Linear relationship between sentinel predation rate and spring weed biomass after corn emergence ................................................................. 94
Figure 3.10. Relationships between sentinel predation rate and significant environmental variables after interseeding ................................................................................................. 95
Figure 4.1. Mean proportion of corn plants damaged by slug feeding (A), with damage to corn whorl (B), and with damage from chewing herbivores (C) in each year of the study .... 133
Figure 4.2. The severity of damage to plants ranked on a 0-4 scale ........................................ 134
Figure 4.3 Mean prevalence of damage due to Helicoverpa zea ear feeding (A), Ostrinia nubilalis tunnels (B), and Spodoptera frugiperda feeding (C) in each year of the experiment ............................................................................................................................. 135
Figure 5.1: The number of Delia platura (A), Hymenoptera (B), and Staphylinidae (C) that emerged per trap per day in corn in ROSE 1.0 ........................................................................... 182
Figure 5.2: The number of Delia platura (A), Hymenoptera (B), and Staphylinidae (C) that emerged per trap per day in corn in ROSE 2.0 ........................................................................... 183
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Chapter 1

Introduction

Abstract

Arthropods can play a number of important roles in agricultural systems, including pest, predator, decomposer, and pollinator. Frequent disturbance and intensive management through chemical usage, tillage, and low crop diversity can shape the diversity, abundance, and composition of arthropod communities present. Here, I review known effects of organic production, incorporation of cover crops, and the use of tillage on pests and natural enemies in agricultural systems. I conclude by suggesting future research directions to improve knowledge of natural enemies, predation, and pest damage in organic cropping systems.

Introduction

Modern agriculture in North America is frequently characterized by frequent disturbance, low crop diversity, and often short growing seasons. Concern over supplying the world with enough food has contributed to intensification of production to grow more food on the same amount of land (Tilman et al. 2011). As management of agriculture has intensified, consequences for arthropod diversity and abundance have gained the attention of numerous researchers (Fahrig et al. 2015, Habel et al. 2019, Hallman et al. 2017, Horváth et al. 2015, Gurr et al. 2017, Pretty and Bharucha 2015, Sánchez-Bayo and Wyckhuys 2019). Declines in arthropod numbers are substantial and have been estimated as a 75% loss in biomass in Europe (Hallman et al. 2017) and affecting up to 40% of species globally (Sánchez-Bayo and Wyckhuys 2019). Agriculture has been named a key driver of these declines, due to reduced habitat, intensive management,
and increased chemical usage, including both pesticides and fertilizers (Habel et al. 2019, Sánchez-Bayo and Wyckhuys 2019). The impacts of crop and soil management practices may cascade to changes in how arthropods affect agriculture through the numerous ecosystem services they provide (Habel et al. 2019, Sánchez-Bayo and Wyckhuys 2019).

Arthropods can have profound effects on agricultural production and ecosystem health as phytophagous pests, seed feeders, pollinators, predators, parasitoids, and decomposers (Losey and Vaughan 2006, Savary et al. 2019). They can also serve as indicators of the stability of an ecosystem. Understanding the impact of disturbance on habitat and available resources for the beneficial organisms that use them is essential to ensure management of crop pests, as well as to maintain biodiversity and the ecosystem services that diverse communities can provide within agroecosystems (Fahrig et al. 2015, Horváth et al. 2015, Gurr et al. 2017).

Agrichemical usage is a major component of intensive agricultural production and has been frequently highlighted for its negative effects on biodiversity, especially beneficial organisms and the ecosystem services they provide (Mazzia et al. 2015). Due to the restrictions on synthetic pest management materials in organic production, management of invertebrate pests relies heavily on cultural methods, such as strategically adjusting planting date, tillage, and crop rotation, and on natural processes, such as biological control (USDA NASS 2015). On average, yields in organic production systems tend to be lower than in conventional systems due in part to lack of chemical pesticides and fertilizers (Cavigelli et al. 2013, de Ponti et al. 2012, Kravchenko et al. 2017). As market demand for organic grain increases in the United States, there is a greater need for approaches that facilitate productivity and profitability (Cavigelli et al. 2013, Crowder and Reganold 2015, Ponisio et al. 2014, Youngberg and DeMuth 2013). Research has often tried to compare entire organic systems to entire conventional systems, yielding mixed results that
frequently fail to identify exactly which component(s) of organic production worked or didn’t work, in terms of production and pest management (Kirschmann et al. 2016, Puech et al. 2014). Thus, research into different facets of production within organic systems can be beneficial to ensuring success of production and a better understanding of the ecology of these production systems (Smith et al. 2008, Fox et al. 2015).

Despite the limitations in comparing organic and non-organic production systems, a number of studies have shown benefits from organic management for arthropods. A study of border strips adjacent to organic fields and conventional fields found that organic management generally supported higher abundance and diversity of arthropods, particularly predators from Lycosidae (Araneae), Coccinellidae (Coleoptera), Cantharidae (Coleoptera), Braconidae (Hymenoptera), Ichneumonidae (Hymenoptera), and Syrphidae (Diptera) (Eyre and Leifert 2011). Organic management favored both carabid beetles (Carabidae: Coleoptera) and Araneae, though specific effects on Araneae varied by family (Pffifner and Luka 2003). Araneae abundance, diversity, and evenness can be greater in organically managed fields than conventional or lower-input conventional fields, though the effects tend to be greater for hunting spiders than for web-building spiders (Caprio et al. 2015, Mazzia et al. 2015). Increasing predator diversity can enhance the effectiveness of pest control (Letourneau et al. 2015, Rivers et al. 2018). Because simplification of landscapes has resulted in less natural pest control, the added biodiversity required by organic management guidelines could contribute to greater pest control (Letourneau and Bothwell 2008, NOP 2005, Rusch et al. 2016). To overcome limitations identified by Kirchmann et al. (2016) in our current understanding of organic agriculture, arthropod research should expand to identify which specific factors of organic production best support beneficial arthropods and the amount of predation they perform.
In addition to studying effects of organic crop management on natural enemies, it will be useful to examine how in-field management factors affect pest populations and the amount of damage they cause. For example, a recommended tactic for managing seedcorn maggot (*Delia platura*) is to delay planting until after the “fly-free” period. However, late planting paired with the restriction on use of transgenic Bt corn in organic production may render organic corn more susceptible to attack by European corn borer (*Ostrinia nubilalis*) (Obopile and Hammond, 2013; Ordas et al. 2013). This pest tends to prefer late-planted corn for larval feeding and utilizes ground vegetation as adults (Bohnenblust and Tooker 2010, Obopile and Hammond 2013, Ordas et al. 2013). Thus, understanding how management practices throughout a cropping season affect pests and the potential for predators to control these pests is needed for growers to maximize productivity and profitability. This dissertation will investigate particular aspects of management within organic systems, including cover crop usage and tillage to better understand arthropod response to organic crop management.

**Tillage as a Management Tool**

Organic growers face tradeoffs when managing their production systems. Without herbicides or synthetic fertility sources, organic growers rely largely on tillage and other mechanical operations for managing weeds and incorporating fertility inputs (Cavigelli et al. 2013). Weed management is often cited as a major factor in determining crop yield, so farmers must be diligent about this aspect of production to be profitable (Cavigelli et al. 2013, Wallace et al. 2018). However, tillage can have detrimental impacts on soil structure, which can limit nutrient availability and water holding capacity, as well as increase erosion potential (USDA 2015). Management of soil also has important implications for greenhouse gas emissions,
eutrophication, and carbon sequestration (Scialabba and Müller-Lindelauf 2010, Tuomisto et al. 2012). It has been highlighted as a practice to avoid when practicing sustainable intensification of agricultural production to balance production needs with conservation needs (Pretty et al. 2014; Pretty et al. 2015). Thus, there are many factors to consider when deciding whether or not to use tillage and the frequency and intensity of tillage practices.

In addition to considering the tradeoffs between tillage for weed management and soil quality, producers need to consider the effects of their management practices on invertebrate pest management. Soil disturbance from tillage can have a profound effect on soil organisms. Often, no-till or reduced-tillage practices promote insect abundance and diversity (Holland and Reynolds 2003, Prasifka et al. 2006). Generalist predators, such as ground beetles (Coleoptera: Carabidae) can be sensitive to disturbance, vegetation, and other factors (Shearin et al. 2007, Woodcock et al. 2010, Kosewska et al. 2014, Rivers et al. 2016). Mechanical disturbance from plowing has been shown to be one of the greatest drivers of diversity and abundance of both Carabidae and Araneae (Hatten et al. 2007, Shearin et al. 2007). As euedaphic decomposers, predators, and prey, Collembola and Acari are also likely to be affected by soil disturbance and available resources underground and could have cascading effects on predators that rely on them for food (Alvarez et al. 2001, Chapman et al. 2013). Some pest species, such as slugs (Gastropoda: Mollusca) and black cutworm (Agrotis ipsilon Hufnagel), may benefit from reductions in tillage through increased plant residue in which to seek shelter (Douglas and Tooker 2012, Mischler et al. 2010). However, some pests such as seedcorn maggot (Delia platura Meigen) may be more abundant and damaging in tilled systems, particularly in high residue environments, through attraction to decomposing residues (Hammond 1990). This may further complicate decisions around tillage as a pest management tool.
Using Cover Crops to Facilitate Reduction in Tillage

One practice used to facilitate reductions in tillage in organic production is the use of winter cover crops, which are planted after cash crop harvest in the fall, grown throughout the winter, and then terminated in the spring before cash crops are sown. Species can be grown alone or in mixtures to achieve a wide range of benefits from reducing soil erosion and nitrogen leaching to supplying nitrogen and organic matter (Murrell et al. 2017, Schipanski et al. 2017). When biomass is sufficient, cover crops may also reduce germination of weeds in the spring by blocking the soil surface from sunlight (Mirsky et al. 2012, Reberg-Horton et al. 2012, Wallace et al. 2018). When comparing non-legume and legume cover crop species, grasses typically achieve higher biomass and provide greater weed suppression and organic matter, as well as scavenge more nitrogen that may have otherwise been leached (Dabney et al. 2001, Meisinger et al. 1991, Staver and Brinsfield 1998). Legumes tend to have comparatively lower biomass but are able to provide nitrogen through fixation of atmospheric nitrogen and in some cases, provide a flowering resource to early-season pollinators or arthropod natural enemies (Murrell et al. 2017). In some cases, a cover crop, such as forage radish (*Raphanus sativus* L.) may aid in weed control and soil aeration without needing management in the spring (Holmes et al. 2017, Murrell et al. 2017). Forage radish is not winter hardy in Pennsylvania, so it dies during the winter and creates an opening in the soil as it decomposes in early spring (Murrell et al. 2017). Additionally, by planting cover crops in bicultures or mixtures rather than choosing a single species, farmers may be able to reap more benefits from the incorporation of cover crops into their cropping systems (Murrell et al. 2017). Studying how these mixtures affect arthropods is important to maximize ecosystem services such as pest control and decomposition.
Habitat enhancement may play a role in preserving arthropod populations that would otherwise be killed or deterred from establishing in intensively disturbed agricultural fields (Landis et al. 2000). Even in areas where disturbance is less frequent and generally favorable to a taxon, the amount of vegetation and litter can shift species assemblages, so actively adding habitat beyond simply leaving areas of a farm unmanaged may be needed to conserve predators in agroecosystems (Horváth et al. 2015). By manipulating an area either within an agricultural field or nearby, farmers can provide refuge and food resources that would otherwise be absent from land used for crop production. Cover crops have the potential to provide habitat across a large area, depending on how much of a farm they cover, but are typically terminated prior to cash crop planting to ensure successful cash crop establishment, either through rolling or mowing in organic systems or through herbicides in conventional systems (Mischler et al. 2010). Studying how arthropod communities change after cover crops are terminated and cash crops are established is an important component to studying their benefits for arthropod dynamics in cropping systems.

Although the species of cover crop can have a range of benefits for nutrient dynamics and weed suppression, species of cover crop may also influence arthropods. Arthropod taxa can differ in their preferences for the type or density of vegetation available to them, even within arthropod families or genera (Eyre and Leifert 2011). For example, Araneae that use active hunting rather than webs to kill prey benefit from increased density of tall grasses and other vegetation as it can help hide them from prey and may harbor higher abundance of prey than sparse vegetation (Caprio et al 2015). Carabids can be omnivorous and are known to feed on seeds, in addition to prey, whereas Araneae are typically carnivorous (Shearin et al. 2007, Woodcock et al. 2010, Caprio et al. 2015). Vegetative cover, density of plants, and composition
of vegetation can affect arthropod assemblages, so having certain arthropod species respond favorably while others do not could alter the benefits realized from habitat addition (Eyre and Leifert 2011, Caprio et al. 2015, Horváth et al. 2015). Although differences between legume cover crops and grass cover crops have been demonstrated, it is less clear how different the response may be to similar plant species or mixtures (Rivers et al. 2018, Jabbour et al. 2016, Fernandez et al. 2008). Investigating the effects of the interaction of cover crops with the presence or absence of tillage on arthropods is important to maximize ecosystem services such as pest control and decomposition.

**Arthropod Diversity and Natural Pest Control**

Biological, including natural, control of invertebrate pests provided by predators and parasitoids has been valued at $4.5 million per year in the United States (Losey and Vaughan 2006). To maximize the benefits of natural control for organic producers, we must understand factors limiting or promoting the establishment of natural enemies, the likelihood that natural enemies will feed on prey within those fields, and the relationship between natural enemy abundance and diversity on damage incurred from pests and yield. Knowing which natural enemies contribute to pest control can help identify crop and soil management strategies that will translate to improved pest suppression and reduction of economic damage.

One of the critical resources needed by arthropod natural enemies is a diverse mixture of food throughout the season (Letourneau et al. 2012). Diversifying diets to include additional prey or non-prey resources can enhance fitness of generalist predators, including Carabidae and Araneae (Harwood et al. 2009). This increased fitness may come with the consequence that predators may not necessarily feed on pests, especially if other food resources, such as pollen
and nectar, are more abundant (Grieshop et al. 2012). Multiple studies have indicated that Collembola can comprise a major portion of Araneae diets, so pests may contribute little to their actual diet (Romero and Harwood 2010, Chapman et al. 2013). In this case, a high abundance of Araneae may not provide a substantial contribution to natural control, which reinforces the need to study which predators do contribute to pest management. Intraguild predation, where predators feed on other predators, may also complicate the level of natural control of pest invertebrates since conservation of predator groups is no longer tied solely to a service through consumption of invertebrate pest prey (Rosenheim et al. 1995).

Many biological control studies rely on estimates of insect feeding behavior without direct observations or without relating predation to changes in pest pressure (Losey and Vaughan 2006). Connecting the diversity and composition of predator communities to the services they provide can be challenging but is important to ensure the pest suppression needs of agriculture are being met (Crowder and Jabbour 2014). Recently, attempts have been made to identify specific trophic linkages between predators and pest consumption ecosystems, including agricultural ecosystems (Harwood et al. 2009, Hagler 2011). Some studies confirm a link between consumption by generalist predators, such as Araneae, and suppression of pest arthropods, such as aphids (Hemiptera: Aphididae) (Chapman et al. 2013). Other studies indicate there may not be a direct relationship between activity density of predators and potential prey (Schmidt et al. 2012). Intraguild predation may limit how informative common predator populations are in terms of estimating predation potential of a cropping system. Intraguild interactions may include direct effects, such as consumption, which would reduce populations caught in traps, or indirect effects, through fear response, which would likely not appear in trapping data (Hermann and Landis 2017, Rosenheim et al. 1995). In addition to predation by
arthropods, Tschumi et al. (2018) demonstrated that rodents can contribute to intraguild predation in agroecosystems and consumed more beneficial arthropods than pest arthropods, leading to a potential ecosystem disservice. These multi-trophic interactions could translate to challenges in implementing conservation biological control. Thus, gaining insight into the relative contribution of generalist predators to pest control, as well as interference by other natural enemies, such as vertebrates, could help improve our ability to understand and predict pest control in agricultural systems, leading to improved recommendations for growers in need of pest control.

Conclusions and Future Directions

Organic management, tillage, and winter cover crop usage can all influence pests and predators within agroecosystems. However, these effects are often studied in isolation. Research is needed to identify how management changes within organic production affect predator diversity, predation services, and pest damage. As grower interest in cover crops and reducing tillage increases among organic farmers, many questions remain as to how to incorporate cover crops, terminate cover crops, which cover crops to plant, and how best to reduce tillage in organic systems without compromising agricultural productivity (Keene et al. 2017, Mischler et al. 2010, Rivers 2016, Wallace et al. 2018). Addressing these questions and the effects these management choices have on pests and predators will be valuable for increasing organic productivity.

In this dissertation, I address the need to study management practices in combination, rather than in isolation, as well as the need to link predator communities, predation, and pest damage. My research investigates the combined effects of winter cover crop species, tillage, and
interseeding of cover crops into corn on arthropod pests, predators, and predation in an organic agronomic crop rotation. Through characterizing the soil-dwelling predator community, estimating the rate of predation, and assessing damage from common early and late season pests of corn, I assessed the risks and benefits to growers of adopting these cover crop establishment and termination practices within their organic agronomic rotations. Additionally, by evaluating the overall costs and benefits of tillage for pests and predators through a meta-analysis, I provide further insight into the role of tillage in pest management. By characterizing predator communities alongside pest damage and predation rate, I was better able to estimate natural biological control potential. My research provides valuable information to help formulate recommendations for organic producers on practices that can maximize predators and predation within their fields to reduce pest damage.

References


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Co-authors include: Elizabeth Rowen*, Karly H. Regan*, Mary E. Barbercheck, John F. Tooker

* E. Rowen and K. H. Regan contributed equally to this project and manuscript.

Abstract

Frequent and intensive tillage can have negative effects on soil productivity, including increasing risk of erosion, oxidizing organic matter, and disrupting life cycles of soil organisms. Even though tillage can negatively influence soil quality, this practice is still used across much of the US to prepare fields for planting and manage pests. Farmers who have adopted reduced-tillage systems can struggle with a suite of invertebrate pests. However, reducing the frequency and intensity of tillage may have beneficial effects on predator populations and biological control. To better evaluate how reduced-tillage practices (e.g., no-till, harrowing, shallow disking) affect the abundance of invertebrate pests and natural enemies relative to high-disturbance tillage practices (e.g., moldboard plowing), we conducted a meta-analysis of studies published between 1983 and 2017. We tested the hypotheses that 1) reducing tillage intensity increases pest herbivore and arthropod predator abundance, 2) soil-associated invertebrates would be more susceptible to tillage than foliar invertebrates, and 3) medium-disturbance tillage practices would have an intermediate effect on pests and arthropod predators relative to high and low disturbance practices. We found that pests were not more abundant in reduced-tillage systems than in high-disturbance tillage. Pest herbivores that spend part of their life-cycle in the soil followed this pattern, but foliar pests were more abundant with more intense tillage practices. Predators that spend part of their life-cycle in the soil were significantly reduced by
tillage, regardless of the intensity of the disturbance, though predators in general were similar in abundance regardless of tillage intensity.

**Introduction**

Tillage has been practiced for over ten thousand years, primarily to prepare soil for planting and to manage weeds (Lal et al. 2007). Tillage can accelerate soil warming, allowing crops to be planted earlier than in undisturbed soil (Bàrberi and Lo Cascia 2001, Cardina et al. 2002, Lal et al. 2007, Stone et al. 1989, van Wijk et al. 1959). Tillage can also help reduce some species of plant pathogens and invertebrate pests (Bockus and Shroyer 1998, Capinera 2017, Douglas and Tooker 2012, Stinner and House 1990). Despite these benefits, tillage increases labor and energy use, erosion risk, oxidation of organic matter, and can reduce habitat for beneficial soil organisms (USDA 2015). Our growing appreciation of the influence of tillage on soil characteristics and microbial communities may reveal other costs of tillage (Zuber and Villamil 2016). In response to these concerns, many farmers are adopting reduced-tillage practices, also called conservation tillage, which has been defined by the U.S. Department of Agriculture’s Natural Resource Conservation Service as operations that leave more than 30% of the previous crop’s residue on the surface after tillage (Census of Ag. 2012). Adoption of these systems are facilitated by technologies such as herbicide-tolerant crops and improved planting equipment for high-residue systems (Lal et al. 2007).

Despite drawbacks associated with high-disturbance tillage systems, many farmers still till their fields. For instance, in the U.S. as of 2012, farmers tilled 38% of cultivated land (Census of Ag. 2012). The reluctance of some farmers to move away from high-disturbance tillage systems originates, at least in part, from concerns that reducing tillage can lead to greater pest
problems, including weeds, pathogens, and insects (Aref and Pike 1998). Indeed, compared to high-disturbance tillage systems, a suite of invertebrate pest species tend to be more problematic in fields managed with reduced-tillage practices than in high-disturbance tillage systems (Capinera 2017, Douglas and Tooker 2012, Stinner and House 1990). For example, slugs tend to be more abundant in no-till systems and can be overwhelming when conditions are cool and rainy during planting season, often causing damage severe enough to require replanting (Douglas and Tooker 2012, Hammond and Stinner 1987a). Further, the availability of vegetation in reduced-tillage systems favors some pest species, resulting in higher populations, e.g., the European corn borer, *Ostrinia nubilalis* Hübner, and the black cutworm, *Agrotis ipsilon* Hufnagel (Bohnenblust and Tooker 2010, Mischler et al. 2010). In contrast, some arthropod pest species, such as the seedcorn maggot, *Delia platura* Meigen, can be more abundant in high-disturbance tillage systems than in reduced-tillage systems (Hammond 1997). This variable effect of tillage raises questions about whether farmers who adopt reduced-tillage practices are simply trading one set of pests for another or if they need to increase their reliance on other pest management tactics, such as the use of insecticides. The key question becomes: “How does tillage alter invertebrate pest populations?”

Tillage can influence populations of invertebrate pests in three primary ways. First, tillage disrupts soil structure and directly disturbs habitat of subterranean and epigeal invertebrates. Soil disturbance is particularly effective for managing some pest species that overwinter as pupae in soil and do not emerge prior to plowing, e.g., the tobacco hornworm *Manduca sexta* L. (Capinera 2001). Second, tillage can indirectly increase pest populations by altering weed populations. Because tillage is often used for weed management, reduced tillage tends to promote greater weed populations (Lal et al. 2007, Wallace et al. 2018). Some
invertebrate species, including *O. nubilalis*, use weedy vegetation as habitat early in the season and disperse to the cash crop from surrounding weeds (Showers et al. 1980, Weber et al. 1990). Greater weed abundance in reduced-tillage systems then can support these pest species within or near fields (Bohnenblust and Tooker 20120, Showers et al. 2010, Wallace et al. 2018). Weeds and crop residue typical of reduced tillage systems can provide moist refuges, buffering environmental fluctuations and improving habitat for invertebrate pests (Bohnenblust and Tooker 2010, Douglas and Tooker 2012, Hammond and Stinner 1987b, Mischler et al. 2010, Thorbek and Bilde 2004, USDA 2015).

While tillage can disrupt pest lifecycles and reduce their populations, reducing tillage can also benefit pest management by supporting natural enemies. Decreasing the intensity and/or frequency of tillage allows crop fields to harbor a greater diversity and abundance of arthropod natural enemies, including predators, parasitoids, and entomopathogens, which can play an important role in reducing invertebrate pest populations (Brust 1986, Brust 1991, House and Stinner 1983, Sosa-Gómez et al. 2001, Stinner and House 1990). Because many predators pass part of their life cycle belowground, predator populations tend to be greater where tillage is reduced (Hatten et al. 2007, Prasifka et al. 2006, Shearin et al. 2007, Stinner and House 1990). In addition, populations of soil-associated predators can be greater in systems that retain weed cover and crop residue from the previous year (Blubaugh and Kaplan 2015, Blubaugh et al. 2017, Halaj et al. 2000, Kosewska et al. 2014, Rivers et al. 2016, Rypstra and Marshall 2005, Shearin et al. 2007, Woodcock et al. 2010). As a result of these factors that promote predator populations, reduced-tillage systems should harbor lower invertebrate pest populations, assuming that greater numbers of predators results in greater predation (Rusch et al. 2017). However, it is
unclear whether the hypothesized increase in predators inevitably increases predation to levels that can control pest populations typical of reduced-tillage systems.

Here we present a quantitative review to determine how tillage intensity influences populations of arthropod pests and natural enemies. Key to this effort was defining tillage intensity, which can vary greatly depending on equipment, frequency of use, and goals of disturbance. Tillage encompasses many mechanical farming practices imposed on the soil surface. The most intensive tillage uses moldboard plowing or heavy disking to invert the soil (hereafter “high-disturbance tillage”, Figure 2.1). Less intensive practices, or “reduced-tillage”, typically do not invert soil, often to reduce soil degradation and include chisel plowing, light disking, strip tillage, harrowing (hereafter “medium-disturbance tillage”), and no-till, which does not use any tillage between harvest and planting (hereafter “low-disturbance tillage”). Further, while we did not quantify these, different tillage practices may be used in sequence; primary tillage may disturb the soil deeply to control weeds and allow greater water infiltration, whereas less intensive secondary tillage practices may break up soil clods left behind by primary tillage and improve seed to soil contact (Wallace et al. 2018).

Using a meta-analysis of published literature, we explored the relationships between these tillage practices and pest and predator populations in grain and vegetable crop fields. Specifically, we investigated the hypothesis that medium- and low-disturbance systems support greater pest populations than high-disturbance systems (House and Alzugray 1989, Wilson and Eisley 1992). For our analysis, we hypothesized that both pests and predators will be more abundant in reduced-disturbance cropping systems than in high-disturbance systems. Because tillage disrupts arthropods directly and indirectly, we expected that as tillage intensity decreases, both predator and pest abundance will increase, so that low-disturbance systems will have the
highest abundances of both. We expected medium-disturbance tillage to have intermediate effects, disrupting soil structure and organisms less than high-disturbance tillage but still offering some weed control and breakdown of plant residues relative to low-disturbance tillage, and somewhat supporting predator populations that may contribute to pest control (Stinner and House 1990). However, because we hypothesized that decreasing disturbance from tillage will support predator populations that can help control invertebrate pests, we may not be able to easily predict the overall effect of different tillage practices on pest abundances. We also expected that arthropod life histories will influence their response to tillage, i.e. we expected a stronger effect of tillage on soil-associated pests and predators compared to foliar arthropods because the former are more likely to be directly affected by soil disturbance.

Materials and Methods

Data Selection

We found papers for the invertebrate herbivore analyses using the following search terms in Web of Science (© 2019, Clarivate Analytics): “tillage and insect*”, “till* insect* pest*”, “tillage herbiv*”, and combinations of “till*”, “plow*”, “plough*”, “arthropod*”, “insect*”, and “predat*” (Figure 2.2). We also found papers by searching previous reviews on the topic: Stinner and House (1990), Lawani (1982), and All (1980). We included papers published before 1 Feb 2019 in this analysis. To be considered in the analysis, studies need to have (1) measured invertebrate abundances in a low- or medium-disturbance and high-disturbance tillage operation, (2) reported means and variance from both reduced and high-disturbance tillage fields to be included in effect size calculations, and (3) focused on annual grain, fiber, and vegetable crops, as we encountered too few studies investigating arthropod response to tillage practices in orchard
crops to include them in our analyses. Tillage was considered any operation that disrupted the soil. High-disturbance included moldboard plowing and heavy disking. Low- or medium-disturbance must be less disruptive management than the high-disturbance tillage operation, and included shallow disking, precision tillage, and no-till (Figure 2.1).

**Database**

We included 32 studies in our pest effect-size calculations and 35 studies in our predator effect size calculations, although 12 studies were included in both analyses (indicated in the reference section for this chapter). For invertebrate pests, nearly all papers focused on insects but we also included one study that measured slug abundance. We did not include plant-parasitic nematodes as an invertebrate pest. All predators were arthropods.

For each study, we recorded site year, crop, reduced-tillage practice, high-disturbance tillage practice, and if the reduced-tillage practice was medium-disturbance or low-disturbance. For the analysis of invertebrate pests, we recorded whether the species spent any life stage in the soil using known natural histories for each species (hereafter “soil-associated” or “foliar”). For the predator analysis, we recorded species, family and order and location of capture (i.e., foliage or soil).

We also quantified the difference in disturbance between the reduced-tillage and high disturbance practices by calculating the NRCS Soil Disturbance Rating (Kemanian and Stöckle 2010, NRCS 2008) based on tillage practices reported in each study. The SDR, which ranges from 0 (least disturbance) to 30 (greatest disturbance) for a field operation, is comprised of the sum of six ratings each with values from 0 to 5 that estimate the relative severity of disturbance. The six component categories of the SDR include soil inversion, soil mixing, soil lifting, soil
shattering, soil aeration, and soil compaction. While we did not consider tillage frequency in our analyses, we did account for repeated tillage in the disturbance rating calculations by summing the SDR values for all practices reported by each paper. A previous use of SDR has dealt with multiple passes of a similar tool by including it in a model that accounts for temperature and soil texture in calculating overall disturbance and turnover of the soil (Kemanian and Stöckle 2010). However, little is known about whether the effect of multiple passes of the same tillage implement has a different effect on arthropods than one pass. Thus, we opted to use all reported passes to calculate a total intensity of disturbance for our usage of SDR.

**Data Analysis**

We used the `escalc` function in the `metafor` package of R to calculate the effect size of each observation (Rowen and Kaplan 2016, Viechtbauer 2010). The effect size, Hedge’s g, is a corrected difference between invertebrate abundances in reduced-tillage treatments and abundances in high-disturbance treatments, weighted by the variance in both treatments (Hedges, 1981).

To calculate a mean effect size, we used a mixed effects model with study as a random factor to account for the non-independence of measurements from the same experiment, using `rma.mv` in `metafor` (Viechtbauer 2010). Additionally, we report the effect size of each study. We calculated 95% confidence intervals around each mean. When these confidence intervals overlap zero, mean effect sizes are not different from zero. When effect sizes are positive, reduced-disturbance treatments had greater abundances than high-disturbance treatments. When effect sizes are negative, high-disturbance tillage treatments had greater abundances. We calculated the effect of any reduced-tillage compared to high-disturbance tillage, whether the intensity of
reduced-tillage practice (medium-disturbance vs. low-disturbance) influences the effect of tillage on herbivores, whether association with soil influences the effect of tillage on abundances and whether this is modulated by tillage intensity.

We evaluated potential publication bias using funnel plots and calculated fail-safe N values using the Rosenthal approach, which indicates the number of studies necessary to render any significant effect sizes non-significant (Jennions et al. 2013). We used a “leave 1 out” analysis to examine undue influence of particular studies on the overall result of our analyses, and did not see evidence of a particular study having undue influence in our calculations.

Diversity Indices

To assess the effects of tillage on differences in pest and predator communities, we extracted species abundances from studies that report community composition from at least one method of sampling (database: Tables S3 and S4). For example, if a study focused on foliar herbivores sampled with sweep nets, the species and abundances were included in our analysis. We did not include studies that specifically targeted a single species. From species composition and abundances, we calculated species richness, a linearized Shannon diversity, Pielou’s evenness index, and the proportion of the community that overlapped for reduced- and high-disturbance tillage systems. We used one-way student’s t-tests to compare these different metrics of diversity for reduced- and high-disturbance tillage systems.
Figure 2.1. Disturbance continuum of tillage practices included in this meta-analysis, based on USDA-NCRS soil disturbance ratings. Examples of soil after tillage practices are shown in pictures. Reduced-tillage practices include all low-disturbance (no-till) and medium-disturbance practices. High-disturbance practices are compared to reduced-tillage practices throughout our analysis.
Results

Pest Herbivores

From our criteria for inclusion in the meta-analysis, we used studies comparing tillage treatments and measuring herbivore pests that were published from 1990 to 2017 on five continents. To account for potential non-independence due to phylogenetic relatedness (Chamberlain et al. 2012), we included study and herbivore taxonomic order as random factors. We found significant heterogeneity between studies ($I^2 = 72\%$). Overall, we did not see a significant difference in pest abundances between systems using high-disturbance tillage or reduced-disturbance tillage ($g = -0.01$, 95% CI = -0.73 to 0.71; Fig. 2.3B). When we separated reduced-tillage practices into either medium disturbance or low disturbance, we do not see a...
difference in effect on pests \( (g = -0.17, 95\% \text{ CI} = -0.88 \text{ to } 0.55; \text{Fig. 2.3B}) \). To explore the range of practices encompassed by reduced-tillage as a source of heterogeneity between studies, we investigated the relationship between soil disturbance rating (SDR) and pest abundance and found a significant negative relationship \( (\Delta = 0.01, P = 0.25; \text{Fig. 2.3A}) \). A positive effect size indicates greater abundance in the reduced-tillage system than in the high disturbance system and we see increasingly positive effect sizes, or greater differences between treatments, as SDR increases.

To further investigate heterogeneity between studies, we looked at pests associated with the soil separately from those not associated with the soil. We had hypothesized that effects of tillage would be greater for pests that spend all or part of their life in the soil than for pests that do not. However, we found that the abundance of soil associated pests were not significantly different between high disturbance tillage environments and reduced tillage environments \( (g = 0.33, 95\% \text{ CI} = -0.42 \text{ to } 1.07; \text{Fig. 2.3D}) \), even when reduced tillage was separated by low and medium disturbance \( (g = -0.34, 95\% \text{ CI} = -1.35 \text{ to } 0.66; \text{Fig. 2.3D}) \). We again detect a significant negative relationship between soil disturbance rating (SDR) and pest abundance in high disturbance systems for soil associated pests \( (\Delta = 0.01, P = 0.32; \text{Fig. 2.3C}) \). We observed greater heterogeneity between studies for soil-associated pests \( (I^2 = 72.5\%) \) than foliar pests \( (I^2 = 57\%) \), though both were high. Foliar pest abundance was significantly greater in high disturbance tillage systems than in reduced tillage systems \( (g = -0.53, 95\% \text{ CI} = -1.06 \text{ to } -0.02; \text{Fig. 2.3F}) \). When reduced-tillage practices were separated into medium and low disturbance there was no effect on foliar pest abundances \( (g = -0.38, 95\% \text{ CI} = -1.31 \text{ to } 0.55; \text{Fig. 2.3F}) \). We do not detect a significant relationship between SDR and foliar pest abundance \( (\Delta = 0.01, P = 0.41; \text{Fig. 2.3E}) \).
To assess the influence of each study on the models, we removed each study sequentially, and recalculated the random effects model with study as random factor. Based on the results of this leave-one-out analysis, we found that Krzic et al. (2001) had a strong influence over the results. When this paper was included, there was a significant relationship between SDR and effect size of pest abundance for the whole group ($\Delta = 0.02, P < 0.01$) and for soil-associated pests ($\Delta = 0.03, P < 0.01$). We excluded this paper from the results we report above as we consider it to be an outlier. We did not find evidence of publication bias (Figure 2.4A, $z = -1.36$, $P = 0.17$; fail-safe $N = 0$).

**Predators**

We used studies comparing tillage treatments and measuring predator abundances that were published from 1983 to 2016 on five continents. Many of the studies on predators featured predators from the order Coleoptera which contains families of generalist predators that vary in their published response to high-residue environments (Eyre et al. 2011, Hatten et al. 2007, Kosewska et al. 2014, Tamburini et al. 2016a), which we expected to add a source of heterogeneity for overall predator response. Thus, we ran predator models with study and predator taxonomic family, rather than predator taxonomic order, included as random factors. We found significant heterogeneity between predator studies ($I^2 = 33\%$), though lower than observed for herbivore studies. We did not detect a significant difference in predator abundances between studies using high disturbance tillage or reduced- tillage ($g = 0.10, 95\% \text{ CI} = -0.19$ to $0.39$; Fig. 2.5B). When we split reduced-tillage practices into either medium-disturbance or low-disturbance, we do not see a difference in effect on predator abundance ($g = -0.15, 95\% \text{ CI} = -$
0.58 to 0.28; Fig. 2.5B). We found no relationship between soil disturbance rating (SDR) and predator abundance ($\Delta < 0.01, P = 0.97; \text{Fig. 2.5A}$).

As with pests, we had hypothesized that effects of tillage would be greater for predators that spend all or part of their life in the soil than for pests that do not. The abundances of soil-associated predators were significantly greater in reduced-tillage systems than in high-disturbance systems ($g = 0.48, 95\% \text{ CI} = 0.12 \text{ to } 0.88; \text{Fig. 2.5D}$), with no difference between low- and medium-disturbance ($g = -0.38, 95\% \text{ CI} = -0.99 \text{ to } 0.21; \text{Fig. 2.5D}$). We again see no relationship between soil disturbance rating (SDR) and predator abundance for soil-associated predators ($\Delta < 0.01, P = 0.70; \text{Fig. 2.5C}$). Heterogeneity between studies was similar for soil-associated predators ($I^2 = 32\%$) and all predators, though foliar predators lacked heterogeneity among studies ($I^2 = 0\%$). Foliar predator abundances did not differ between high-disturbance tillage systems and reduced-tillage systems ($g = -0.23, 95\% \text{ CI} = -0.64 \text{ to } 0.17; \text{Fig. 2.5F}$). When reduced-tillage practices were separated into medium- and low-disturbance there was still no effect on foliar predator abundances ($g = -0.43, 95\% \text{ CI} = 0.86 \text{ to } 1.72; \text{Fig. 2.5F}$). We do not detect a significant relationship between SDR and foliar pest abundance ($\Delta = 0.04, P = 0.82; \text{Fig. 2.5E}$).

To assess the influence of each study on the models, we removed each study sequentially, and recalculated the random effects model with study as random factor. For predators, we did not find any studies with strong influence on the overall results. We did not find evidence of publication bias (Figure 2.4B; $z = 1.64, P = 0.10$).
Diversity Indices

Neither pest nor predator species richness was affected by the intensity of tillage (pest: \( t_{19} = 0.13, P = 0.89 \); predator: \( t_{20} = 0.77, P = 0.45 \); Fig. 2.6A). Shannon diversity of pests (\( t_{19} = 0.88, P = 0.39 \); Fig. 2.6B) and evenness (\( t_{19} = 0.24, P = 0.81 \); Fig. 2.6C) also did not differ between reduced- and high-disturbance systems. However, predator communities had greater linearized Shannon diversity indices (\( t_{20} = 2.22, P = 0.04 \); Fig. 2.6B) and were more even (\( t_{20} = 2.55, P = 0.02 \); Fig. 2.6C) in reduced- compared to high-disturbance systems. Both pests and predators had significantly different communities between reduced- and high-disturbance systems, as the mean proportion overlap was significantly less than 1 (pest: \( t_{19} = -4.66, P < 0.001 \); predator: \( t_{20} = -5.75, P < 0.001 \); Fig. 2.6D).
Figure 2.3. The relationship between soil disturbance rating and pest abundance is shown for all pests (A), soil-associated pests (C), and foliar pests (E) in both medium-disturbance and low-disturbance systems. Points represent effect size for individual studies with lines showing 95% confidence intervals. Additionally, we show the mean effect size for all reduced tillage systems,
medium disturbance systems, and low-disturbance systems for all pest studies (B), soil-associated pests (D), and foliar pests (F). Error bars represent 95% confidence intervals.

Figure 2.4. Funnel plots of pest (A) and predator (B) data. If studies with non-significant results are not published (publication bias) funnel plots will be asymmetric where small studies with non-significant results are missing from the “mouth” of the funnel (Jennions et al. 2013).
Figure 2.5. The relationship between soil disturbance rating and predator abundance is shown for all predators (A), soil-associated predators (C), and foliar predators (E) in both medium-disturbance and low-disturbance systems. Points represent effect size for individual studies with line showing 95% confidence intervals. Additionally, we show the mean effect size for all reduced tillage systems, medium disturbance systems, and low-disturbance systems for all
predator studies (B), soil-associated predators (D), and foliar predators (F). Error bars represent 95% confidence intervals.

Figure 2.6. Differences in predator and pest diversity as a result of soil disturbance. Differences in number of species for pests and predators (A), differences in transformed Shannon diversity indices as a result of differences in soil disturbance (B), differences in Pielou’s evenness of predators and pests in high disturbance and reduced-tillage systems (C), and the overlap in community composition in reduced- and high-disturbance tillage. P values for A-C represent the probability that differences between tillage systems will overlap zero. For D, P-values represent the probability that community overlap is not equal to 1 or full overlap between communities. All error bars represent standard error.

Discussion

We hypothesized that abundances of invertebrate pests and arthropod predators would increase with lower disturbance from tillage. Contrary to our expectations, both pest and predator
abundances were similar in reduced- and high-disturbance tillage systems, even when the reduced-tillage practice was low-disturbance (Fig. 3B and 4B). Previously, mechanical soil disturbance has been hypothesized to disrupt arthropod life cycles and decreases habitat quality (Edwards 1975). However, our results are consistent with those of Stinner and House (1990) who indicate that pest abundances in no-till systems are similar or lower than those in high-disturbance systems. This suggests that rather than supporting higher pest populations, no-till has emergent properties that can replace mechanical disturbance for managing arthropod pests.

Because no-till systems are relatively physically undisturbed, habitat stability, reduced plant susceptibility, and biological control may work in combination to reduce invertebrate pest populations. For instance, low-disturbance systems often have greater abundances of weeds than high-disturbance tillage systems, which can decrease apparency of host crops, leading to lower abundances of certain arthropod species (Carballo 1979, Shenk and Saunders 1984). However, weeds can contribute to greater abundances of certain invertebrate pest species (Johnson et al. 2984, Musick and Beasley 1978).

Compared to high-disturbance tillage, low-disturbance systems have greater stratification of plant residues and fertility amendments on or near the surface of the soil profile (Feng and Balkcom 2017, House et al. 1984, Mattson 1980), possibly altering nutrient availability and thus plant susceptibility to pests. In addition, low-disturbance systems can support greater abundances of soil-borne entomopathogens (Bing and Lewis 1993, Brust 1991, Hummel et al. 2002, Sosa-Gómez et al. 2001) and have greater abundances of beneficial soil microbes that can stimulate plant defense responses against invertebrate pests (Bowles et al. 2017, Jansen et al. 2002, Jung et al. 2012, Koricheva et al. 2009). Finally, the influence of tillage on predator populations can alter biological control in those systems (Rivers et al. 2016). These various mechanisms have been
well explored in no-till systems where they interact to regulate invertebrate pest populations, but their roles in medium-disturbance tillage systems are less clear.

In addition to the overall effect of tillage intensity on pest abundances, we expected that pests that spend any part of their life cycle in the soil would be more sensitive to disturbance from tillage than those that do not spend time in the soil. However, we did not observe any difference in soil-associated pests, relative to all pests. In contrast to soil-associated pests, abundances of foliar pests were greater in high-disturbance tillage systems compared to reduced-tillage systems, perhaps due to changes in plant quality in reduced-tillage systems. Plant-mediated effects of tillage on pests have been rarely examined and this is a potentially fruitful avenue of future research. The intensity of disturbance encompassed by the tillage treatments we compared is important to consider when evaluating effects of tillage.

For pests, we see a positive relationship between the intensity of tillage, as represented by soil disturbance rating, and the abundance of pests, including soil-associated pests, driven by inclusion of one paper (Krzic et al. 2001), which studied slug abundance in response to spring tillage practices. Without this paper, we detect no relationship between SDR and pest abundance. Their tillage treatments consisted of eight or more tillage passes in the spring, which contributed to a very high SDR rating, versus zero spring tillage passes in their reduced-tillage treatment. They observed a substantial difference in slug populations between tillage treatments, with lower slug abundance in their high disturbance treatment (Krzic et al. 2001). Although eight tillage passes is higher than the number in most other studies we examined, they also provided greater detail about their tillage treatments than did other papers, including the exact number of passes, depth of passes, in addition to the type of equipment used for each pass. Thus, we suggest future
studies on tillage provide equivalent detail into the number and depth of their tillage treatments to allow better comparisons between tillage frequencies and intensities.

Predators are widely considered to benefit from decreasing disturbance in agriculture (Rusch et al. 2017, Stinner and House 1990, Wardle 1995). We found that for predators as a whole, there was no difference between high-disturbance tillage systems and reduced-tillage systems. However, soil-associated predators were significantly more abundant in reduced-tillage systems compared to high-disturbance systems. Soil-associated predators can be harmed directly by mechanical disturbance (Blubaugh and Kaplan 2015, Gailis et al. 2013, Thorbek and Bilde 2004, Wardle 1995) or indirectly by altering habitat (van der Laat et al. 2015). Because we observed increased predator activity for soil-associated but not for foliar-collected predators, a combination of direct effects through reduced mortality and indirect effects through habitat alteration of soil-surface residues seem more likely to be contributing than changes in above-ground vegetation or weed communities.

We did not see a significant relationship between soil disturbance rating and predator abundance, even for soil-associated predators. Predators are not all equally sensitive to disturbance, and some groups prefer relatively simple habitats, while others prefer more complex environments (Halaj et al. 2000, Rypstra and Marshall 2005, Sunderland and Samu 2000). For some groups, the disturbance from tillage increases activity and trapping efficiency, which could influence the number of predators detected in each treatment (Lang 2000, Lundgren et al. 2006, Rivers et al. 2018). Thus, the mix of habitats possible in agricultural systems may favor a wider range of predator species and greater overall predator abundances regardless of the level of disturbance in these systems. Alternatively, the influence of tillage on predators may be driven by their prey, with abundance of predators tracking prey abundance. Because pest abundance
was similar regardless of tillage treatment, predators may able to find sufficient food resources in high disturbance systems. Field experiments that manipulate prey abundance may help to discriminate between these two alternatives.

We summarized the effects of tillage practices on invertebrate pest and predator abundances, but previous work on tillage suggests that disturbance affects diversity and community structure as well as abundance (Stinner and House 1990). Not all studies included in our meta-analysis identified pests and predator to species; hence, we used a subset of papers that identified all arthropods to species to understand how tillage affected community composition. Agricultural pests are often r-selected with short generation times and high fecundity, which would be favored under high levels of disturbance, particularly where annual re-colonization is necessary (Price 1976). We found no difference in pest diversity, richness, or evenness. However, although we detected substantial overlap in the pest community between reduced disturbance and high disturbance systems, the communities were significantly different. Since we did not detect differences in effect size between disturbance levels, it appears that tillage has little effect on herbivore communities overall, either in diversity or abundance.

In contrast to our results with invertebrate pest diversity, arthropod predator communities were more diverse and even in reduced-disturbance systems compared with high-disturbance systems. Predator habitat and alternative prey resources are greater in reduced-disturbance compared with high-disturbance systems, and we expected a greater number of species utilizing those resources. Instead, while predator community diversity was greater in systems with reduced-disturbance tillage, tillage did not affect the number of predator species. The greater evenness of predator communities in reduced-disturbance compared to high-disturbance systems may have been due to greater habitat complexity (Holland and Reynolds 2003). Minimizing
tillage has been observed to promote more complex soil-surface conditions, which supports greater diversity of predators (Altieri 1999, Brust and House 1990, Meyling 2007, Quintanilla-Tornel et al. 2016, Tscharntke et al. 2005). The increase in predator diversity and evenness is beneficial for ecosystem services, as evenness has been shown to better predict biological control potential than species richness (Crowder et al. 2010) Further, sentinel prey assays indicate that predators in low-disturbance systems may be more effective at suppressing pests than in medium-disturbance systems (Brust and House 1988, Cromar et al. 1999), promoting stronger biological control (Rusch et al. 2017).

Meta-analysis offers a quantitative approach to understanding large patterns in the published literature, without being limited by site or time (Douglas and Tooker 2016, Koricheva and Gurevitch 2013). We are, however, limited to studies that have been published. Bias in publishing significant results has been identified as an issue in meta-analyses (Jennions et al. 2013). We detected no prejudice against non-significant results in the studies we included, and no publication bias. Nevertheless, we were unable to include many published studies in our analysis because authors did not report variance in the abundances of pests from each treatment. Thus, we strongly encourage authors to include variance in their publications. In addition, some authors did not include sufficient detail about the type of tillage they considered “conventional” or high-disturbance to allow assignment to one of the categories of our analysis. This restricted our ability to analyze finer-scale differences between specific types of tillage on arthropods.

Finally, because this meta-analysis is a summary of the literature, rather than a detailed review of specific pest responses to tillage, our recommendations based on this summary must be general. Tillage intensity affects pests and predators in species-specific ways. Thus, while pest
abundances may be equivalent between reduced and high-disturbance tillage, composition of the pest communities, rather than abundances, may vary among tillage systems (Figure 6A).

Conclusions

We found that abundance of invertebrate pests is similar in low-disturbance and other reduced-tillage systems relative to high disturbance systems, contrary to what many farmers appear to believe (Aref and Pike 1998). Notably, foliar pests were more abundant in systems with more disturbance, emphasizing that pest species, which are often r-selected, can thrive in more disturbed settings.

Soil-associated predators were more abundant in reduced-tillage systems, indicating the potential for any reduction in tillage to conserve this valuable group of natural enemies and potentially the ecosystem services they provide. Predator populations in conservation tillage systems can provide meaningful biological control (Douglas et al. 2015, Stinner and House 1990). Moreover, the abundance of resident arthropod predators in no-till systems can be so great that it can compensate for insufficient landscape-based biological control in homogeneous landscapes (Tamburini et al. 2016a). As agricultural professionals promote adoption of conservation-tillage agriculture, they can communicate that these low-physical-disturbance systems have greater potential for biological control due to the greater abundances of soil-associated arthropod predators. Importantly, for this predation potential to be realized in systems that are not organically managed, no-till farming should be paired with Integrated Pest Management to ensure that unnecessary insecticide use does not counteract biological control (Douglas et al. 2015, Pearsons and Tooker 2017, Stenberg 2017, Stern et al. 1959).
References

\( ^h \) included in herbivore dataset

\( ^p \) included in predator dataset

\( ^c \) included in community composition dataset


Carballo, M. 1979. Incidencia de plagas en maiz (Zea mays L.) bajo diferentes sistemas de manejo de malezas. CIMMYT 1–89.


structure on biomass, species richness and functional diversity of ground beetles.

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Chapter 3
Arthropod Predators and Predation in Response to a Cover Crop-Based Reduced-Tillage Organic Feed Grain System

Abstract

Soil-dwelling arthropod communities are both valuable and vulnerable components of agroecosystems due to the many ecosystem services they provide and the many field operations that disturb the soil environment. This chapter investigates the effect of cover crop mixtures preceding corn, the use of tillage to terminate and incorporate cover crop residue, and the use of interseeding to establish cover crops mid-season on soil arthropod communities, with a focus on predators. In addition to investigating effects of cropping system on activity density of predators, I also tested effects on diversity and composition of predator communities, as well as on the rate of predation. I found that spring cover crops, tillage, and interseeding of cover crops all affect predators, but these effects are often taxon-specific and mediated by indirect changes to available habitat and resources through changes in soil quality or surrounding weeds. Carabidae activity density, taxonomic richness, and Shannon diversity were greater in treatments with standing hairy vetch and triticale than with red clover and timothy. After tillage, there were more Staphylinidae and fewer Araneae, lower richness, and lower Shannon diversity in tilled than in no-till treatments. After interseeding cover crops into standing corn, Staphylinidae activity density differed by treatment, but it is unclear whether environmental conditions influenced this result. Despite differences in activity density of specific predator taxa associated with cropping system, predator richness, evenness, diversity, predation rate were unaffected. Predation rate appears to be influenced by ground cover throughout the season and by disturbance late in the season. These results indicate that the four cropping systems support similarly abundant and even predator communities, as well as potential biological control services.
Introduction

Within cropping systems, soil-dwelling arthropod communities can provide numerous benefits such as predation of invertebrate pests, consumption of weed seeds, and decomposition of plant residues and other organic materials (Losey and Vaughan 2006). They can also be a highly vulnerable component of agroecosystems due to the many components of agricultural production that target soil. With few cost-effective rescue treatments available to organic agronomic producers, there is heavy reliance on cultural and biological control of pests (USDA NASS 2015). Often, the cultural practices that kill pests are detrimental to natural enemies, however. Thus, understanding the effect of cultural practices that promote natural enemies and the ability to render control services is essential to ensuring productive and profitable field crop growth.

Particularly in organic production, the soil surface can be heavily disturbed through multiple tillage passes to incorporate manure and control weeds. Ammonium in manure can volatilize when it is applied to the soil surface, leading to economic losses and air pollution, making tillage important for mitigating these losses (Cavigelli et al. 2013). Additionally, the lack of herbicides renders weed management particularly challenging for organic producers (Cavigelli et al. 2013). Tillage, however, can have negative impacts on arthropods. Understanding how disturbance accumulates under different tillage regimes and the effects of disturbance for arthropod predators is essential to identifying practices that can conserve beneficial arthropods and the ecosystem services they may provide (Stinner and House 1990).

One strategy used to facilitate reductions in tillage in organic production is the use of winter cover crops, which are typically planted after cash crop harvest in the fall, grown
throughout the winter, and are then terminated in the spring before cash crops are sown. Species can be grown alone or in mixtures to achieve a wide range of benefits including reducing soil erosion, adding organic matter, and suppressing weed growth (Schipanski et al. 2014). They can also provide habitat to arthropods (Rivers et al. 2018). Very little research has evaluated differences in arthropod response to specific cover crop species or mixtures, alone or in mixtures. One study found differences in arthropod community composition between a hairy vetch and triticale mixture relative to cereal rye, but these are dramatically different in structure, biomass, and nutrient ratios (Rivers et al. 2018). However, even more closely related species can differ from one another in terms of biomass or nutrient content, so they could also translate to differences in arthropod conservation (Jabbour et al. 2016). Studying how similar cover crop species interact with the presence or absence of tillage to affect arthropods is important to maximize ecosystem services such as pest control and decomposition.

It is also important to consider individual arthropod taxa, as well as the overall community, because responses may differ by taxa. For example, carabid species may differ in how they respond to tillage depending on whether they are fall or spring breeders (Kosewksa et al. 2014). Araneae can differ in how much they benefit from vegetation, depending on whether or not they use webs to hunt (Caprio et al. 2015, Pfiffner and Luka 2003). Other common predator species, such as Opiliones and Staphylinidae have not been studied as often but may be less favored by no-till systems than Carabidae and Araneae (Tamburini et al. 2015).

In addition to understanding effects of crop management on natural enemies, it is also important to consider the impact on the ecosystem services they render. Studies have shown benefits to pest control of increased activity density and/or diversity of predators (Letourneau et al. 2009, Rivers 2016). This trend, however, does not always hold true. Generalist predators can
vary in the types of prey they consume and may not always consume pests. For example, common predators, such as Araneae, can feed heavily on pests such as aphids (Romero and Harwood 2010); however, a much greater proportion of their diet may consist of non-pest items, such as Collembola (Romero and Harwood 2010, Chapman et al. 2013). Intraguild predation is another factor that may limit how informative common predator populations are in terms of estimating predation potential of a cropping system. These could include direct consumption, which would reduce populations caught in traps, or indirect effects, e.g., through fear response, which would likely not appear in trapping data. Vertebrates may also contribute to intraguild predation, further complicating the implementation of conservation biological control, particularly when they contribute to a disservice by consuming natural enemies as well as pests (Tschumi et al. 2018). Thus, it is important to consider interactions within the community, in addition to estimating predation conducted by the predator community.

In this project I investigated the effect of winter cover crop mixtures, tillage regime, and the use of interseeded cover crops on soil-dwelling arthropod predator communities. I hypothesized that predator activity density and diversity would be 1) similar between two legume-grass cover crop bicultures preceding corn, as they have similar structural complexity, 2) have lower activity early in the season in systems experiencing spring tillage relative to systems no-till planted in the spring due to negative impacts of disturbance, and 3) be greater where interseeding of cover crops mid-season occurs, due to a more consistent supply of plant resources across the soil surface. I also hypothesized that predator communities would 1) differ in composition between two different mixtures of functionally similar cover crops, 2) between tilled and no-till treatments early in the season, and 3) between interseeded and non-interseeded treatments later in the season. Lastly, I hypothesized that predation rate would be 1) similar
between different standing cover crop mixtures, 2) greater in no-till planted corn than corn established after tillage shortly after corn planting, and 3) greater in interseeded treatments than in treatments without interseeding later in the season.

Methods

Site Description

Sampling was conducted over three years at the Russel E. Larson Agricultural Research Center at Rock Springs, PA within the Reduced-Tillage Organic Systems Experiment (hereafter, ROSE, for convenience; Barbercheck et al. 2014, Champagne 2017). The ROSE site is approximately 4 hectares, has been managed organically since 2011, and received organic certification in 2014 (NOP 2005). Soil at this site consists primarily of a Hagerstown silt loam (fine, mixed, semiaactive, mesic Typic Hapludalfs) with zones of Opequon-Hagerstown soil (clayey, mixed, active, mesic Lithic Hapludalfs) scattered within. This site is in Zone 6b of USDA Plant Hardiness Zones and has temperate humid climate with ~2146 mm of combined rainfall and snowfall precipitation per year. Annual temperature ranges between 5º and 25º C throughout the year.

Experimental Design

This experiment was conducted from 2015-2017. All sampling occurred in maize plots (Zea mays L.) in a three-year rotation with soybean (Glycine max (L.) Merr) and spelt (Triticum spelta L.). Winter cover crops were planted between the spelt and corn growing seasons, as well as between the corn and soybean growing seasons. Each of four cropping systems are replicated
four times with plots measuring 9 meters (30 ft) by 49 meters (160 ft) in a full-entry experiment with all crops present in all years.

Differences among cropping systems included cover crop species, their planting date and termination method, manure management, and tillage intensity and frequency. Short planting windows between cash crop harvest and reduction in temperatures can make establishing cover crops challenging in the fall, especially when harvesting corn for grain rather than silage (Curran et al. 2018). The cover crop treatments in this experiment were chosen to overcome this short planting window in some cases, as well as to improve cover crop benefits to soil, weed management, and arthropod communities.

In all four systems, corn variety ‘MC4050’ (Masters Choice, Anna, IL) was planted on 76-cm rows at 81,510 seeds ha\(^{-1}\) using a four-row John Deere 7200 planter equipped with Dawn Biologic ZRX residue managers, typically in late May or early June, depending on the cropping system (Table 3.1). In System 1, liquid dairy manure was broadcast at 74,831 L ha\(^{-1}\) and incorporated via moldboard plow following spelt harvest. A winter cover crop mixture of hairy vetch (\textit{Vicia villosa} Roth ‘Groff’) plus triticale (\textit{x Triticosecale} Wittm. ‘815’) was planted at a seeding rate of 34 kg ha\(^{-1}\) each. Cover crops were terminated by rolling with a roller-crimper in the spring to form a mulch on the soil surface into which corn was no-till planted immediately after. The timing of rolling occurred at Mischler 7 growth stage to optimize weed suppression (Mischler et al. 2010). A second rolling pass a week after planting, as well as two passes with a John Deere 886 high-residue cultivator at four and five weeks after planting were employed to ensure cover crop termination and enhance weed control.

In System 2, no manure was applied in the fall and a chisel plow was used after spelt harvest to prepare the soil for cover crop planting. Hairy vetch and triticale were planted at the
same rates as in System 1. In Systems 3 and 4, a cover crop mixture of medium red clover
(*Trifolium pratense* L. ‘VNS’) and timothy (*Phleum pratense* L. ‘VNS’) was no-till drilled into
standing spelt at 12 and 4.5 kg ha\(^{-1}\), respectively in early spring. This allowed establishment prior
to spelt harvest, added economic benefit through a fall forage cutting, suppressed weeds, and
allowed a longer growing season and period without tillage than the hairy vetch and triticale
mixture in the other two systems. After spelt was harvested in early August and the cover crop
was able to grow, the cover crop was mowed and prepared for use as forage and to prevent weed
seed production. After continued growth through the fall and winter, this mixture and the hairy
vetch and triticale in System 2 were mowed and incorporated using a moldboard plow. For
fertility needs of the corn, manure was broadcast at 74,831 L ha\(^{-1}\) prior to plowing. Corn was
planted 7-10 days following tillage. A mix of blind cultivation and inter-row cultivation was
used in these systems to control weeds early in the season. Although the frequency varied from
year to year depending on weed severity and environmental conditions, all three tilled systems
received the same treatment as each other in a given year.

In Systems 2 and 4, cover crops were drill interseeded between corn rows using a 3-row
high clearance no-till drill (Interseeder Technologies, Woodward, PA) in early July at the V6
stage of corn to allow harvest of corn for grain in late fall without compromising cover crop
establishment. A three-species mixture of annual ryegrass (*Lolium perenne* L. ssp. *multiflorum*
(Lam.) Husnot) ‘KB Supreme’, orchardgrass (*Dactylis glomerata* L.) ‘Potomac’, and forage
radish (*Raphanus sativus* L.) ‘Tillage’ was drill-planted at 11, 11, and 3.4 kg ha\(^{-1}\), respectively.
In Systems 1 and 3, corn was harvested for silage production in September using a plot-scale
silage harvester, and cover crops were seeded afterward. In Systems 2 and 4, corn grain was
harvested using a small plot combine. For specific dates of planting and termination of cover
crops and corn, see Table 3.1. Comparison of these four cropping systems allows us to assess effects of the species and management of preceding cover crops, timing of tillage, and mid-season interseeding of cover crops on the composition of soil-dwelling arthropod communities.

**Arthropod Sampling**

To characterize soil-dwelling arthropod communities, I placed two pitfall traps consisting of a plastic container measuring 129 mm deep and 114 mm wide buried level with the soil surface in each treatment plot (Catalog # 89009-668, VWR, Radnor, PA). I placed these traps 3 crop rows, or 2.3 m, from each edge of the plot. I placed one 118-mL specimen cup (Catalog # 15704-092, VWR, Radnor, PA) filled with 40 mL ethylene glycol (50% ethylene glycol, 50% diethylene glycol, Prestone Products Corporation, Lake Forest, IL) in each specimen cups as a killing agent. To improve capture of arthropods entering the trap, I created a funnel by cutting the top off of a 2-liter soft drink bottle and inverted above each specimen cup. Additionally, a 150 mm by 15 mm petri dish (Catalog # 25373-187, VWR, Radnor, PA) propped up by three attached bolts (Catalog # 812626, The Hillman Group, Cincinnati, OH) was placed above the trap to shield the trap from rain. Traps were left open for 72 hours starting on 8 May 2015, 22 June 2015, 6 May 2016, 27 June 2016, 19 August 2016, 8 May 2017, 7 July 2017, and 25 August 2017. After trapping, I returned the pitfall samples to the laboratory, removed all arthropods from the ethylene glycol mixture, preserved them in 80% ethanol, and counted and identified them. I identified each arthropod to family in the following groups, as these groups were most likely to contain different feeding guilds within an insect order: Cantharidae, Carabidae, Coccinellidae, Elateridae, Histeridae, Staphylinidae (Coleoptera); Geocoridae, Nabidae, Reduviidae (Hemiptera); Formicidae (Hymenoptera); and Gryllidae (Orthoptera) (Johnson and
Diptera specimens were identified to suborder. All other groups were identified to order. I classified the dominant trophic group of each arthropod using literature descriptions, into the categories of predatory, or animal-feeders; herbivorous, plant-feeders; and decomposers, feeding primarily on decomposing plant matter and other detritus (Johnson and Triplehorn, 2004; Lundgren, 2009). Although the differences in level of taxonomic identification by group limits my ability to accurately measure richness or other diversity measures, it was sufficient to allow me to group arthropods by feeding guild which enabled me to focus on groups of interest driven by ecosystem services provided, especially predation. I collected taxa that could be considered omnivorous, or feeding on both animal and plant materials, including species within Gryllidae, Formicidae, and Carabidae (Lundgren 2009). However, I consider arthropods which may feed on weed seeds as predators because they could provide a beneficial ecosystem service to organic agriculture. I classified microarthropods as Collembola and mites (Acari) and macroarthropods as all other groups due to the different ecological roles and environmental sensitivities of these groups (Alvarez et al. 2001, Kladivko 2001, Weeks and McIntyre, 1997).

To measure predation rate, I attached five last-instar *Galleria mellonella* (Lepidoptera: Pyralidae) larvae to index cards to serve as sentinel prey (The Nature’s Way, Ross, OH). Baited index cards were surrounded by a 19 cm high by 14.5 cm diameter trap constructed from 19-gauge hardware cloth and covered with a 150 mm x 15 mm white plastic petri dish to allow entry by invertebrate predators but exclude vertebrates, such as mice, and rain and secured in place with a single carriage bolt (Rivers et al. 2016) (cloth: 840178, Lowe’s Companies, Inc., Mooresville, NC, lids: 25373-187, VWR, Radnor, PA, bolts: 812626, The Hillman Group, Cincinnati, OH). In 2015 and 2016, I placed two baited sentinel cards in each plot for 24 hours.
In 2017, I placed four baited cards instead of two. In all cases, I placed cards at least 3 crop rows, or 2.3 m, in from the edge and randomly placed lengthwise along the plot. Assays began on 7 May 2015, 25 June, 2015, 5 May 2016, 27 June 2016, 15 August 2016, 9 May 2017, 10 July 2017, and 24 August 2017. After 24 hours, I counted the number of damaged and undamaged larvae to determine the proportion of waxworms that were fed upon.

Environmental Conditions

I used several biotic and abiotic environmental variables from existing data sets affiliated with the ROSE project to help understand how each cropping system affected the habitat and to better understand environmental factors contributing to corn damage across the experimental site. At the time of early-season pest damage assessments, I estimated the corn population using two 5.3 meter (17.5 ft) transects and recorded the height and growth stage of three corn plants per transect. To understand the contribution of vegetation and residue to habitat and distribution of pests and damage, I used pre-termination above-ground cover crop biomass collected in all four systems from nine 0.25m² quadrats per treatment plot that were dried at 65°C, converted to kg ha⁻¹ dry weight, and averaged for each plot (Champagne 2017). Cover crop biomass was assessed 1 week prior to termination, resulting in System 1 being sampled 7-10 days later than in Systems 2 - 4. In System 1, this biomass contributed to residue at the soil surface while in Systems 2 - 4, the cover crop biomass was incorporated into the soil using a moldboard plow prior to corn planting, altering the soil environment of these systems early in the season. Weeds were identified to species (Champagne 2017), from which I calculated species richness and Smith-Wilson evenness of the weed communities in each plot using the Evar function in the Vegan package in R (Smith and Wilson, 1996). When cover crop termination was not complete,
cover crop re-growth (“volunteers”) was also assessed as part of the weed community. Weed biomass was measured in the field in mid-August, using nine 0.5m² quadrats placed randomly in each treatment plot, dried at 65°C, converted to kg ha⁻¹ dry weight, and averaged for each plot (Champagne 2017). At the same time, the density of the interseeded cover crop in Systems 2 and 4 was measured and the proportion of grass species and forage radish in the mixture was calculated.

I used soil characteristics measured in the early summer (17 June 2015, 28 June 2016, and 21 June 2017) and late summer (17 Aug 2015, 2 Sep 2016, and 1 Aug 2017) for each cropping system to understand the effect of soil characteristics on arthropods. In each treatment plot, 9 randomly distributed soil cores (2.5 x 15 cm) were collected, from which gravimetric moisture (Gardner 1986), matric potential (Hamblin 1981), pH, electrical conductivity, and permanganate oxidizable (‘active’) carbon (Weil et al. 2003) were measured. I also used the inorganic N reported as mg N per kg of dry soil, measured from the same soil dates (Isbell, unpublished). A portion of each composite soil sample was submitted to the Pennsylvania State University Agricultural Analytical Services Laboratory (AASL) for analysis of total organic matter by loss-on-ignition, soil textural class, phosphorus, potassium, magnesium, calcium, zinc, copper, sulfur, and cation exchange capacity (CEC). Additionally, 15 last-instar Galleria mellonella larvae were added to a 250 mL portion of each soil sample for 10 days, then assessed for infection from Metarhizium spp. (Hypocreales: Clavicipitaceae) infection, which is a soil-borne entomopathogen common to these cropping systems (Randhawa 2017).

To assess the influence of mechanical disturbance through tillage and other field operations on arthropod communities, I summed the number of disturbance events that occurred in each plot from the start of each experiment until the time of sampling, as well as within a
single season from January 1 until the time of sampling. I split these into above-ground events, such as mowing or planting, and soil events to represent the frequency of tillage events. Additionally, to represent the intensity of disturbance, I used a USDA Natural Resources Conservation Service soil disturbance rating (SDR) obtained through RUSLE2 (NRCS 2008). This measurement is a simpler version of the STIR index, which also accounts for erosion potential but requires speed and depth of each tillage event, which were not available to me through organic field records. The SDR, which ranges from 0 (least disturbance) to 30 (greatest disturbance) for a field operation, comprises the sum of six ratings each with values from 0 to 5 that estimate the relative severity of disturbance. The six component categories of the SDR include soil inversion, soil mixing, soil lifting, soil shattering, soil aeration, and soil compaction. The field operation that we employed with the highest SDR was tillage with a moldboard plow with an SDR of 29, and one of the lowest was flail mowing with an SDR of 3. As with frequency of events, these were summed in each plot from the start of each experiment until the time of sampling, as well as within a single season from January 1 until the time of sampling.

A previous study dealt with multiple passes of a similar tool by including it in a model that accounts for temperature and soil texture in calculating overall disturbance and turnover of the soil (Kemanian and Stöckle 2010). Although repeated passes of the same tillage implement may have redundant impacts on soil, little is known about whether the effect of multiple passes of the same tillage implement has a different effect on arthropods than one pass. Thus, I opted to use all reported passes to calculate a total intensity of disturbance for this usage of SDR and am relying on it as a means to compare each cropping system standardized by the weed management used in each year, rather than a true measure of disturbance.
**Data Analysis**

I analyzed all data in R Version 3.5.1 (R Core Team 2018), except where otherwise noted. Data from pitfall traps are reported as activity density to account for the fact that likelihood of capture will not be equal for all taxa, depending on their behavior (Rivers et al. 2018). I used plot totals in all analyses by summing the two traps in each plot for a given date. I calculated Smith-Wilson Evenness using the Evar function in the Vegan package (Arnott 2011) and Shannon Diversity using the diversity function in Vegan (Oksanen 2019). I used linear-mixed models (function lme) in the nlme package (Pinheiro et al. 2013) to assess the effect of cropping system on total predator activity density, sentinel predation rate, and the activity density of the four most common generalist predator taxa: Araneae, Opiliones, Carabidae, and Staphylinidae. I also used linear mixed models to assess the effect of cropping system on taxonomic richness, evenness, and diversity for the predator community. All count data were square root transformed where needed to meet assumptions of the model, including normal distribution and homogeneity of variance and sentinel predation rate was arcsine square root transformed to account for being proportion data (Gotelli and Ellison, 2004, Kutner et al., 2005). I analyzed sampling dates individually and specified a model using block nested within year as a random effect and cropping system as a fixed effect. I conducted post hoc, pairwise tests of means between each treatment level using Tukey’s honest significant difference test. I used the varcomp function in the ape package to partition the variance explained by each model by year and block.

To assess the overlap in community composition between each cropping system at each sampling date, I used non-metric dimensional scaling (NMDS) to plot dissimilarity of arthropod communities and overlaid these with ellipses representing the 95% confidence interval for each
cropping system in the Vegan package (Mazzia et al. 2015). Additionally, I ran pairwise permutation MANOVAs to assess differences in community composition among cropping systems using the adonis function in the RVAideMemoire package (Hervé 2019).

To assess relationships between environmental conditions and the predator community, I conducted redundancy analyses (RDA) in CANOCO for Windows version 5.0 (Lepš and Šmilauer 2014), using a forward-selection to reduce the number of environmental variables. I next conducted partial canonical correlation analyses (CCAs) that were constrained by cropping system with environmental variables included as supplemental variables. I conducted separate analyses for each sampling date within the season: pre-termination, post-emergence, and post-interseeding. In addition to taxa classified as predators, I also included activity densities of slugs, mites, and Collembola to represent prey availability in each system. The environmental variables in each analysis included standing crop characteristics (height, cash crop population), characteristics of the cover crop (biomass, proportion of legume), characteristics of the weed community (total biomass, species richness, species evenness), soil quality parameters, (nutrients, moisture, active carbon, cation exchange capacity, aggregate stability, and pH), and infection rate of sentinel prey by Metarhizium. RDA and CCA results are displayed graphically with bi-plot scaling focused on centered inter-taxon distances, where arthropod species with a fit to the model of at least 20% are represented as solid line vectors. Significant environmental variables were projected as vectors onto the bi-plots as passive supplementary response variables. To measure differences in arthropod activity densities in each cropping system across the dates in each year, I used principle response curves, which are a type of redundancy analysis (Lepš and Šmilauer 2014).
In addition to the univariate analysis of sentinel predation rate shown above, I conducted multiple regressions with environmental variables and with predator taxa to understand factors influencing predation rate. I included Araneae, Opiliones, Carabidae, Staphylinidae, Hymenoptera, and Formicidae as explanatory variables, as well as standing crop characteristics (height, cash crop population), characteristics of the cover crop (biomass, proportion of legume), characteristics of the weed community (total biomass, species richness, species evenness), soil quality parameters, (nutrients, moisture, active carbon, cation exchange capacity, aggregate stability, and pH), and infection rate of sentinel prey by *Metarhizium*. I used stepwise selection using the stepAIC function in the MASS package in R to reduce the complexity of the full models (Schmidt and Rypstra, 2010; Venables and Ripley, 2002). Models were compared by Akaike’s Information Criteria (AIC), with the final model having the lowest AIC (Kutner et al., 2005; Murtaugh, 2009). For significant environmental variables, I conducted individual linear regressions to allow plotting of the relationship between the factor and predation rate.
Table 3.1 Field operations and sampling dates for each experimental system in each year. Dates for interseeding of cover crops in Systems 2 and 4 are denoted by I. HV denotes hairy vetch and triticale and RC denotes red clover and timothy. NT denotes no-tillage planting, while T denotes tilled systems.

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<th>System 1 (HV-NT)</th>
<th>System 2 (HV-T-I)</th>
<th>System 3 (RC-T)</th>
<th>System 4 (RC-T-I)</th>
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<td>Plant Cov. Crop</td>
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<td>Pitfall trapping</td>
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Results

Activity Density of Macroarthropods

Across all three years of the experiment, I collected 10,407 macroarthropods and 129,606 microarthropods. Of the macroarthropods, the most common taxa were Araneae (caught in 93% of traps), Hymenoptera (92% of traps), Carabidae (91% of traps), and Staphylinidae (90% of traps), all of which I classified as predators. These four taxa also accounted for four of the five most abundant taxa caught by traps, representing 9%, 12%, 10%, and 12% of total macroarthropod trap catch. Overall, predators composed 52% of total trap catch, making predators the dominant trophic guild. Because foliar predators composed less than 1% of arthropods caught in pitfall traps, they were included in the overall analysis rather than analyzing them separately from soil predators.

Total predator activity density was not significantly affected by cropping system prior to cover crop termination (F$_{3, 33} = 1.12$, P = 0.36) or after corn emergence (F$_{3, 33} = 1.81$, P = 0.16) (Fig. 3.1A). After cover crops were interseeded into corn, there was significantly greater activity density in System 3, which was preceded by red clover and timothy, tilled following cover crop termination, and not interseeded than in either of the two systems preceded by hairy vetch and triticale (Sys 1 and Sys 2). Patterns in predator activity density were driven more by annual variation than spatial variation, with year explaining 82% of conditional variation in activity-density prior to cover crop termination and 60% of variation after corn emergence. Spatial variation was more prevalent after cover crops were interseeded, with block explaining 32% of the conditional variation in predator activity density at the third sampling date.
Diversity of Predatory Macroarthropods

Predator richness was significantly affected by cropping system throughout the season, though the effect differed by sampling date (Fig. 3.1B). There was significantly greater predator richness in the two systems preceded by hairy vetch and triticale (System 1 and System 2) than in System 4, which was preceded by red clover and timothy prior to cover crop termination ($F_{3, 33} = 4.21, P = 0.01$). After corn emergence, predator richness was significantly greater in the no-till system (System 1) than in two of the three tilled systems (System 2 and System 4) ($F_{3, 33} = 3.70, P = 0.02$). After cover crops were interseeded, predator richness was significantly greater in System 3, which was tilled but not interseeded after red clover and timothy termination, than either system preceded by hairy vetch and triticale (System 1 and System 2), regardless of later season management ($F_{3, 33} = 4.04, P = 0.02$). Predator evenness was not affected by cropping system at any of the three sampling dates (Fig. 3.1C). Shannon diversity of predators largely mirrored the effects of cropping system on richness, though there was no effect after interseeding occurred (Fig. 3.1D).

Specific generalist predator taxa were significantly affected by cropping system, though these effects differed by taxa. For Carabidae, activity density was significantly greater when averaged across all years in systems preceded by hairy vetch and triticale (Sys 1 and Sys 2) than systems preceded by red clover and timothy (Sys 3 and 4) prior to cover crop termination ($F_{3, 33} = 5.34, P = 0.004$; Fig 3.2A). For Araneae, I detected significantly greater activity density after corn had emerged in no-till planted corn (Sys 1) than in tilled corn (Sys 2-4) ($F_{3, 33} = 12.34, P < 0.001$; Fig. 3.2B) while Staphylinidae had the opposite response ($F_{3, 33} = 6.49, P = 0.001$; Fig. 3.2B). After cover crops were interseeded, Staphylinidae were significantly greater in System 3, which was tilled after red clover and timothy termination and not interseeded, than System 2,
which was tilled after hairy vetch and triticale termination and then interseeded (\(F_{3, 33} = 3.51, P = 0.03; \text{Fig. 3.2C})\).

**Community Composition of Predatory Arthropods**

The NMDS analysis comparing community composition among cropping systems indicated strong overlap among cropping systems at all three sampling dates (Appendix A3.1). Stress values were relatively high at 0.22 prior to cover crop termination, 0.25 after corn emergence, and 0.16 after interseeding cover crops, indicating a poor fit of the models’ ability to measure dissimilarity. The permutation MANOVAs prior to cover crop termination (\(F_{3, 44} = 4.46, P < 0.01\)) and after corn emergence (\(F_{3, 44} = 2.80, P = 0.01\)) indicated significant differences in community composition. Pairwise comparisons indicated significant differences in community composition prior to cover crop termination between System 1 (\(P = 0.01\)) and 2 (\(P = 0.02\)), which were both preceded by hairy vetch and triticale from System 4, which was preceded by red clover and timothy. I was unable to detect differences between individual systems using pairwise comparisons after corn emergence. After corn was interseeded, there was no significant difference in overall community composition among cropping systems (\(F_{3, 28} = 1.40, P = 0.19\)).

Using forward-selection RDA prior to cover crop termination, I observed strong relationships between environmental variables and the activity density of all predator groups, except for ants, which had a weaker relationship (Figure 3.3A). Hymenoptera and Carabidae demonstrated a strong relationship with weed richness and relationship with mite activity density. Araneae responded most strongly to Collembola activity density. Staphylinidae was related to the number of days since last disturbance event and to caterpillar prey. Opiliones
responded most strongly to soil characteristics, specifically N and pH. No predator in the system was strongly related to slugs, which are a potential source of prey and a key pest of corn.

The RDA model prior to termination explained 64.2% of variance in arthropod activity densities (Table 3.2). When constrained by cropping system through partial CCA, relationships between environmental variables and the majority of predators are no longer detected. The only taxa remaining are Coccinellidae, a foliar-dwelling predator, and caterpillars and slugs, both of which were included in the model as potential prey (Fig. 3.3B). Slugs and Coccinellidae were not strongly related to any environmental variable, whereas caterpillars were associated with the accumulated SDR over the rotation and weed richness. The partial CCA only explained 9.5% of variation prior to cover crop termination (Fig. 3.3B). Both axes were significant in the partial CCA (Axis 1: pseudo-F = 1.2, P < 0.001; Axis 2: pseudo-F = 2.5, P < 0.001).

After cover crops were terminated and corn had emerged, there were no positive associations between environmental variables and predator taxa (Fig. 3.4B). Araneae showed a strong negative response to SDR within the season, days since planting, and corn height. Formicidae showed a weak positive relationship with these same factors. Staphylinidae were negatively associated with slugs. The RDA model after corn emergence explained 57.2% of variance in arthropod activity densities (Table 3.2). When constrained by cropping system, few relationships between taxa and environmental variables were detected, though slugs seemed to be associated with System 1 (Fig. 3.4B). The partial CCA explained 16.4% of variation after corn emergence. Both axes were significant in the partial CCA (Axis 1: pseudo-F = 3.3, P < 0.001; Axis 2: pseudo-F = 3.9, P < 0.001).
After cover crops were interseeded, Carabidae and Hymenoptera share a strong relationship and respond weakly to SDR within the season (Fig. 3.5A). Opiliones were negatively related to slugs, which are a potential prey source. Neither Araneae nor Staphylinidae remain in the RDA model, using the 20% fit threshold for inclusion. The RDA model after interseeding explained 42.4% of variance in arthropod activity densities (Table 3.2). When constrained by cropping system through partial CCA, there is a positive relationship between phosphorus and Systems 2 and 4. There is also a negative relationship between SDR within the season and System 1, which makes sense given the lower amount of tillage in this cropping system. The partial CCA explains only 3.4% of variance and neither axis is significant, indicating that treatments do not significantly affect taxa at this sampling date (Axis 1: pseudo-F = 0.9, P = 1; Axis 2: pseudo-F = 1.3, P = 1).

Based on the principle response curves, the arthropod communities in System 1 differ from Systems 2, 3, and 4 (Fig. 3.6). The differences increase after corn emergence and after interseeding. Few taxa were strongly influenced by cropping system. However, slugs are more strongly associated with System 1 compared with Systems 2, 3, and 4 and Formicidae were less strongly associated with System 1 compared with Systems 2, 3, and 4.

**Predation and the Role of Generalist Predators**

There was no significant effect of cropping system on sentinel predation rate prior to cover crop termination, after corn emergence, or after interseeding occurred (Fig. 3.7). Relationships between common predator taxa and predation rate were consistently weak. Prior to cover crop termination, the regression between environmental conditions and predation rate was reduced to only contain spring cover crop biomass, which explained 35% of variation in
predation rate ($F_{46,47} = 26.56, P < 0.001$; Fig. 3.8). After corn emergence, the regression model was reduced to spring weed biomass, which explained 40% of variance ($F_{46,47} = 32.37, P < 0.001$; Fig. 3.9). After interseeding, the regression model contained three environmental variables which had positive relationships with days since last disturbance ($F_1 = 28.93, P < 0.001$) and interseeded cover crop biomass ($F_1 = 12.69, P = 0.001$) and a negative relationship with SDR within the season ($F_1 = 59.62, P < 0.001$). The model explained 66% of variation in predation rate ($F_{3,28} = 20.89, P < 0.001$). Although SDR within the season ($F_{2,29} = 7.90, P = 0.001$; Fig. 3.10A) and days since last disturbance ($F_{2,29} = 13.53, P < 0.001$; Fig. 3.10 B) are also significant on their own, interseeded cover crop biomass is not ($F_{1,30} = 0.004, P = 0.94$; Fig. 3.10C).
Figure 3.1. Activity density and diversity metrics of arthropod predator community caught prior to cover crop termination. The total activity density (A), taxonomic richness (B), Smith-Wilson evenness, and Shannon diversity of predators are shown. Letters within plots represent cropping systems that significantly differed at the 0.05 level for that sampling date. Purple points represent systems preceded by hairy vetch and triticale (Sys 1 and Sys 2), red points represent systems preceded by red clover and timothy (Sys 3 and Sys 4), the diamond point is the no-till planted system (Sys 1), and the two triangle points represent systems where cover crops were interseeded (Sys 2 and Sys 4).
Figure 3.2. The activity density per plot of the four most common generalist predator taxa are shown prior to cover crop termination (A), after corn emergence (B), and after cover crops were
interseeded (C). Systems shown in purple were preceded by hairy vetch and triticale while red was preceded by red clover and timothy. Cross-hatched bars represent the no-till planting in System 1 while the diagonal bars represent interseeding in Systems 2 and 4. Letters represent means that are significantly different at the 0.05 level for that taxa. Arthropod icons are credited to Maxime Dahirel, Garreth Monger, and T. Michael Keesey via phylopic.org, and to Nicholas Sloff.
Figure 3.3. Multivariate relationships between taxa and environmental variables prior to cover crop termination. The biplot resulting from interactive forward selection RDA represents relationships between predator and prey taxa with model fit > 20%, shown in black italic text, and environmental factors identified as significant at 0.05 level of significance in a forward-selection RDA, shown in red bold text (A). The partial CCA constrained by cropping system shows taxa, shown in black italic text, and environmental variables, shown in blue text, that show significant relationships with cropping system, shown in bold red text (B). Systems 1 and 2 represent systems with hairy vetch and triticale while Systems 3 and 4 represent red clover and timothy.
Figure 3.4. Multivariate relationships between taxa and environmental variables after cover crops are terminated and corn has emerged. The biplot resulting from interactive forward selection RDA represents relationships between predator and prey taxa with model fit > 20%, shown in black italic text, and environmental factors identified as significant at 0.05 level of significance in a forward-selection multiple regression, shown in red bold text (A). The partial CCA constrained by cropping system shows taxa, shown in black italic text, and environmental variables, shown in blue text, that show significant relationships with cropping system, shown in bold red text (B). System 1 represents no-till planted corn in rolled cover crop residue while cover crops in Systems 2-4 were terminated and incorporated by tillage prior to corn planting.
Figure 3.5. Multivariate relationships between taxa and environmental variables after cover crop interseeding has occurred. The biplot resulting from interactive forward selection RDA represents relationships between predator and prey taxa with model fit > 20%, shown in black italic text, and environmental factors identified as significant at 0.05 level of significance in a forward-selection multiple regression, shown in red bold text (A). The partial CCA constrained by cropping system shows taxa, shown in black italic text, and environmental variables, shown in blue text, that show significant relationships with cropping system, shown in bold red text (B). Systems 2 and 4 have cover crops established by interseeding, while Systems 1 and 3 have not been interseeded.
Table 3.2. Summary statistics for forward-selection RDA models of predator taxa and significant environmental variables.

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Significance codes: * < 0.05, ** < 0.01, *** < 0.001, --- indicates factors that were not included in the regression model.
Figure 3.6. Principal response curve of arthropod activity density in Systems 2-4, relative to System 1. Negative values indicate arthropods that are less abundant in System 1 than the other systems, while positive values indicate arthropods that are more abundant in System 1 than the other three systems.
Figure 3.7. The proportion of waxworms eaten at each sampling date. Purple points represent systems preceded by hairy vetch and triticale (Sys 1 and Sys 2), red points represent systems preceded by red clover and timothy (Sys 3 and Sys 4), the diamond point is the system planted no-till (Sys 1), and the two triangle points represent systems where cover crops were interseeded (Sys 2 and Sys 4).
Figure 3.8. Linear relationship between sentinel predation rate and spring cover crop biomass prior to cover crop termination. The shaded area represents a 95% confidence interval of the regression.

Figure 3.9. Linear relationship between sentinel predation rate and spring weed biomass after corn emergence. The shaded area represents a 95% confidence interval of the regression.
Figure 3.10. Relationships between sentinel predation rate and significant environmental variables after interseeding. The bivariate relationship between SDR within the season and predation rate (A), bivariate relationship between days since last disturbance and predation (B), and the linear relationship between interseeded cover crop biomass and predation rate (C) are shown. Shaded areas represent 95% confidence intervals of the regression.


Discussion

Including cover crops in crop rotations can provide numerous services to growers, including reduced erosion and regulation of soil moisture, by taking up water as they grow and protecting the soil surface from evaporation once killed (Murrell et al. 2017, Schipanski et al. 2017). Additionally, cover crops can facilitate reduced-tillage crop production through weed suppression and nutrient provisioning (Schipanski et al. 2017, Wallace et al. 2018). Including cover crops and reducing tillage may also support more abundant and diverse communities of arthropods that can provide enhanced ecosystem services, such as biological control (Fernandez et al. 2008, Hatten et al. 2007, Rivers 2016, Shearin et al. 2007). I hypothesized that activity density of predators and predation rate would be similar between two functionally similar cover crop mixtures, greater in no-till planted corn than in tilled corn, and greater in systems that used cover crops interseeded into V6 corn compared to those that did not have interseeded cover crops. However, I expected that community composition would differ among cropping systems, even when activity densities were similar, which may translate to differences in predator diversity among cropping systems throughout the season.

As hypothesized, I detected no difference in total predator activity density prior to cover crop termination. However, Carabidae activity density was greater in systems preceded by hairy vetch and triticale, indicating a taxon-specific response to crop management. Carabidae activity density was greater in corn following hairy vetch and triticale than in soy following cereal rye in a previous study at this site (Rivers 2016). Though my study featured a legume-grass mixture
rather than a grass as a second cover crop, the hairy vetch and triticale treatment may have still been able to provide more resources needed by carabids (Rivers et al. 2018).

Most predator taxa collected prior to cover crop termination were influenced by environmental conditions, as observed in the RDA model. Araneae responded most positively to availability of Collembola. Collembola have been documented as a major component of spider diets (Romero and Harwood 2010, Chapman et al. 2013), so this relationship is unsurprising. Carabids responded most positively to weed richness and mite activity densities. In addition to feeding on an array of invertebrate prey, carabids can be seed predators (Blubaugh and Kaplan 2016, Blubaugh et al. 2016), and greater weed richness may have provided a greater diversity of food for these beetles. The positive relationship of Hymenoptera with Carabidae, both early in the season and late in the season, may indicate intraguild predation as some Hymenoptera species in this system have been identified as parasitoids of Carabidae (István Mikó, pers. comm.). The response of Staphylinidae to increasing time since last disturbance was surprising because multiple studies have indicated no response or greater activity density of Staphylinidae in disturbed systems relative to lower-disturbance agricultural systems (Eyre and Leifert 2011, Jabbour et al. 2016, Tamburini et al. 2015). However, the opposite has also been demonstrated in an experiment studying strip tillage in sugar beets, where Staphylinidae was more abundant in strip tilled plots than conventionally tilled plots (Pretorius et al. 2018). Opiliones responded positively to pH and nitrogen in the soil, which may indicate a response to higher quality prey and other food resources in these systems. A previous study found that complex diets rich in amino acids and lipids positively impact Opiliones fitness, relative to feeding on sugar-rich aphids (Allard and Yeargan 2005). As response of predators to environmental conditions was taxon-specific prior to cover crop termination, there does not appear to be a clear benefit of
choosing one cover crop bicululture over the other for conserving predator populations in terms of conserving particular predator groups.

In addition to cover crop mixtures, the method used to terminate cover crops and the corresponding disturbance intensity (SDR) may affect arthropods. I compared termination with a roller-crimping followed by no-till planting of corn into the rolled cover crop to mowing and incorporating residue using tillage prior to planting, which resulted in substantially different SDR within the early season, ranging from 9 in the no-till system in each year to between 132-215 in each of the three tilled systems, depending on year. My hypothesis of greater activity density in no-till plots than in tilled plots was not supported. This may have been driven by taxon-specific responses to tillage. Whereas activity density of Araneae was lower in tilled than in no-till systems, Staphylinidae responded positively to disturbance. Thus, the combined activity density of these two taxa and others may have contributed to the equivalent activity densities observed among systems. Araneae can benefit strongly from residue to use as habitat or as sources of prey, which was likely more available in no-till compared with tilled treatments (Caprio et al. 2015, Pffifner and Luka 2003, Thorbek and Bilde 2004). Araneae activity density was lower and Staphylinidae activity density was greater in full-tillage systems than in reduced-tillage during transition to organic management (Jabbour et al. 2016). The response of Staphylinidae to tillage aligns with other research showing they preferred open space with less vegetation in comparisons of conventional and organic agriculture (Eyre and Leifert 2011, Tamburini et al. 2015). These effects of tillage can be long-lasting, as evidenced by the continued separation of Systems 2-4, which were tilled, from the no-till conditions in System 1 at both later planting dates in the principal response curves.
In the interactive forward selection RDA model, Araneae showed a strong negative response to SDR, corn height, and days since planting, which confirms the results of lower activity density in tilled plots which were planted earlier and had larger plants at the time of sampling than did no-till plots. Formicidae had an opposite response to these conditions. Formicidae activity density was greater in tilled, low-residue systems relative to reduced-tillage high-residue systems in a study comparing a conservation agriculture feed grain system to a conventional tilled system (Rivers et. al. 2016). Staphylinidae, a known predator of slugs (Douglas and Tooker 2012), showed a strong negative response to slugs, which may indicate that staphylinids were contributing to predation of slugs after tillage occurred. However, it could instead indicate that slugs and staphylinids preferred differing conditions, as staphylinids have previously been shown to have greater activity density in tilled systems (Jabbour et al. 2016) whereas slugs tend to be more abundant in systems with high moisture and abundant residue (Douglas and Tooker 2012).

After interseeding, activity density of Staphylinidae was lower in one of the interseeded systems relative to one of the systems with no interseeded cover crops. The other two systems experienced intermediate Staphylinidae activity density. This result indicates a slight trend towards lower activity density in interseeded plots than those where cover crops were not interseeded. As with conditions earlier in the season, this may indicate a preference by Staphylinidae for more open areas of bare soil (Eyre and Leifert 2011). However, Staphylinidae did not appear to respond to any of the environmental variables included in the final multivariate models and was excluded from the models for having less than 20% fit. Thus, factors driving the decrease in activity density of Staphylinidae in interseeded plots relative to treatments in which no cover crops were interseeded require further research. Carabids and Hymenoptera had a weak
positive relationship with the SDR accumulated through the season, which may be driven by the particular carabid species present. The last sampling date was dominated by large species, such as *Harpalus pensylvanicus* and *Pterostichus melanarius* whereas carabid species of a wider range of sizes occurred earlier in the season (unpublished data). Large carabid species tend to be less vulnerable to tillage than medium-sized species (Thorbek and Bilde 2004). Opiliones, which can be a predator of slugs (Douglas and Tooker 2012), were negatively related with slugs. Thus, they may have been contributing to natural control of slugs at this point in the season. In this area, slugs of various species are present and feeding throughout the season, though they spend more time hiding late in the season as daytime temperatures rise (Douglas and Tooker 2012).

In addition to the activity density of predators, it is important to consider the diversity of their communities when evaluating ecosystem services (Letourneau et al. 2012). Cover crops can support diversity and activity of predator communities (Fernandez et al. 2008). I observed lower taxonomic richness in samples from standing cover crops than in post-termination samples, which corresponded to lower activity density. We terminated cover crops in the spring prior to planting corn, which is likely earlier in the season than peak populations for many arthropods found in agricultural fields (Kosewska et al. 2014, Welch et al. 2011). Cover crops can provide resources early in the season and late in the season to arthropods when in-crop resources are absent (Rivers et al. 2018). However, both cover crop mixtures included a legume and a grass, so resources provided by each biculture may have been similar. In addition to resources provided directly by cover crops, their presence and management can change soil characteristics and weed communities, which has can have cascading effects on habitat and prey (Fernandez et al. 2008).

I measured greater taxonomic richness and Shannon diversity of predator communities in no-till compared with in tilled treatments, indicating negative effects of tillage for diversity of
predator communities, even though activity density was unchanged. This may indicate that communities in tilled systems are dominated by disturbance-tolerant taxa, such as Staphylinidae. However, there were no differences in evenness among no-till and tilled systems. Richness of predator communities was greater in interseeded systems than in systems where cover crops were not interseeded, which corresponded to the greater activity density measured in these systems. Although interseeded cover crops remain small throughout the corn-growing season, they may still provide resources through added vegetation that would otherwise be missing in these systems. It should be noted that this study focused on soil-surface arthropod activity after interseeding, rather than shifting sampling efforts into the plant canopy, so there may have been additional changes aboveground that I was unable to capture through my sampling methods.

Increases in activity density of one taxon may be able to compensate for decreases in other taxa in terms of natural control as long the remaining predator community is sufficiently diverse and abundant (Letourneau et al. 2009, Lundgren and Fergen 2014, Nyffeler and Sunderland 2003). This appears to be the case in my study, as well. There were no significant differences in predation rate at any of the three sampling dates, despite changes in activity density of common generalist predators, total activity density, and composition and diversity of predator communities throughout the season. Crowder et al. (2010) found that predator evenness is the best predictor of biological control. In my experiment, evenness was unchanged among cropping systems at all dates, which may correspond to the lack of effect of diversity and activity density on predation rate.

Ground cover appears to be an important factor regulating predation rate in these cropping systems, as cover crop biomass prior to termination, weed biomass after corn emergence, and interseeded cover crop biomass after interseeding all emerged as significant
factors for explaining variation in predation rate in multiple regressions. With the exception of the third sampling date, which occurred after interseeding, no other environmental variable was significant, further highlighting the importance of ground cover. Ground cover has been demonstrated as an important environmental factor promoting predation in previous studies (Lundgren and Fergen 2011, Prasifka et al. 2006). After interseeding occurred, disturbance also affected predation rate, with both SDR accumulated within the season and time since last disturbance associated with reductions in predation rate. Disturbance from tillage can be an important driver of predator community structure and activity (Holland and Reynolds 2003, Kosewska et al. 2014, Thorbek and Bilde 2004), which can then influence predation services by arthropods (Brust and House 1988, Cromar et al. 1999, Rivers et al. 2016, Tamburini et al. 2015). Relationships between specific predator taxa and predation rate were consistently weak, indicating that the community is important for achieving natural biological control. It is also possible that intraguild predation played a role in these relationships, where more diverse and active predator communities resulted in lower activity densities of specific predators vulnerable to competition or predation (Muller and Brodeur 2002, Rosenheim et al. 1995).

Overall, the four organic cropping systems I evaluated in this study were equivalent in their ability to conserve predators and predation of sentinel caterpillars. This study is one of few that evaluate specific differences in management within established organic systems, instead of comparing organic management to conventional management (Caprio et al. 2015, Eyre and Leifert 2011, Fusaro et al. 2016, Kirchmann et al. 2016) or tracking arthropod response during the transition to organic management (Rivers 2016, Jabbour et al. 2016). However, another study evaluating different border strips in organic systems also found no effect on arthropod activity density, concluding that organic management provided enough habitat complexity and resources
to support arthropods regardless of management nuances (Fox et al. 2015). Although carabid activity density, taxonomic richness and Shannon diversity were greater in hairy vetch and triticale in my study, these differences did not extend into the following corn crop and did not translate to differences in predation rate. Instead, there was greater richness in treatments preceded by red clover and timothy later in the season. Although communities in tilled differed than those in no-till systems and had lower richness, Shannon diversity, and Araneae activity density, there were no carry-over effects of cover cropping into the following corn crop and species composition of the cover crop biculture did not affect predation rate. Interseeding into V6 corn supported greater activity density of Staphylinidae and total predator activity density but did not result in differences in predation. Thus, my results indicate that organic growers can support predator communities which differ according to their tillage and winter cover cropping practices as a result of these management changes without changing ecosystem services, such as predation.

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https://fargo.nserl.purdue.edu/rusle2_dataweb/RUSLE2_Index.htm


Abstract

Invertebrate agricultural pests exhibit an array of responses to management practices and can present significant challenges for producers. Within organic production, where the use of synthetic insecticides is not allowed, producers rely on tillage, crop rotation, and other cultural practices to manage pests. I investigated the prevalence of damage to corn from multiple pests, including slugs (Gastropoda: Mollusca), European corn borer (*Ostrinia nubilalis* Hübner), corn earworm (*Helicoverpa zea* Boddie), and fall armyworm (*Spodoptera frugiperda* Smith), early and late in the growing season in four cropping systems varying in tillage frequency and intensity and winter cover crop species in organic corn. Specific management tactics included two cover crop bicultures preceding corn, the use of roller-crimping or tillage to terminate cover crops preceding corn, and the use of interseeded cover crops after corn establishment. Prevalence of early season damage was often widespread but severity of damage was low and not related to corn yield. The use of tillage to terminate cover crops reduced the number of corn plants affected by chewing pests early in the season. Cropping system did not affect the numbers of late-season caterpillar pests or corn yield. Natural enemies including spiders (Arachnidae: Araneae) and predation by natural enemies appeared to effectively maintain damage from chewing pests below yield-damaging levels. These results support the inclusion of winter and interseeded cover crops in organic agronomic crop rotations to gain environmental benefits while not increasing risks to damage from insect pests.
Introduction

Yield is a critical factor influencing the profitability of agronomic crop production, and therefore, yield-limiting factors are critical to consider. Weeds and soil fertility management are persistent and challenging yield-limiting factors in organic systems (Cavigelli et al. 2013) and organic yields are consistently lower than conventional yields (Kravchenko et al. 2017, Ponisio et al. 2014, Youngberg and DeMuth 2013). Insect pests also can have detrimental effects on yield and profitability in organic cropping systems (Losey and Vaughan 2006, Mischler et al. 2010, Savary et al. 2019).

In conventional production systems, key pests of corn, including corn earworm, *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae), European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae), and fall armyworm, *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae), are usually managed with insecticidal transgenic varieties, modified to express *Bt* toxins to control feeding by lepidopteran pests (Bohnenblust et al. 2013, Bohnenblust et al. 2014, Lundgren et al. 2015). Because transgenic varieties and use of synthetic insecticides are prohibited in organic production systems, and the costs of allowable pest control materials are relatively high, organic agronomic crop producers largely rely on integrating non-chemical tactics such as cultural and biological control practices (NOP 2015, USDA NASS 2015). Crop rotation, which is required in organic production systems (NOP 2005) can contribute to interrupting pest lifecycles and is one example of a cultural practice critical for an effective integrated pest management program (Prasifka et al., 2006, Rivers et al. 2016b, Thierfelder and Wall, 2010).
Tillage is another commonly used cultural practice that can influence pest populations, both directly through disruption of pest life cycles or indirectly through enhanced conservation biological control (Gurr et al. 2017). For many early-season pests, including slugs (Gastropoda: Mollusca) (Douglas and Tooker 2012), true armyworm, *Pseudaletia unipuncta* Haworth (Lepidoptera: Noctuidae) (Tooker and Calvin 2009), *O. nubilalis* (Capinera 2017), and black cutworm, *Agrotis ipsilon* Hufnagel (Lepidoptera: Noctuidae) (Stinner and House 1990), tillage can be an effective management tool. Slugs have been a primary concern among conventional corn and soybean growers in the Mid-Atlantic US as no-till production has been increasingly adopted, though true armyworm and black cutworm damage have been problematic in no-till systems (Mischler et al. 2010, Myer 2018). Thus, organic feed grain producers may face tradeoffs in pest management as they reduce tillage, needing to conserve enough predators to prevent damaging pest populations, while also potentially facing increased pest populations to control (House and Alzugaray 1989, Willson and Eisley 1992). Much research on pests of corn, both early and late in the season, have focused on conventional systems (Archer et al. 2001, Bohnenblust et al. 2013, Bohnenblust et al. 2014, Hutchison et al. 2010, Lundgren et al. 2015, Walter et al. 2018). Less is known about organic systems, where predator populations may be higher and natural biological control potentially more effective.

Cover crops, usually grown between cash crops over the winter to conserve soil and regulate soil fertility, can have similarly confounding effects as tillage on pest management. Adding cover crops to a rotation can provide numerous benefits to growers but also challenges. The addition of resources and refugia through increased structural complexity can benefit both herbivores and predators (Landis 2000, Rivers 2016a, Rivers et al. 2016b). Because synthetic herbicides are not allowed in organic production, other practices are used to terminate cover
crops before planting the cash crop. Winter cover crops in organic systems are often terminated before they flower or during flowering to prevent them from becoming weedy, which could limit their benefits to natural enemies (Keene 2017).

One approach to managing winter cover crops in organic systems is to terminate them with a roller-crimper, which leaves a thick mat of mulch on the soil surface. Cover crops may need to be rolled multiple times to effectively terminate them, increasing above-ground disturbance (Keene et al. 2017, Wallace et al. 2017). For growers in northern temperate climates, an additional challenge to including winter cover crops in an annual feed grain rotation are short fall planting windows after cash crops are harvested (Curran et al. 2018). Agronomists and growers have pursued interseeding cover crops into standing cash crop rows to overcome fall planting limitations and to help manage weeds through competition in the cash crop (Curran et al. 2018). Interseeding may have benefits for predator populations through increased vegetation and plant diversity (Bickerton and Hamilton 2012, Nyffeler 2003). An increase in plant diversity through interseeding may limit the ability of pests to locate host plants (Hummel et al. 2009, Grettenberger and Tooker 2015). It is unclear how strong the impacts of interseeding may be for late-season pests, as timing of establishment of the interseeded cover crop is chosen to balance successful cover crop germination and establishment and minimize competition with the cash crop rather than for benefits to pest management. The optimal timing to interseed cover crops into corn is between V4 and V6, resulting in low, non-competitive growth of the cover crop until the cash crop is removed (Curran et al. 2018).

Organic systems tend to face weed management challenges throughout the growing season (Wallace et al. 2017). This is particularly true for reduced-tillage systems as tillage has been an important tool for managing weeds for over ten thousand years (Aref and Pike 1998, Lal
et al. 2007). Weediness can influence the occurrence and abundance of arthropod pests, such as *O. nubilalis*, whose adults rest on grassy vegetation during the day (Bohnenblust et al. 2014). Moist, high residue environments created by reduced-tillage systems, though greater weed populations or through cover crops, can experience increased damage from early pests, such as *A. ipsilon* and slugs (Douglas and Tooker 2012, Mischler et al. 2010, Myer 2018).

Annual feed grain crops in organic systems are typically planted later than in conventional systems to avoid damage from early season pests and weeds (Hammond 1990, Schmidt et al. 2017). This practice may elevate the risk of damage from second generation *O. nubilalis*, which preferentially oviposits on late-planted corn and may cause more damage to corn that develops later in the season (Obopile and Hammond 2013, Ordas et al. 2013). Additionally, late planting may render corn more attractive and vulnerable to *H. zea* and *S. frugiperda* as populations migrate north into the Mid-Atlantic US later in the season (Changnon et al. 2010, Diffenbaugh et al. 2008, Morey et al. 2012, Nagoshi et al. 2009). The later planting of sweet corn relative to field corn has been suggested as one reason that damage from *H. zea* and *S. frugiperda* is more severe for sweet corn (Buntin 2008, Johnson et al. 1975, Xinzhi et al. 2007). The caterpillars of *H. zea*, *O. nubilalis*, and *S. frugiperda* have broad host ranges, but prefer corn (Burkness et al. 2010, Chitkowski et al. 2003, Nagoshi et al. 2009, Molina-Ochoa et al. 2010, Rutschky 1950, Westbrook and Lopez 2010). Thus, populations and damage from these pests in organic corn are important to evaluate.

This study aims to assess the combined effects of winter cover crop species, tillage, and interseeded cover crops on damage from early- and late-season invertebrate pests. I hypothesized that 1) early season pest damage will be similar between two functionally similar cover crop bicultures, 2) pests will be more abundant in no-till planted corn than corn established and
managed with tillage, and 3) damage from late-season pests, such as *Ostrinia nubilalis*, *Spodoptera frugiperda*, and *Helicoverpa zea*, will be similar between plots that have been interseeded with a cover crop mixture and those that have not.

**Methods**

*Site Description*

Sampling was conducted over three years at the Russell E. Larson Agricultural Research Center at Rock Springs, PA within the Reduced-Tillage Organic Systems Experiment (hereafter, ROSE, for convenience; Barbercheck et al. 2014, Champagne 2017). The ROSE site is approximately 4 hectares, has been managed organically since 2011, and received organic certification in 2014 (NOP 2005). Soil at this site consists primarily of a Hagerstown silt loam (fine, mixed, semiactive, mesic Typic Hapludalfs) with zones of Opequon-Hagerstown soil (clayey, mixed, active, mesic Lithic Hapludalfs) scattered within. This site is in Zone 6b of USDA Plant Hardiness Zones and has temperate humid climate with ~2146 mm of combined rainfall and snowfall precipitation per year. Annual temperature ranges between 5º and 25º C throughout the year.

*Experimental Design*

This experiment was conducted from 2015-2017. All sampling occurred in corn plots (*Zea mays* L.) in a three-year rotation with soybean (*Glycine max* (L.) Merr) and spelt (*Triticum spelta* L.). Winter cover crops were planted between the spelt and corn growing seasons, as well as between the corn and soybean growing seasons. Each of four cropping systems are replicated
four times with plots measuring 9 meters (30 ft) by 49 meters (160 ft) in a full-entry experiment with all crops present in all years.

Differences among cropping systems included cover crop species, their planting date and termination method, manure management, and tillage intensity and frequency. Short planting windows between cash crop harvest and reduction in temperatures can make establishing cover crops challenging in the fall, especially when harvesting corn for grain rather than silage (Curran et al. 2018). The cover crop treatments in this experiment were chosen to overcome this short planting window in some cases, as well as to improve cover crop benefits to soil, weed management, and arthropod communities.

In all four systems, corn variety ‘MC4050’ (Masters Choice, Anna, IL) was planted on 76-cm rows at 81,510 plt ha\(^{-1}\) using a four-row John Deere 7200 planter equipped with Dawn Biologic ZRX residue managers, typically in late May or early June, depending on the cropping system (Table 3.1). In System 1, liquid dairy manure was broadcast at 74,831 L ha\(^{-1}\) and incorporated via moldboard plow following spelt harvest. A winter cover crop mixture of hairy vetch (\textit{Vicia villosa} Roth ‘Groff’) plus triticale (\textit{x Triticosecale} Wittm. ‘815’) was planted at a seeding rate of 34 kg ha\(^{-1}\) each. Cover crops were terminated by rolling with a roller-crimper in the spring to form a mulch on the soil surface into which corn was no-till planted immediately after. The timing of rolling occurred at Mischler 7 growth stage to optimize weed suppression (Mischler et al. 2010). A second rolling pass a week after planting, as well as two passes with a John Deere 886 high-residue cultivator at four and five weeks after planting were employed to ensure cover crop termination and enhance weed control.

In System 2, no manure was applied in the fall and a chisel plow was used after spelt harvest to prepare the soil for cover crop planting. Hairy vetch and triticale were planted at the
same rates as System 1. In Systems 3 and 4, a cover crop mixture of medium red clover
(*Trifolium pratense* L. ‘VNS’) and timothy (*Phleum pratense* L. ‘VNS’) was no-till drilled into
standing spelt at 12 and 4.5 kg ha$^{-1}$, respectively in early spring. This allowed it to establish prior
to spelt harvest, added economic benefit through a fall forage cutting, suppressed weeds, and
allowed a longer growing season and period without tillage than the hairy vetch and triticale
mixture in the other two systems. After spelt was harvested in early August and the cover crop
was able to grow, the cover crop was mowed and prepared for use as forage to prevent weed
seed production. After continued growth through the fall and winter, this mixture and the hairy
vetch and triticale in System 2 were mowed and incorporated using a moldboard plow. For
fertility needs of the corn, manure was broadcast at 74,831 L ha$^{-1}$ prior to plowing. Corn was
planted 7-10 days following tillage. A mix of blind cultivation and inter-row cultivation was
used in these systems to control weeds early in the season. Although the frequency varied from
year to year depending on weed severity and environmental conditions, all three tilled systems
received the same treatment as each other in a given year.

In Systems 2 and 4, cover crops were drill interseeded between corn rows using a 3-row
high clearance no-till drill (Interseeder Technologies, Woodward, PA) in early July at the V6
stage of corn to allow harvest of corn for grain in late fall without compromising cover crop
establishment. A three-species mixture of annual ryegrass (*Lolium perenne* L. *ssp. multiflorum*
(Lam.) Husnot) ‘KB Supreme’, orchardgrass (*Dactylis glomerata* L.) ‘Potomac’, and forage
radish (*Raphanus sativus* L.) ‘Tillage’ was drilled at 11, 11, and 3.4 kg ha$^{-1}$, respectively. In
Systems 1 and 3, corn was harvested for silage production in September with a cover crop
seeded afterward. In Systems 2 and 4, corn grain was harvested in late October or early
November. Corn yield was collected by harvesting six rows for the entire length of the treatment
plots using either a small silage harvester (Systems 1 and 3) or small plot combine (Systems 2 and 4) and used to connect damage from herbivores to crop performance. For specific dates of planting and termination of cover crops and corn, see, see Table 4.1. Comparison of these four cropping systems allowed me to assess effects of the preceding cover crop biculture, tillage, and mid-season interseeding of cover crops on the prevalence of early-season damage by multiple invertebrate groups, including early season slugs and arthropods, and late-season caterpillars.

**Early Season Damage to Corn**

I assessed early-season damage to corn from invertebrate pests through visual observations approximately two weeks after corn emergence (Table 4.1). I measured damage along two 5.3 meter (17.5 ft) transects in each treatment plot and assessed each corn plant along the transect individually. I classified damage from plant-feeding pests common to the Mid-Atlantic US, including: slugs (Gastropoda), cutworms (Lepidoptera: Noctuidae), first generation European corn borer (*Ostrinia nubilalis* Hübner), stink bugs (Hemiptera: Pentatomidae), billbugs (*Sphenophorus aequalis* Gyllenhal), and other chewing insects into three categories: slug damage, whorl damage, or chewing damage. Whorl damage included any damage that appeared to have occurred prior to the leaf unfurling that then appears as a line of small holes on fully expanded leaves. In 2016 and 2017, I assigned each corn plant a rating of damage severity from 0-4, with 0 indicating no damage, 1 indicating 1-25% of the plant damaged, 2 indicating 26-50% of plant damaged, 3 indicating 51-75% damaged, and 4 indicating greater than 75% of plant damaged. I also recorded corn populations along these transects.
Late Season Damage to Corn

I conducted two late-season assessments of damage from common caterpillar pests of corn. One assessment targeted fall armyworm (*Spodoptera frugiperda* Smith) and consisted of five randomly located transects of 20 plants each along a crop row. I inspected each corn plant for damage typical to this caterpillar (Bohnenblust and Tooker 2012) and collected and identified any caterpillars found. I assessed corn plants in mid-August to target the time when this pest is typically most abundant in central Pennsylvania (Table 4.1). The second assessment targeted *O. nubilalis* and corn earworm (*Helicoverpa zea* Boddie). European corn borer has two flights in central Pennsylvania (Bohnenblust et al. 2014). I assessed damage from both *O. nubilalis* and *H. zea* in early September after the conclusion of the second generation flight of *O. nubilalis* to provide a cumulative measure of damage from both generations of *O. nubilalis* and later instars of *H. zea*. I collected twelve plants per plot and examined them for boring tunnels on corn stalks or feeding damage on the corn ear. I collected and identified larvae found in tunnels or on the ear using Cooperative Extension identification guides (Foster 2016, Krupke et al. 2010, Passoa 1991, Royer and Arnold 2017).

Environmental Conditions

I used several biotic and abiotic environmental variables from existing data sets affiliated with the project to help understand how each cropping system affected the habitat and to better understand environmental factors contributing to corn damage across the experimental site. At the time of early-season pest damage assessments, the corn population was estimated using the same 5.3 m transects described above. I also recorded the height and growth stage of three corn plants per transect. To understand the contribution of non-crop vegetation and surface residue to
habitat and distribution of pests and damage, I used pre-termination above-ground cover crop biomass in all four systems from nine 0.25m$^2$ quadrats per treatment plot that were dried at 65°C, converted to kg ha$^{-1}$ dry weight, and averaged for each plot (Champagne 2017). Cover crop biomass was assessed 1 week prior to termination, resulting in System 1 being sampled 7-10 days later than in Systems 2 - 4. In System 1, this biomass contributed to residue at the soil surface while in Systems 2 - 4, the cover crop biomass was incorporated into the soil using a moldboard plow prior to corn planting, altering the soil environment of these systems early in the season. Weeds were identified to species (Champagne 2017) and used to calculate species richness and Smith-Wilson evenness of the weed communities in each plot (Smith and Wilson, 1996). When cover crop termination was not complete, cover crop re-growth (“volunteers”) was also assessed as part of the weed community. Weed biomass was measured in the field in mid-August, using nine 0.5m$^2$ quadrats placed randomly in each treatment plot, dried at 65°C, converted to kg ha$^{-1}$ dry weight, and averaged for each plot (Champagne 2017). At the same time, the density of the interseeded cover crop in Systems 2 and 4 was measured and the proportion of grass species and forage radish in the mixture was calculated. For Systems 1 and 3, which were not interseeded, I used 0 for these parameters.

I used soil characteristics measured in the early summer (17 June 2015, 28 June 2016, and 21 June 2017) and late summer (17 Aug 2015, 2 Sep 2016, and 1 Aug 2017) for each cropping system to understand the effect of soil characteristics on arthropods. In each treatment plot, 9 randomly distributed soil cores (2.5 x 15 cm) were collected, from which gravimetric moisture (Gardner 1986), matric potential (Hamblin 1981), pH, electrical conductivity, and permanganate oxidizable (‘active’) carbon (Weil et al. 2003) were measured. I used soil inorganic N reported as mg N per kg of dry soil, measured from the same soil dates (Isbell,
unpublished). A portion of each soil sample was submitted to the Pennsylvania State University Agricultural Analytical Services Laboratory (AASL) for analysis of total organic matter by loss-on-ignition, soil textural class, phosphorus, potassium, magnesium, calcium, zinc, copper, sulfur, and cation exchange capacity (CEC). Additionally, 15 last-instar *Galleria mellonella* (Lepidoptera: Pyralidae) larvae were added to a 250 mL portion of soil from these same soil collections and held for 10 days, then assessed for infection from *Metarhizium* spp. (Hypocreales: Clavicipitaceae) infection, which is a soil-borne entomopathogen common to these cropping systems (Randhawa 2017).

To assess the influence of mechanical disturbance through tillage and other field operations on early-season pests, I summed the number of disturbance events that occurred in each plot from the start of each experiment until the time of sampling, as well as within a single season from January 1 until the time of sampling. I split these into above-ground events, such as mowing or planting, and soil events to represent the frequency of tillage events. Additionally, to represent the intensity of disturbance, I used a USDA Natural Resources Conservation Service soil disturbance rating (SDR) obtained through RUSLE2 (NRCS 2008). The SDR, which ranges from 0 (least disturbance) to 30 (greatest disturbance) for a field operation, is comprised of the sum of six ratings each with values from 0 to 5 that estimate the relative severity of disturbance. The six component categories of the SDR include soil inversion, soil mixing, soil lifting, soil shattering, soil aeration, and soil compaction. The field operation that we employed with the highest SDR was tillage with a moldboard plow with an SDR of 29, and one of the lowest was flail mowing with an SDR of 3. As with frequency of events, these were summed in each plot from the start of each experiment until the time of sampling, as well as within a single season from January 1 until the time of sampling. A previous study dealt with multiple passes of a
similar tool by including it in a model that accounts for temperature and soil texture in calculating overall disturbance and turnover of the soil (Kemanian and Stöckle 2010). Although repeated passes of the same tillage implement may have redundant impacts on soil, little is known about whether the effect of multiple passes of the same tillage implement has a different effect on arthropods than one pass. Thus, I opted to use all reported passes to calculate a total intensity of disturbance for this usage of SDR and am relying on it as a means to compare each cropping system standardized by the weed management used in each year, rather than a true measure of disturbance.

Data Analysis

I analyzed all data in R Version 3.5.1 (R Core Team 2018). For each category of early-season damage, the number of plants with damage was divided by the number of plants along that transect to give one proportion of damaged plants per transect. Ratings for damage intensity were also averaged along transects. Transect means were then averaged by plot. Late-season damage was calculated as a proportion by dividing the number of plants with damage or with caterpillars present by the number of plants sampled. Early and late season damage prevalence was analyzed using linear-mixed models (function lme) in the nlme package (Pinheiro et al. 2013). Data were square root transformed where needed to meet assumptions of the model, including normal distribution and homogeneity of variance. I specified a model using block nested within year as a random effect and cropping system as a fixed effect. I conducted post hoc, pairwise tests of means between each treatment level using Tukey’s honest significant difference test. I used the varcomp function in the ape package to partition the variance explained by each model by year and block. I measured no damage from European corn borer in 2017, and
therefore excluded 2017 from late-season damage analyses. Because the effects of cropping system varied by year in most cases, I also analyzed data within individual years using block as a random effect and system as a fixed effect. Damage ratings were analyzed using the clm2 function in ordinal package to build a cumulative logit model to account for multinomial ordinal data (Christenson 2019).

To identify relationships between damage to corn and environmental conditions, I used multiple linear regression with the proportion of damaged corn plants as the response variable and environmental characteristic values as explanatory variables, and used stepwise selection using the stepAIC function in the MASS package in R to reduce the complexity of the full models (Schmidt and Rypstra, 2010, Venables and Ripley, 2002). For soil and weed characteristics that were sampled multiple times per season, the sampling date closest to the date of damage sampling was used. All models for arthropod damage to corn included corn growth and population, weed parameters, soil physical characteristics, soil disturbance parameters, and soil nutrients. Early season damage regressions included characteristics of the preceding spring cover crop to represent available plant residue while late season damage regressions included characteristics of the interseeded cover crop. Models were compared by Akaike’s Information Criteria (AIC), with the final model having the lowest AIC (Kutner et al., 2005, Murtaugh, 2009).

To assess association between damage to corn from invertebrate pests and corn yield, I conducted a multiple linear regression between corn yield, damage from both early and late season pests, as well as other yield-limiting factors including soil moisture, soil texture, soil nutrients and total weed biomass. In this case, the nutrient samples, including nitrogen, from mid-June was used to estimate differences in availability to the corn across the field site. Silage yields and grain yields were analyzed separately. Lastly, I conducted multiple linear regressions
to assess the association between predation rate, total predator activity density, and activity
density of common generalist predators on the proportion of corn damage by invertebrate pests
to understand whether generalist predators contribute to biological control of common pests in
these cropping systems.
Table 4.1 Field operations and damage assessments for each experimental system in each year. Dates for interseeding of cover crops in Systems 2 and 4 are denoted by I. HV denotes hairy vetch and triticale and RC denotes red clover and timothy. NT denotes no-till planting, while T denotes tilled systems.

<table>
<thead>
<tr>
<th></th>
<th>System 1 (HV-NT)</th>
<th>System 2 (HV-T-I)</th>
<th>System 3 (RC-T)</th>
<th>System 4 (RC-T-I)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant Cov. Crop</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2016-2017</td>
<td>8/30/16</td>
<td>8/30/16</td>
<td>3/4/16</td>
<td>3/4/16</td>
</tr>
<tr>
<td>Plant corn</td>
<td>5/15/15</td>
<td>5/15/15</td>
<td>5/17/16</td>
<td>5/17/16</td>
</tr>
<tr>
<td>Early Dmg Assessment</td>
<td>6/25/15</td>
<td>6/25/15</td>
<td>6/21/17</td>
<td>6/21/17</td>
</tr>
<tr>
<td>Sampling</td>
<td>6/22/15</td>
<td>6/22/15</td>
<td>7/7/17</td>
<td>7/7/17</td>
</tr>
<tr>
<td>Interseed FAW</td>
<td>7/8/15</td>
<td>6/24/16</td>
<td>7/12/17</td>
<td>7/12/17</td>
</tr>
<tr>
<td>Assessment</td>
<td>8/14/15</td>
<td>8/14/15</td>
<td>8/10/17</td>
<td>8/10/17</td>
</tr>
<tr>
<td>Predator</td>
<td>8/16/16</td>
<td>8/24/17</td>
<td>8/24/17</td>
<td>8/24/17</td>
</tr>
<tr>
<td>Harvest corn</td>
<td>9/17/15</td>
<td>9/17/15</td>
<td>9/17/15</td>
<td>9/17/15</td>
</tr>
</tbody>
</table>

127
Results

**Damage Prevalence and Severity in Corn**

Slug feeding was consistently the most common type of early-season damage to corn. Damage was particularly frequent in 2017 with approximately 90% of plants affected, though cropping system was not significant ($F_{3,33} = 1.24, P = 0.31$, Fig. 4.1A). Year accounted for 71.9% of the variation explained by the mixed model. The only year in which cropping system had a significant effect was in 2016, where slug damage was significantly greater in System 4 than in Systems 1 and 3 ($F_{3,9} = 6.39, P = 0.01$). System had a marginal but insignificant effect on whorl damage ($F_{3,33} = 2.27, P = 0.09$), while block and year contributes 7% and 4%, respectively, of the variance explained by the model. This marginal significance was driven by the proportion of damage in 2015, where proportion of damaged corn plants was greater in System 1 than in Systems 2 and 4 ($F_{3,9} = 11.37, P < 0.01$) (Fig. 4.1B). Chewing damage was significantly greater in System 1, which was planted no-till, than Systems 2–4 overall ($F_{3,33} = 23.68, P < 0.01$), in 2016 ($F_{3,9} = 24.34, P < 0.01$), and in 2017 ($F_{3,9} = 14.38, P < 0.01$). This difference in system appears to increase over time and 16.7% of the variation explained by the model is attributed to year. Overall damage ratings measured in 2016 (Fig. 4.2A) and 2017 (Fig. 4.2B) were low and averaged 1 out of 4 on the rating scale used, indicating damage to less than 25% of above-ground damage to corn. However, the damage rating was significantly greater in System 1, which was planted no-till, than in the tilled Systems 2–4 ($X^2_3 = 8.78, P = 0.03$).

Cropping system had little effect on late-season damage from *Spodoptera frugiperda*, *Ostrinia nubilalis* and *Helicoverpa zea*. In 2016, there was a trend toward fewer plants with *H. zea* feeding damage in plots where interseeding had occurred (Systems 2 and 4) than in plots without interseeded cover crops (Systems 1 and 3) (Fig. 4.3A). Cropping system did not
significantly affect the proportion of plants damaged by *H. zea* (*F*<sub>3,33</sub> = 1.77, *P* = 0.17). Year explained 17% of the variation in damage from *H. zea* explained by the mixed model. There were no significant effects on the amount of damage by caterpillar feeding or tunnel formation, however (*F*<sub>3,33</sub> = 0.50, *P* = 0.68). No tunnels were found in 2017 and year accounted for 26.2% of the variance explained by the model. Damage from *S. frugiperda* was greatest in 2016 with year explaining 76.8% of the variance explained by the mixed model but there was no difference in damage among the four cropping systems (*F*<sub>2,33</sub> = 0.85, *P* = 0.48). In 2017, there was significantly more damage from *S. frugiperda* in System 1 than in Systems 2 and 3. For all three caterpillar species, block explained a low amount of variation, ranging from 0% to 4%.

**Environmental Conditions Contributing to Damage**

Multiple regressions indicate that environmental conditions are associated with prevalence of damage to corn, both early and late in the season. Models including cash crop growth, cover crop, weed, soil characteristics, and nutrient levels explained between 49% and 78% of variation in pest pressure and all models were significant at the 0.05 level.

Variation in early season damage was explained by soil and cover crop characteristics (Table 4.2). The strongest relationships in the model for the proportion of corn plants damaged by slugs included the proportion of legume in the preceding cover crop mixture and gravimetric soil moisture content (*F*<sub>14,33</sub> = 12.83, *P* < 0.001). Both relationships were positive. Other factors that were significant in the model, but had weaker relationships, included positive relationships with the number of days since disturbance and pH and negative relationships with soil clay and zinc content. Whorl damage was most strongly influenced by gravimetric moisture, followed by proportion of legume in the preceding cover crop, and soil pH and zinc content (*F*<sub>13,34</sub> = 5.32, *P* <
Relationships with soil gravimetric moisture and pH were negative while proportion of legume and soil zinc content were positive (Table 4.2). There were various other vegetation and soil characteristics that were significant in the model but had relationship strengths near zero. The strongest factors in the model for variation in chewing damage included soil gravimetric moisture and the number of above-ground disturbance events ($F_{16,31} = 8.5, P < 0.001$). The relationship with soil moisture was positive while the relationship with above-ground disturbance was negative (Table 4.2). Other weak but significant factors in the model include soil organic matter and pH, and weed species richness. Interestingly, none of the three models for damage included soil nitrogen.

I detected weaker relationships in most of the models for late-season damage than those for early season damage (Table 4.3). For *H. zea* damage, all factors had low estimate values but the strongest factors were a negative relationship with the number of soil disturbances and a positive relationship with soil zinc content ($F_{12,35} = 4.84, P < 0.001$). The strongest factor in the model built for *O. nubilalis* damage was a negative relationship with soil gravimetric moisture ($F_{26,3} = 4.93, P = 0.04$). For *S. frugiperda* damage, all factors had low estimate values but the strongest factors were a positive relationship with the number of above-ground disturbances and a negative relationship with corn height early in the season ($F_{18,39} = 16.96, P < 0.001$) (Table 4.3).

**Impacts of Pest Damage on Corn Yield**

Yields of corn for grain and silage were analyzed separately to account for the difference in biomass harvested in each method, represented by different units of measure. Multiple regressions investigating the relationship between early-season pest damage, late-season pest
damage, soil nutrients, and weed biomass with corn yield, explained a high percentage of variation, from 90% of variation in silage yield to 88% of variation in grain yield. Silage yield had the strongest relationships with year. Silage corn produced less harvestable biomass in 2016 than in other years. Silage yield was positively associated with soil copper content ($F_{12,11} = 18.21, P < 0.001$, Table 4.4). The model for grain included fairly strong relationships with multiple variables but the two strongest were a positive relationship with soil zinc content and a negative relationship with soil phosphorus content ($F_{10,13} = 17.95, P < 0.001$, Table 4.4). When pest damage factors were significantly related to yield, these relationships were positive, indicating that pests may not have contributed substantially to yield loss in corn relative to other factors.

**Predators, Sentinel Predation Rate, and Corn Damage**

Significant relationships were observed between generalist predators, sentinel predation rate, and early-season damage (Table 4.5). Although Araneae activity density and total activity density of predators were significant in the model for slug damage, neither had very strong negative relationships. Whorl damage was weakly but significantly related to activity density of Araneae ($F = 0.24, P = 0.02$), though this relationship was positive. Chewing damage was strongly and positively related to sentinel predation rate ($F = 27.46, P < 0.01$) and weakly, though significantly and positively related to Araneae activity density ($F = 2.13, P = 0.04$). Interestingly, relatively few of these relationships were negative, indicating that more predators did not consistently translate to reduced damage from pests in these cropping systems.

No relationship was observed between sentinel predation rate and damage from the late-season pest for *H. zea* ($F_{1,46} = 0.61, P = 0.44$) or *S. frugiperda* ($F_{1,46} = 0.72, P = 0.40$). Nor was a
relationship observed between common generalist predator activity densities and late-season damage from these two caterpillar species. However, a strong negative relationship was observed between sentinel predation rate and *O. nubilalis* damage (*F* = -33.54, *P* < 0.01).
Figure 4.1. Mean proportion of corn plants damaged by slug feeding (A), with damage to corn whorl (B), and with damage from chewing herbivores (C) in each year of the study. Error bars represent standard errors and asterisks represent means within years that are significantly
different at P< 0.05. Purple bars represent systems preceded by hairy vetch and triticale (HV), red bars represent systems preceded by red clover and timothy (RC), and crosshatched bars represent no-till planted corn. (NT).

Figure 4.2. The severity of damage to plants was ranked on a 0-4 scale, with 1 indicating less than 25% of the plant was damage. Error bars represent standard errors and asterisks represent means within years that are significantly different at the 0.05 level. Purple bars represent systems preceded by hairy vetch and triticale (HV), red bars represent systems preceded by red clover and timothy (RC), and crosshatched bars represent no-till planted corn into rolled cover crop residue (NT).
Figure 4.3. Mean prevalence of damage due to *Helicoverpa zea* ear feeding (A), *Ostrinia nubilalis* tunnels (B), and *Spodoptera frugiperda* feeding (C) in each year of the experiment. Error bars represent standard errors and asterisks represent means within years that are significantly different at the 0.05 level. Purple bars represent systems preceded by hairy vetch.
and triticale (HV), red bars represent systems preceded by red clover and timothy (RC),
crosshatched bars represent no-till planted corn into rolled cover crop residue (NT), and dashed
bars represent plots into which cover crops were interseeded (I).

Table 4.2: Regression models showing significant association of environmental characteristics on
prevalence of early-season pest damage categories.

<table>
<thead>
<tr>
<th>Overall Model</th>
<th>Slug Damage</th>
<th>Whorl Damage</th>
<th>Chewing Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>Pr &lt; 0.05</td>
<td>Estimate</td>
</tr>
<tr>
<td>Variance Explained</td>
<td>R^2 adj = 0.78</td>
<td></td>
<td>R^2 adj = 0.54</td>
</tr>
<tr>
<td>Cover crop biomass</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cover crop height</td>
<td>NS</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Proportion legume</td>
<td>26.25</td>
<td>*</td>
<td>38.13</td>
</tr>
<tr>
<td>Cumulative SDR</td>
<td>~0</td>
<td>**</td>
<td>0.03</td>
</tr>
<tr>
<td>Seasonal SDR</td>
<td>~0</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Num. above-ground disturbance events</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days since last disturbance</td>
<td>0.03</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>Weed seedbank</td>
<td>~0</td>
<td>*</td>
<td>~0</td>
</tr>
<tr>
<td>Weed species richness</td>
<td></td>
<td>1.73</td>
<td>**</td>
</tr>
<tr>
<td>Weed biomass</td>
<td>~0</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>Soil clay content</td>
<td>-0.01</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Gravimetric moisture</td>
<td>2.42</td>
<td>**</td>
<td>-158.5</td>
</tr>
<tr>
<td>Organic matter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active carbon</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>0.26</td>
<td>**</td>
<td>-23.59</td>
</tr>
<tr>
<td>Electrical conductivity</td>
<td>~0</td>
<td>**</td>
<td>-0.09</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>~0</td>
<td>**</td>
<td>-1.01</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td></td>
<td>-0.09</td>
</tr>
<tr>
<td>Zinc</td>
<td>-0.44</td>
<td>***</td>
<td>20.51</td>
</tr>
</tbody>
</table>

Significance codes: --- dropped from stepwise model, NS > 0.05, * < 0.05, ** < 0.01, *** < 0.001.
Table 4.3: Regression models showing significant association of environmental characteristics on prevalence of damage from late season lepidopteran pests.

| Overall Model | \textit{Helicoverpa zea} | | \textit{Ostrinia nubilalis} | | \textit{Spodoptera frugiperda} | |
|---|---|---|---|---|---|
| Variance Explained | \text{Estimate} | \text{Pr < 0.05} | \text{Estimate} | \text{Pr < 0.05} | \text{Estimate} | \text{Pr < 0.05} |
| Early corn height | -0.03 | *** | NS | | -0.02 | *** |
| Corn population | --- | | NS | | -0 | * |
| Cumulative SDr | -0 | ** | NS | | -0 | * |
| Seasonal SDr | -0.02 | ** | NS | | -0 | * |
| Num. above-ground disturbance events | --- | | --- | | 0.68 | ** |
| Num. soil disturbance events | -0.35 | ** | --- | | --- | --- |
| Days since last disturbance | NS | | -0.05 | ** | --- | --- |
| Weed biomass | NS | | -0 | ** | --- | --- |
| Soil clay content | 0.04 | ** | NS | | --- | --- |
| Soil silt content | --- | | -0.05 | * | --- | --- |
| Gravimetric moisture | --- | | -10.26 | * | --- | --- |
| Nitrogen | --- | | 0.03 | ** | --- | --- |
| Potassium | -0 | *** | NS | | NS | NS |
| Zinc | 0.33 | ** | NS | | NS | NS |
| CEC | --- | | -0.17 | * | --- | --- |

Significance codes: --- dropped from stepwise model, NS > 0.05, * < 0.05, ** < 0.01, *** < 0.001.
Table 4.4. Relationship between yield of corn harvested as grain, damage by pests, weed biomass, and soil fertility measures.

<table>
<thead>
<tr>
<th>Overall Model</th>
<th><strong>Estimate</strong></th>
<th><strong>P value</strong></th>
<th><strong>Estimate</strong></th>
<th><strong>P value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Silage Yield</strong></td>
<td>F_{12,11} = 18.21 &lt; 0.001 ***</td>
<td>R_{adj}^2 = 0.90</td>
<td>F_{10,13} = 17.95 &lt; 0.001 ***</td>
<td></td>
</tr>
<tr>
<td><strong>Grain Yield</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Variance Explained**

<table>
<thead>
<tr>
<th>Year</th>
<th><strong>Estimate</strong></th>
<th><strong>P value</strong></th>
<th><strong>Estimate</strong></th>
<th><strong>P value</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>-35.3</td>
<td>***</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>2017</td>
<td>NS</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Slug damage</td>
<td>-0.49</td>
<td>**</td>
<td>55.77</td>
<td>***</td>
</tr>
<tr>
<td>Whorl damage</td>
<td>---</td>
<td>*</td>
<td>21.34</td>
<td>*</td>
</tr>
<tr>
<td>Chewing damage</td>
<td>---</td>
<td>**</td>
<td>81.48</td>
<td>***</td>
</tr>
<tr>
<td>Corn earworm damage</td>
<td>0.38</td>
<td>**</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>European corn borer damage</td>
<td>---</td>
<td>NS</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Weed biomass</td>
<td>0.01</td>
<td>***</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.41</td>
<td>**</td>
<td>10.31</td>
<td>*</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>NS</td>
<td>-103.26</td>
<td>***</td>
<td></td>
</tr>
<tr>
<td>Magnesium</td>
<td>---</td>
<td>-12.16</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>-0.03</td>
<td>*</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Copper</td>
<td>5.27</td>
<td>*</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Zinc</td>
<td>NS</td>
<td>3544.56</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

Significance codes: --- dropped from stepwise model, NS > 0.05, * < 0.05, ** < 0.01, *** < 0.001.

Table 4.5: Regression models showing significant association of predator activity-density and predation rate on prevalence of early-season pest damage categories.

<table>
<thead>
<tr>
<th>Overall Model</th>
<th><strong>Slug Damage</strong></th>
<th><strong>Pr &lt; 0.05</strong></th>
<th><strong>Whorl Damage</strong></th>
<th><strong>Pr &lt; 0.05</strong></th>
<th><strong>Chewing Damage</strong></th>
<th><strong>Pr &lt; 0.05</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Estimate</strong></td>
<td>F_{3,44} = 9.19 ***</td>
<td>R_{adj}^2 = 0.34</td>
<td>F_{2,45} = 3.22 *</td>
<td>R_{adj}^2 = 0.09</td>
<td>F_{4,43} = 6.90 ***</td>
<td>R_{adj}^2 = 0.33</td>
</tr>
<tr>
<td><strong>Variance Explained</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predation Rate</td>
<td>---</td>
<td>---</td>
<td>27.46 **</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Araneae</td>
<td>-0.38</td>
<td>*</td>
<td>0.26</td>
<td>*</td>
<td>2.13</td>
<td>*</td>
</tr>
<tr>
<td>Staphylinidae</td>
<td>---</td>
<td>---</td>
<td>-0.17 ***</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Total Predators</td>
<td>-0.14</td>
<td>**</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Significance codes: --- dropped from stepwise model, NS > 0.05, * < 0.05, ** < 0.01, *** < 0.001.
Discussion

Winter cover crops provide numerous services to cropping systems, including soil nutrient provisioning, weed suppression, and habitat for beneficial arthropods (Murrell et al. 2017, Rivers et al. 2018, Schipanski et al. 2017, Wallace et al. 2018). There is concern that adding habitat for insect pests through winter cover crops and reducing tillage could elevate the severity of damage from insect pests (House and Alzugaray 1989, Willson and Eisley 1992). I hypothesized that early-season damage to corn from arthropods and slugs would be similar between two functionally similar cover crop mixtures, that early-season pests would be more abundant in no-till planted corn than in tilled corn due to reduced mortality from tillage equipment, and that damage from late-season arthropod pests and slugs would be similar between systems that used cover crops interseeded into V6 corn compared to those that did not have interseeded cover crops. Additionally, I used multiple regressions to investigate the association of environmental factors with damage by arthropod pests and slugs. I found that preceding cover crop mixture did not affect damage from herbivores, tillage affected damage in some cases, and interseeding had relatively little effect on damage.

Early Season Damage Prevalence in Corn

As hypothesized, the proportion of damage to corn from arthropod pests was similar following the two winter cover crop bicultures of hairy vetch and triticale and of red clover and timothy. Damage to corn from slugs was consistently the most common early-season damage across years and cropping systems in this study and affected up to 90% of plants. Slugs are a common concern of growers in the Mid-Atlantic region, particularly in no-till systems (Douglas and Tooker 2012, Le Gall and Tooker 2017). Interestingly, I saw no evidence for greater slug
damage in System 1, which was planted no-till, relative to Systems 2 – 4, in which cover crops were terminated with a moldboard plow. This unexpected result could be due to the experiment being managed through rotational no-till, rather than continuous no-till (Wallace et al. 2017). In organic, cover crop-based rotational no-till, corn is established by no-till planting into a mat of rolled cover-crop residue, but tillage occurs to establish winter cover crops in the fall.

Additionally, the use of experimental treatment plots may have limited my ability to accurately measure effects of cropping system on slug damage. Cantelo (1986) suggests 30 m$^2$ as a minimum plot size for accurate assessments of pest damage in corn. Our plots exceed that size, but are relatively long and narrow (9 m by 49 m) rather than square, which may have allowed some recolonization of both pests and natural enemies from plot edges following tillage and crop establishment.

Slug damage affected a greater percentage of plants in 2017 than in 2015 and 2016. The proportion of corn plants damaged by slugs was especially high across the region in 2017, so this is not unique to any aspect of our study (Myer 2018). Slug damage responded positively to increasing soil moisture which is unsurprising as slugs require moisture for survival (Douglas and Tooker 2012, Myer 2018). Slug damage was also greater when the proportion of legume in the preceding cover crop mixture, either *Vicia villosa* or *Trifolium pretense*, was greater. This may indicate that slugs used legume cover crop species as hosts prior to termination or that the residue rolled down or tilled in was able to provide more nutrients to the corn as the cover crops began to decompose. Slugs have been shown to regulate nutrient intake through foraging, preferring legumes over grasses (Douglas and Tooker 2012, Le Gall and Tooker 2017). Slugs may have been regulating their nutrient intake in this study, as well.
Whorl damage from arthropods was similar in all three years. The effects of cropping system on whorl damage were not consistent, indicating that the environment within each cropping system and its susceptibility to pests may be influenced by year-to-year changes in weather or by area-wide abundance of arthropod pests. Area-wide suppression of *O. nubilalis* has been detected in the United States following Bt adoption (Hutchison et al. 2010, Bohnenblust et al. 2014, Walter et al. 2018). First generation *O. nubilalis* larvae contribute to whorl feeding and tend to prefer weedy areas but may exhibit preferences for certain vegetation over others (Bohnenblust et al. 2014, Stamps et al. 2007). Early-instar black cutworm, *A. ipsilon*, can also feed on the whorl and can be problematic in high-residue no-till environments, such as System 1 of this study (Mischler et al. 2010). Whorl damage increased as proportion of legume in the preceding cover crop mixture increased, which may indicate that herbivores used these cover crops as a host early in the season and benefitted from the structural complexity and nitrogen provided. Whorl damage was also lower as soil moisture increased, which may indicate moisture exceeded optimal conditions for the herbivores contributing to this damage and forced them to seek alternate habitat (Erb and Lu 2013). Late-season damage from *O. nubilalis* was also lower later in the season as soil moisture increased, suggesting that drier conditions could have been more favorable for this pest throughout the season in this study. One study relating *O. nubilalis* damage to weather conditions hypothesized that heavy rain washes eggs off of leaves, reducing survival of the populations (Sarajlić et al. 2017). Whorl damage was negatively associated with soil pH, which could be an indirect result of decreased plant quality as pH increased. Although the range in soil pH was narrow (6.02-7.79), it did exceed the optimal range for corn in some cases, which is between 6 and 7 to maximize uptake of micronutrients (Beegle 2015). Lastly, whorl damage was greater where soil zinc content was higher. This is consistent with previous
observations of the Colorado potato beetle, *Leptinotarsa decemlineata*, where increased soil zinc content correlated with greater damage to plants (Aloykhin et al. 2015). Zinc can provide a mechanical benefit to chewing herbivores by reducing mandibular wear from tough leaf tissues (Acevedo 2016).

Chewing damage was significantly greater in the no-till-planted system (System 1) compared with the three systems in which corn was established with tillage (Systems 2 – 4), and the percentage of damaged corn plants increased in each year in System 1. Although chewing damage was not attributed to specific pests, this elevated frequency of damaged corn plants through time in System 1 is consistent with other research demonstrating the negative effects of tillage on early-season pests (Mischler et al. 2010, Stinner and House 1990). This interpretation is supported by a negative relationship between chewing damage and an increase in the number of above-ground field operations, which corresponded with the cropping systems receiving tillage. The true armyworm, *Pseudaletia unipuncta*, is one potential cause of chewing damage in our region and is known to be more problematic in no-till fields (Tooker and Calvin 2009). The true armyworm overwinters in tropical and subtropical areas and migrates north, and therefore, late-planted corn is especially vulnerable to damage by this pest (Fields and McNeil 1984, Tooker and Calvin 2009). Chewing damage was positively related to soil moisture. The greatest frequency of chewing damage occurred in 2017, which also had the wettest conditions in spring (Champagne 2017, Myer 2018). Thus, conditions may have been particularly favorable for true armyworm and other early-season chewing pests favored by moist environments. One possible way in which armyworm could have been favored is through increased migration opportunities through increased storm frequency, if these storms moved into the region from the south (Nagoshi et al. 2009, Sparks 1979). There was a strong negative relationship between chewing
pests and predation rate in our study, which aligns with previous research showing ground-dwelling generalist predators to be effective at reducing damage from *P. unipuncta* (Clark et al. 1994). Predators and predation may have aided in keeping damage severity low in all systems.

**Late Season Damage Prevalence in Corn**

There was great year-to-year variation in late season damage for all three sampled taxa, but especially damage from fall armyworm. This is likely related to the migratory patterns and broad host ranges of these lepidopteran pests. Neither corn earworm nor fall armyworm overwinter in Pennsylvania (Changnon et al. 2010, Diffenbaugh et al. 2008, Morey et al. 2012, Nagoshi et al. 2009).

There was a trend for damage from corn earworm in 2016 to be lower in Systems 2 and 4, which were interseeded with cover crops, than in the two systems that were not interseeded. Otherwise, there was no effect of cropping system on damage from this pest. Multiple regressions suggesting a weak relationship between measured environmental variables and variation in frequency of corn earworm damage. Corn earworm damage was weakly and positively related to soil zinc content. The Colorado potato beetle, *Leptinotarsa decimlineata*, was also observed to have weak positive relationships with zinc. This relationship contributed to lower abundance of *L. decimlineata* in manure-fertilized plots where zinc was lower than in treatments in which synthetic fertilizer was used and where zinc concentration was greater (Aloykhin et al. 2015). Zinc is an important element for fortifying caterpillar mandibles against wear from tough plant leaves, including corn and rice (Acevedo 2016). Corn earworm was negatively related to the frequency of tillage events, though this relationship was not particularly
strong. As this pest does not overwinter in Pennsylvania, it is unlikely to be directly affected by early-season crop management.

European corn borer responded negatively to increased soil moisture, which is consistent with a European study that found the lowest damage in the year with the highest precipitation (Sarajlić et al. 2017). They hypothesized that heavy rains wash eggs off of leaves, reducing populations (Sarajlić et al. 2017). I did not detect strong relationships between damage from European corn borer and weediness or any other vegetation metric. Although European corn borers commonly use weedy vegetation as resting spots during the day (Bohnenblust et al. 2014), other studies have shown no effect of weed management or surrounding vegetation on crop damage from European corn borer and oviposition (Stamps et al. 2007, Wilson et al. 2004).

Fall armyworm damage to corn was not strongly related to any environmental factor, though it was positively related to the number of above-ground disturbances. The increase in above-ground disturbances corresponded to cropping systems that were tilled, compared to those established no-till. Establishment of corn, particularly in no-till plots was challenging, and populations of corn were lower in System 1 than in Systems 2 – 4, which may have prevented our ability to detect differences due to cropping system on plant damage between tilled and no-till treatments in other years (Champagne 2017). Although fall armyworm is polyphagous, corn is a preferred host, so lower corn plant populations in no-till planted System 1 may have appeared less attractive to migrating moths compared to the greater corn populations in Systems 2-4 (Sparks 1979). As with corn earworm, the fall armyworm is unable to overwinter in northern regions of the US and their populations will be largely determined by moth oviposition behavior (Nagoshi et al. 2009).
Effects of Pest Damage on Yield

Historically, biological control potential as determined by sentinel predation assays has been high at this site, so the overall low amount of early season damage is unsurprising (Rivers et al. 2018). One factor reducing the likelihood of economically significant damage from arthropod pests in our system is the use of crop rotation, which is an important tactic within integrated pest management programs for reducing buildup of pest populations (Prasifka et al. 2006, Thierfelder and Wall, 2010). Corn can tolerate substantial defoliation from early-season pests, such as *P. unipuncta*, before experiencing loss in yield (Mulder and Showers 1986). The proportion of plants with damage late in the season corresponds to recent assessments across Pennsylvania, with *H. zea* damage affecting from 0.5% to 33% of sampled corn plants and *O. nubilalis* affecting fewer than 25% in most cases (Bohnenblust et al. 2013, Bohnenblust et al. 2014). It should be noted that the amount of damage per ear that I observed for *H. zea* far exceeded that measured in recent studies of both these pests across Pennsylvania, where damage rarely exceed 1 kernel per ear (Bohnenblust et al. 2013). Although not analyzed, most of the ears on which I found *H. zea* feeding exceeded 1 kernel per ear (unpublished data). This indicates that I measured damage within a range that could potentially lead to detectable losses in yield. The lack of a negative relationship between pest damage and yield in our study likely indicates that other factors, such as weed populations and nitrogen deficiencies are greater limitations for corn yield in organic systems (Cavigelli et al. 2018, Wallace et al. 2018).

Interestingly, soil nitrogen was not strongly related to pest damage either early in the season or late in the season. Nitrogen is known to affect herbivore performance (Mattson 1980). However, the effects of individual nutrients, even important nutrients such as N, can depend on the ratio with other nutrients, with interactions between nutrients altering herbivore response, as
well as the variation in responses associated with different herbivorous species (Busch et al. 1999). The weakness of nitrogen effects may have been due to insufficient N in all the systems. Because decomposition of the preceding cover crop residue was slow and we primarily relied on manure for soil fertility, corn tended to be nitrogen-limited regardless of cropping system (S. Isbell, pers. communication). Thus, the ability of nitrogen to improve corn yield in the amounts it was available may have been weak. Silage yield was much more strongly affected by year than any other environmental factor, including pest damage, with the lowest yield in 2016. This year corresponded to wet spring conditions and drier than typical conditions in early summer, resulting in challenges planting corn and managing weeds. Thus, it had the lowest corn yields of any of the three years (Champagne 2017). The challenges establishing corn at planting likely reduced detectable effects of any other environmental factors.

With the exception of chewing damage to corn, cropping system had little effect on damage to corn from invertebrate pests. In previous experiments focused on organic cover crop-based rotational no-till at this site (Rivers 2016a), damage to corn from invertebrates was greater in corn planted into rolled hairy vetch and triticale than in soybean planted into rolled cereal rye, though it is unclear to what extent this was due to cover crop species versus cash crop species. In the current experiment, we compared two grass-legume cover crop bicultures, which resulted in similar levels of damage in the following corn crop. Tillage is often an important pest management tactic in organic production (USDA NASS 2015) and pests have been more problematic in conventionally managed no-till systems in some cases (Douglas and Tooker 2012, House and Alzugray 1989). Indeed, we observed a greater prevalence of chewing damage in corn in the no-till system compared to the tilled systems. However, the impacts of tillage are not uniform for all pests (Stinner and House 1990, also see Ch. 2 of this dissertation). We did not
observe greater frequency or severity of damage in no-till-established corn compared to corn established with tillage for other early- or late-season invertebrate pests.

We studied the effects of increasing plant diversity on herbivore damage in two of our systems in which winter cover crops were interseeded into V6 corn. Crop cultivar mixtures have contributed to improved management of pests in other studies (Grettenberger and Tooker 2015, Pettersson et al., 1999, Smithson and Lenne 1996, Tooker and Frank 2012, Underwood, 2009) and may even lead to greater plant productivity (Cook-Patton et al. 2011, Crutsinger et al. 2006). However, those studies focused on increasing cultivar diversity within the same crop or otherwise mixing stands of similar maturity, whereas our study examined newly emerged cover crop seedlings planted between rows of maturing corn plants. The timing of cover crop mixture establishment in V5 corn in our study is intended to prevent rapid growth and cover crop competition with corn (Curran et al. 2018). Therefore, it is possible that the cover crops established in corn were too small to provide any of the benefits of planting diverse mixtures observed in other studies.

**Conclusions**

Overall, specific nuances of crop management within organic cover crop-based reduced-tillage systems appear to have little impact on damage to corn by herbivores. The legume-grass cover crop mixtures which preceded corn in my study did not affect the frequency or severity of damage by early-season pests and did not have consistent impacts on damage by late-season pests. In our study, tillage effectively suppressed damage by early-season pests. As hypothesized, my results suggest that interseeding cover crops into corn has neither beneficial nor detrimental effects on damage to corn by late-season arthropod pests. Thus, using cover crops and reducing
tillage within organic rotations adequately regulates arthropod pests and their associated damage to crops.
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Chapter 5
Seedcorn Maggot Response to Planting Date, Cover Crops, and Tillage in Organic Cropping Systems

Abstract

One of the most important pests for corn and soybean in the northern United States is seedcorn maggot, *Delia platura* (Diptera: Anthomyiidae). Adults flies are known to be attracted to decomposing plant residues, creating potential management issues as growers employ tillage to manage weeds and invertebrate pests and cover crops to improve their crop rotations. Growing degree day models are an important tool for effectively managing this pest but have not been evaluated recently. Cultural tools such as cover crops, tillage, and degree day models are especially important in organic systems where chemical tactics are limited. This chapter studies two organic cropping systems incorporating cover crops and reducing tillage to investigate how planting date, cover crop species mixture and biomass, and intensity and timing of tillage affect seedcorn maggot emergence in corn and soy in central Pennsylvania. I found that delaying planting date reduced fly emergence in corn but not in soy. Emergence was much lower in soy than corn over three years of trapping. Significantly more flies emerged from corn planted into tilled plots than into no-till planted corn with rolled cover crop residue. Fly emergence also exhibited a negative relationship with increasing proportion of legumes in the preceding cover crop mixture. These results can help inform decisions on cover crop mixtures, termination method, and planting date for corn growers in the Mid-Atlantic US.
Introduction

Pests of agricultural crops can have detrimental effects on yield and profitability. Seedcorn maggot (*Delia platura* Meigen) is a pest of large-seeded crops, including corn (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.). They tend to be occasional pests in Pennsylvania, but can have detrimental effects on young seedlings, leading to delayed seedling emergence, reduced plant populations, and increased risk of soil-borne pathogens (Hammond 1991, Hesler et al. 2018). A review of crop pests of soybeans in the Midwest found that despite being one of their most important pests, fewer than 1% of considered seedcorn maggot in their management decisions (Hesler et al. 2018). When conditions are cool and wet in the spring after cash crop planting, there is greater risk of crop injury from seedcorn maggot feeding and subsequent pathogen infection (Hesler et al. 2018). When temperatures exceed 84°F, seedcorn maggot may begin to experience mortality and risk is reduced (Hesler et al. 2018).

Mechanical control through the use of tillage has been shown effective for some insect pests and slugs (Capinera 2017, Douglas and Tooker 2012, Le Gall and Tooker 2017, Mischler et al. 2010, Stinner and House 1990). However, seedcorn maggot has been shown to be more abundant as tillage increases (Hammond 1997). In their review, Hesler et al. (2018) found that damage by seedcorn maggot is rare in no-till fields. No-till and other conservation tillage practices have been increasingly adopted in Pennsylvania (Douglas and Tooker 2012, Myer 2018), which may influence the prevalence of this pest in our region. There is also strong interest in reducing tillage in Pennsylvania and surrounding states among organic growers, who have more challenges to consider when reducing tillage, such as weed, invertebrate pest, and nutrient management (Wallace et al. 2018). In addition to reducing tillage, there has been strong interest in planting winter cover crops in the mid-Atlantic region among both organic and conventional
producers (Wallace et al. 2018, Myer 2018). This added residue could influence host plant location by ovipositing flies and subsequent pest damage.

The increase of damage from seedcorn maggots in tilled systems is likely influenced by olfactory cues from incorporated plant residue. As interest in using winter cover crops increases among organic growers, increasing the amount of residue being incorporated, management of *D. platura* will be increasingly important (USDA NASS 2015). *Delia platura* are attracted to decaying plant residues and exhibit a preference for fields with incorporation of living cover crop residue over residue from a previous crop, which may indicate these freshly killed plants give off stronger cues than older plant residue (Hammond 1991). A related species, the cabbage root fly (*D. radicum*), was effectively managed by a cabbage trap crop supplemented with one of five different repellants that resulted in up to 45% less damage to the target crop of broccoli (Lamy et al. 2017). Damage by *D. radicum* was also reduced when canola, a suitable host plant, was intercropped with wheat, which is not used as a host (Hummel et al. 2008). As the proportion of wheat in the intercropped mixture increased, damage to canola and adult fly abundance decreased, indicating there may have been reduced apparancy of the host plant.

Timing of cash crop planting, particularly after incorporating cover crop residue, can be important to consider. In soybean, *D. platura* damage was reduced when planting was delayed three weeks after tillage (Hammond and Cooper 2003) while damage to soybean was greater when planting was delayed in another instance (Hammond 1991). This effect was attributed to accumulation of growing-degree days prior to the time of planting and the life stage of *D. platura*. Timing of cover crop termination can influence the success of termination in organic reduced-tillage systems and the benefits gained from use of cover crops may further complicate
decisions around planting timing (Keene et al. 2017, Mischler et al. 2010). This may create seedcorn maggot management challenges for growers.

If conditions are favorable, adult seedcorn maggot flies deposit eggs over a brief time period, which can be as short as a few days (Hammond 1991). Maggots will feed on planted seeds as they germinate, preventing successful emergence. After completing larval development, pupation occurs underground and adult flies emerge from the soil seeking mates and oviposition sites (Hammond 1991, Hammond 1997). In Pennsylvania, D. platura can have 4-5 generations per year but the generation that overlaps with corn and soybean planting is the most detrimental (Schmidt et al. 2017). Damage from Delia feeding tends to be greatest one to two weeks after peak emergence (Throne and Eckenrode 1985). Shifting planting date is a cultural practice that has been recommended for D. platura (Schmidt, et al. 2017, Schwarting et al. 2015). By delaying planting until after peak activity of insects, growers can minimize risk to germinating plants. Another pest managed through delayed planting is the Hessian fly (Mayetiola destructor Say) but recent trapping has indicated that “fly-free dates” should be reassessed periodically to ensure their continued accuracy (Scharting et al. 2015). This tactic is particularly critical in organic crops, where synthetic chemicals are restricted and allowed chemical tactics are not often not economical to use in agronomic crops (NOP 2005, USDA NASS 2015). Therefore, organic growers must rely on other tactics such as cultural, mechanical, and biological control. For example, planting in optimal conditions to accelerate germination is an important cultural tactic for both D. platura and the onion maggot, D. antiqua (Wilson et al. 2015.)

In addition to adjusting the timing of planting and residue management, biological control can contribute to management of seedcorn maggot damage. A study that compared conventional and organic brassica fields found similar oviposition by D. radicum but reduced
survival to adulthood, potentially due to increased predation in the organic systems (Meyling et al. 2013). Natural enemies known to feed on species of Delia include two species of rove beetle (Coleoptera: Staphylinidae) that parasitize fly pupae and wasps that parasitize larvae (Hymenoptera) (Hummel et al. 2010). It is not clear how closely tied these groups of natural enemies may be to pest density or crop management. As intercropping wheat with canola reduced pressure from D. radicum, abundance of parasitoids Aleochara verna and A. bilineata were reduced, while abundance of Hymenoptera were unaffected in one study (Hummel et al. 2010). Another study found reduced density of A. bilineata as weed biomass increased (Broatch et al. 2010), which may pose an issue for natural control of seedcorn maggot in organic systems where weeds are a major challenge (Cavigelli et al. 2013, Wallace et al. 2018). Intraguild predation may pose another limitation to the effectiveness of natural control of Delia spp., as small carabid beetles (Carabidae: Coleoptera) and staphylinids are known to consume Delia eggs but also to be consumed by larger carabids commonly present in agricultural systems (Prasad and Snyder 2004). Thus, a greater understanding of how cultural tactics influence natural enemies and their likelihood to contribute to subsequent management of Delia is needed.

As interest in using winter cover crops increases among organic growers alongside interest in reducing tillage, decisions around timing of planting will be further complicated (Keene et al. 2017). My research investigates the combined effects of planting date, cover crop mixture, and tillage a 3-year crop rotation focused on reducing tillage on D. platura emergence in organically managed corn and soybean, as well as impacts on potential natural enemies. I hypothesized that 1) damage will decrease in severity as planting date is delayed, 2) cover crop biomass will be positively correlated with Delia emergence in tilled systems, 3) Delia emergence
will be positively correlated with increased disturbance through tillage practices, and 4) Delia emergence will be reduced where natural enemy abundance is greatest.

**Methods**

**Site Description**

Sampling for *D. platura* was conducted over two three-year periods at the Russel E. Larson Agricultural Research Center at Rock Springs, PA in two different experiments occurring at the same site in different years. Both experiments took place within the Reduced-Tillage Organic Systems Experiments (hereafter, ROSE, for convenience) (Barbercheck et al. 2010, Barbercheck et al. 2009, Champagne 2017, Keene 2015). The ROSE site is approximately 4 hectares, the site has been managed organically since 2011, and it received organic certification in 2014 (NOP 2005). Soil at this site consists primarily of a Hagerstown silt loam (fine, mixed, semiactive, mesic Typic Hapludalfs) with zones of Opequon-Hagerstown soil (clayey, mixed, active, mesic Lithic Hapludalfs) scattered within. This site is in Zone 6b of USDA Plant Hardiness Zones and has temperate humid climate with ~2146 mm of combined rainfall and snowfall precipitation per year. Annual temperature ranges between 5º and 25º C throughout the year.

**Experimental Design and Field Operations**

The first of two three-year experiments was conducted from 2011 to 2013 (hereafter, “ROSE 1.0”) and consisted of four blocks of a full-entry design with all crops present in all years (Barbercheck et al. 2009, Keene 2015). The total cropping area in each block amounted to 6020 m² (2006 m² per crop). Each of the four blocks within the experiment contained three cropping
strips each representing one of three cash crops, corn (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), and wheat (*Triticum aestivum* L.). Crops were rotated annually with cover crops with a complete rotation as follows: corn, cereal rye (*Secale cereale* L.), soybean, winter wheat, and a hairy vetch (*Vicia villosa* Roth) and triticale (*× Triticosecale* Wittmack) biculture.

Winter cover crops were planted in the fall following tillage and seedbed preparation, with a seeding rate of 34 kg seed ha\(^{-1}\) each for hairy vetch and triticale, and 189 kg seed ha\(^{-1}\) for cereal rye. In the spring, we rolled the cover crops prior to planting of corn or at the time of planting of soybean. Corn and soybean were no-till planted into the rolled cover crop mat at 84,000 seeds ha\(^{-1}\) and 556,000 seeds ha\(^{-1}\), respectively, at three dates: early, middle, and late. Cash crop served as main plot with planting date as a subplot. Both corn and soybean were sampled for seedcorn maggot.

Within each planting date subplot, the plots were further split into sub-subplots measuring 18.3 m by 9.1 m (167 m\(^2\)) to compare the effects of high-residue inter-row cultivation to no cultivation in the corn and soybean (335 m\(^2\) per cultivation treatment). The high residue cultivator is equipped with a no-till coulter to cut the residue, which is followed by a single 50 cm wide sweep to sever emerged weeds while leaving the surface residue relatively undisturbed (Keene et al. 2017). The cultivation treatment was used to supplement the weed suppression provided by the cover crop mulch and each plot was cultivated twice about one week apart. In soybean, the treatments receiving cultivation were planted in 76 cm rows, while the no-cultivation treatments were planted in 38 cm rows. Both corn treatments were planted in 76 cm rows.

The second project in which we sampled from 2014-2017 consisted of a full-entry experiment with corn in a three-year rotation with soybean and spelt (*Triticum spelta* L.)
(hereafter, referred to as “ROSE 2.0”; Barbercheck et al. 2014, Champagne 2017). All sampling occurred in corn plots. Crops were arranged in a randomized complete block, split-plot design with four replications. Within each block, the three crops comprised two main plots, separated by grass alleyways to allow field operations, with two split-plots in each main plot (totaling four split-plots per block). Each split-plot measured 9m by 49 m and was managed under one of four cropping systems. Cropping systems differences included cover crop species, their planting date and termination method, manure management, and tillage intensity and frequency. Short planting windows between cash crop harvest in the fall and reduction in temperatures can make establishing cover crops challenging in the fall, especially when harvesting corn for grain rather than silage (Curran et al. 2018). The cover crop treatments in this experiment were chosen to overcome this short planting window in some cases, as well as to improve cover crop benefits to soil, weed management, and arthropod communities.

In all four systems, corn variety ‘MC4050’ (Masters Choice, Anna, IL) was planted on 76-cm rows at 81,510 plt ha⁻¹ using a four-row John Deere 7200 planter equipped with Dawn Biologic ZRX residue managers, typically in late May or early June, depending on the cropping system (Table 3.1). In System 1, liquid dairy manure was broadcast at 74,831 L ha⁻¹ and incorporated via moldboard plow following spelt harvest. A winter cover crop mixture of hairy vetch (Vicia villosa Roth ‘Groff’) plus triticale (x Triticosecale Wittm. ‘815’) was planted at a seeding rate of 34 kg ha⁻¹ each. Cover crops were terminated by rolling with a roller-crimper in the spring to form a mulch on the soil surface into which corn was no-till planted immediately after. The timing of rolling occurred at Mischler 7 growth stage to optimize weed suppression (Mischler et al. 2010). A second rolling pass a week after planting, as well as two passes with a
John Deere 886 high-residue cultivator at four and five weeks after planting were employed to ensure cover crop termination and enhance weed control.

In System 2, no manure was applied in the fall and a chisel plow was used after spelt harvest to prepare the soil for cover crop planting. Hairy vetch and triticale were planted at the same rates as System 1. In Systems 3 and 4, a cover crop mixture of medium red clover (Trifolium pratense L. ‘VNS’) and timothy (Phleum pratense L. ‘VNS’) was no-till drilled into standing spelt at 12 and 4.5 kg ha\(^{-1}\), respectively in early spring. This allowed it to establish prior to spelt harvest, added economic benefit through a fall forage cutting, suppressed weeds, and allowed a longer growing season and period without tillage than the hairy vetch and triticale mixture in the other two systems. After spelt was harvested in early August and the cover crop was able to grow, the cover crop was mowed and prepared for use as forage to prevent weed seed production. After continued growth through the fall and winter, this mixture and the hairy vetch and triticale in System 2 were mowed and incorporated using a moldboard plow. For fertility needs of the corn, manure was broadcast at 74,831 L ha\(^{-1}\) prior to plowing. Corn was planted 7-10 days following tillage. A mix of blind cultivation and inter-row cultivation was used in these systems to control weeds early in the season. Although the frequency varied from year to year depending on weed severity and environmental conditions, all three systems received the same treatment as each other in a given year.

Although Systems 3 and 4 were managed similarly from the previous season until the time of sampling in this experiment, I am analyzing them separately to account for any long-term legacy effects from the three-year rotation.
**Sampling Methods**

To assess effects of management system on seedcorn maggot populations, seedcorn maggot emergence traps were placed at the soil surface shortly after corn emergence to capture emerging adult flies (Hammond 1991). For details on trapping dates and their relation to planting dates in each experiment, see Table 5.1. In ROSE 1.0, one trap was placed in each sub-subplot for both corn and soybean. In ROSE 2.0, two traps were placed in each plot for corn only. Traps consist of a plastic window box (0.6 m x 0.3 m x 0.2 m) with a hole cut in the base that is fitted with a 0.5 L glass mason jar (box: 932-839, Home Depot, Atlanta, GA; jar: 1033891, Jarden Home Brands, Fishers, IN). The traps are placed base side-up with edges buried below soil surface to achieve darkness under the trap so that as flies emerge from the soil, they move towards sunlight visible through the jar and are trapped by a mesh funnel in the opening in the jar (Hammond 1991). Jars were removed and emptied weekly from corn emergence until true leaf formation (V2). If cultivation or other field operations needed to be performed to control weeds, jars were collected and traps were removed until the operation was complete then traps were replaced with fresh jars to complete the sampling period. All arthropods caught were returned to the lab, preserved in 70% ethanol, and identified using established keys (Brooks 1951, Griffiths 1985). As some species of Hymenoptera and Staphylinidae are known to parasitize seedcorn maggot, any of these two taxa caught as by-catch were also used in analysis to investigate effects of crop management on potential natural enemies (Hummel et al. 2010).

**Environmental Conditions**

I used several biotic and abiotic environmental variables from existing data sets affiliated with the ROSE projects to help understand the habitat associated with cropping system and better
understand factors contributing to *Delia platura* abundance across the experimental site. To understand the influence of cover crop biomass and species on oviposition, I used whole plant, above-ground cover crop biomass collected immediately prior to termination in both cover crop treatments using two 0.5m$^2$ quadrats per treatment plot in ROSE 1.0 and nine 0.25m$^2$ quadrats per treatment plot in ROSE 2.0, and converted all to a dry-weight in kg ha$^{-1}$ (dried at 65°C) (Champagne 2017; Keene 2015). Cover crop biomass was assessed 1 week prior to termination. In the no-till plots, which includes all of ROSE 1.0 and System 1 of ROSE 2.0, this biomass contributed to residue at the soil surface while in the other three systems of ROSE 2.0, cover crop biomass was tilled under prior to corn planting, altering the soil environment of these systems early in the season. At the time of cover crop sampling, weed biomass measurements were also taken and reported in kg ha$^{-1}$ (Champagne 2017). For ROSE 2.0, cover crop biomass was split by species, and therefore I was able to use the proportion of the mixture that was legume as a covariate.

To assess the influence of mechanical disturbance through tillage and other field operations on emergence of *D. platura* and its parasitoids, I summed the number of disturbance events that occurred in each plot from the start of each experiment until the time of sampling, as well as within a single season from January 1 until the time of sampling. I split these into above-ground events, such as mowing or planting, and soil events to represent the frequency of tillage events. Additionally, to represent the intensity of disturbance, we used a USDA Natural Resources Conservation Service soil disturbance rating (SDR) obtained through RUSLE2 (NRCS 2008). The SDR, which ranges from 0 (least disturbance) to 30 (greatest disturbance) for a field operation, is comprised of the sum of six ratings each with values from 0 to 5 that estimate the relative severity of disturbance. The six component categories of the SDR include
soil inversion, soil mixing, soil lifting, soil shattering, soil aeration, and soil compaction. The field operation that we employed with the highest SDR was tillage with a moldboard plow with an SDR of 29, and one of the lowest was flail mowing with an SDR of 3. As with frequency of events, these were summed in each plot from the start of each experiment until the time of sampling, as well as within a single season from January 1 until the time of sampling. A previous study dealt with multiple passes of a similar tool by including it in a model that accounts for temperature and soil texture in calculating overall disturbance and turnover of the soil (Kemanian and Stöckle 2010). Although repeated passes of the same tillage implement may have redundant impacts on soil, little is known about whether the effect of multiple passes of the same tillage implement has a different effect on arthropods than one pass. Thus, I opted to use all reported passes to calculate a total intensity of disturbance for this usage of SDR and am relying on it as a means to compare each cropping system standardized by the weed management used in each year, rather than a true measure of disturbance.

Because *D. platura* populations are highly regulated by growing degree days, I recorded the growing degree days accumulated (GDD) at the start and end dates for trapping for each year (NEWA 2018) and used these in analysis. I used Julian date for cash crop planting (Table 5.1). Because the traps I used are designed to catch adults, I expected the number of flies caught in traps to be influenced by the growth stage of flies at the time of trapping. I used reports of GDD required for *D. platura* to complete its life cycle to estimate which life stage the insect would be at the time of sampling (Cullen 2010, Funderburk et al. 1984; Table 5.1).
Data Analysis

I analyzed all data in R Version 3.5.1 (R Core Team 2018). Samples from each plot were summed across the entire sampling period for both experiments and divided by the length of the sampling period to obtain a mean number of emerged arthropods per day. For ROSE 2.0, I calculated the mean of the daily captured arthropods of the two traps in each plot. *Delia platura* abundance was analyzed using linear-mixed models (function lme) in the nlme package (Pinheiro et al. 2013). Additionally, Hymenoptera and Staphylinidae abundance were analyzed to account for their potential to parasitize *Delia* (Hummel et al. 2010). Data were either square root transformed or cube root transformed where needed to meet assumptions of the model, including normal distribution and homogeneity of variance. For each arthropod group, experiment, and cash crop, I specified a model using block nested within year as random effects and treatment (either planting date for ROSE 1.0 or cropping system for ROSE 2.0) as a fixed effect. I conducted post hoc, pairwise tests of means between each treatment level using Tukey’s honest significant difference test. I used the varcomp function in the ape package to partition the variance explained by each model by year and block.

To identify relationships between arthropod relative abundance and environmental conditions, I used multiple linear regressions with either *D. platura*, Hymenoptera, or Staphylinidae abundance as the response variable and various environmental values as explanatory variables, and used stepwise selection using the stepAIC function in the MASS package in R to reduce the complexity of the full models (Schmidt and Rypstra, 2010; Venables and Ripley, 2002). For *D. platura*, both Hymenoptera and Staphylinidae were included in the model to gauge relationships with potential natural enemies. For either of the parasitoid groups, the other parasitoid group was included to gauge competitive effects and *D. platura* was included
to gauge the effect of prey abundance. Models were compared by Akaike’s Information Criteria (AIC), with the final model having the lowest AIC (Kutner et al., 2005; Murtaugh, 2009). Environmental variables for all models included day of year for planting, start of trapping, and end of trapping, GDD at the start of trapping and end of trapping, year, tillage treatment, preceding cover crop species, cash crop, cover crop biomass, weed biomass, cumulative SDR, annual SDR, cumulative number of soil disturbance events, annual number of soil disturbance events, and the estimated growth stage of *D. platura* during sampling, as calculated using growing degree day models (Cullen 2010, Funderburk et al. 1984; Table 5.1). Models for ROSE 1.0 also included planting date treatment while models for ROSE 2.0 included cropping system treatment, annual number of above-ground disturbance events, and the proportion of legume in the cover crop species mixture.
Table 5.1. Cash crop planting and emergence trapping dates for ROSE 1.0 and 2.0, growing degree days reported by NEWA at the time of trapping and planting included, and an estimation of the growth stage *Delia platura* would be in during trapping based on degree days required to complete life cycle.

<table>
<thead>
<tr>
<th>ROSE 1.0</th>
<th>Corn Planting</th>
<th>Soy Planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date</td>
<td>GDD</td>
</tr>
<tr>
<td>Late 2011</td>
<td>6/16/11</td>
<td>1787</td>
</tr>
<tr>
<td>Early 2012</td>
<td>5/31/12</td>
<td>1645</td>
</tr>
<tr>
<td>Middle 2012</td>
<td>6/7/12</td>
<td>1794</td>
</tr>
<tr>
<td>Late 2012</td>
<td>6/15/12</td>
<td>2032</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ROSE 2.0</th>
<th>Corn Planting</th>
<th>Soy Planting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Date</td>
<td>GDD</td>
</tr>
<tr>
<td>Tilled (Sys 2-4) 2015</td>
<td>5/29/15</td>
<td>1046</td>
</tr>
<tr>
<td>No-Till (Sys 1) 2016</td>
<td>6/9/16</td>
<td>1406</td>
</tr>
<tr>
<td>Tilled (Sys 2-4) 2016</td>
<td>5/26/16</td>
<td>991</td>
</tr>
<tr>
<td>Tilled (Sys 2-4) 2017</td>
<td>6/2/17</td>
<td>1364</td>
</tr>
</tbody>
</table>

176
Results

Effects of Planting Date and Cash Crop

Within ROSE 1.0, I investigated the effect of delayed cover crop termination and subsequent cash crop planting on emergence of *Delia platura* in both corn and soy. In corn, planting date had a significant effect on fly emergence with fewer flies emerging in the Late planting date than in Early or Middle planting (F\textsubscript{2,22} = 7.64, P < 0.01; Fig. 5.1A). Year explained 25% of the variation in fly emergence in corn and emergence was lower in 2013 than in the previous two years. Spatial variability, accounted for through inclusion of block as a random factor, explained 11% of the variation in fly emergence in corn. In soybean, there was no significant effect of planting date on fly emergence (F\textsubscript{2,22} = 1.39, P = 0.27; Fig. 5.1D). Neither year nor block explained a substantial amount of variation, explaining 3% and 0% of variation in fly emergence in soybean plots, respectively.

Fly emergence in soybean was significantly lower than in corn (F\textsubscript{1,55} = 11.67, P < 0.01; Fig 5.1). In multiple regressions of environmental factors influencing *D. platura* emergence from corn and soybean plots, the planting date treatments had the strongest relationship for both cash crop models (F = 2.71, P = 0.03 for corn, F = 0.42, P = 0.02 for soy; Table 5.2). In corn in ROSE 1.0, the date trapping began, year, and estimating *D. platura* to be in larval stage during trapping all had significant but weak negative relationships with fly emergence while estimating flies to be in pupal stage had a significant but weak positive relationship with adult fly emergence during the trapping period (Table 5.2).
Effects of Cover Crops and Tillage

Within ROSE 2.0, I investigated the effect of two different grass-legume bicultures preceding corn and the use of tillage prior to corn planting on emergence of *D. platura*. Cropping system significantly affected fly emergence with the lowest emergence in System 1, which was planted no-till into rolled hairy vetch and triticale, intermediate numbers of flies emerging in Systems 2 and 3, which differ in preceding cover crop but were both tilled, and highest emergence in System 4, which was tilled and preceded by red clover and timothy (*F*₃,₃₃ = 4.50, *P* < 0.01; Figure 5.2A). Although the species composition of cover crop biculture did not have a significant effect on fly emergence in a multiple regression, the proportion of each biculture that was a legume was negatively related to fly emergence (*F* = -3.24, *P* < 0.001; Table 5.3).

Neither the intensity of disturbance, as measured by SDR, nor frequency of disturbance in each plot during the season and during the preceding time throughout the experiment influenced *D. platura* emergence in individual crop and experiment regression models, but had weak significant relationships with fly emergence when corn plots were combined over both experiments (Table 5.4). In this case, both cumulative SDR from the beginning of each ROSE and annual SDR within each growing season had slight negative relationships with fly emergence in corn.

Year-to-year variation had a substantial effect on fly emergence in ROSE 2.0, explaining 66% of variation in variance partitioning of the ANOVA model and producing the largest F value in the multiple regression model (*F* = 4.93, *P* < 0.001; Table 5.3). Block explained 5% of variation in fly emergence in ROSE 2.0 corn plots. Year also had the greatest F value when emergence from corn was combined over both ROSE 1.0 and ROSE 2.0 in a multiple regression.
model \(F = -3.22, P < 0.001; \text{Fig.} \ 5.4\), exhibiting a stronger relationship with \textit{D. platura} emergence than either cover crop biculture or disturbance.

\textit{Natural Enemy Response to Crop Management}

Abundances of parasitoid wasps (Hymenoptera) and rove beetles (Staphylinidae: Coleoptera) caught in \textit{Delia platura} emergence traps were investigated in response to cropping system. Although I did not identify specific natural enemies of \textit{D. platura}, I was interested in effects of crop management on the abundance of natural enemies co-occurring in the traps. Planting date did not significantly affect wasp emergence from either corn or soybean or rove beetle emergence from soybean during ROSE 1.0. Rove beetle emergence was significantly different between planting dates in ROSE 1.0 corn, though these effects were inconsistent by year with significantly fewer beetles emerging in the Middle planting date than either Early or Late in 2012 and significantly fewer in the Early date than either Middle or Late in 2013.

With the exception of Hymenoptera emergence in 2015, preceding cover crop biculture had no impact on natural enemy emergence in ROSE 2.0 corn. In 2015, there was a trend for more wasps to emerge from the two systems (System 1 and 2) preceded by hairy vetch and triticale compared to red clover and timothy (System 3 and 4). System 2, which was tilled, was not significantly different from System 3 and 4. Although ROSE 1.0 had very different cover crops preceding the cash crops, with hairy vetch and triticale before corn and cereal rye before soy, any effect of rye could not be separated from effects of cash crop since it was only planted prior to soybean. Rove beetle emergence significantly and positively related to the proportion of legume in the cover crop biculture preceding corn in ROSE 2.0 \(F= 0.50, P = 0.02; \text{Table} \ 5.3\). No strong relationships with cover crops or weeds emerged for either natural enemy group in
ROSE 1.0 corn or soybean regressions, nor with the model combining corn for ROSE 1.0 and 2.0.

Tillage had a consistent negative impact on both Hymenoptera ($F_{3,33} = 17.68, P < 0.001$; Figure 5.2B) and Staphylinidae ($F_{3,33} = 7.60, P < 0.001$; Figure 5.2C) emergence in ROSE 2.0. Wasp emergence was significantly greater in System 1, which was no-till planted, than in the three tilled systems in 2016 and 2017 (Fig. 5.2B). Emergence of rove beetles was greatest in System 1 in 2015 and 2017 (Fig. 5.2C). In 2015, significantly more wasps emerged from System 1 than Systems 3 and 4, which were both tilled and preceded by red clover and timothy but System 2 had intermediate emergence between these different systems. Year and block explained only 1% and 6% of variation in wasp emergence from corn in ROSE 2.0 through variance partitioning of the ANOVA model. Rove beetle emergence was low for all four systems in 2016 and year explained 12% of variation in Staphylinidae emergence from corn in ROSE 2.0. Cropping system had the strongest relationship with rove beetle emergence in ROSE 2.0 ($F = -1.08, P < 0.001$; Table 5.3). In the model combining corn in ROSE 1.0 and 2.0, tillage had the strongest relationship with rove beetle emergence ($F = 10.81; P < 0.001$; Fig. 5.4).

In multiple regressions to determine the association of environmental factors with arthropod emergence, I included *D. platura* abundance in models for each natural enemy and natural enemies in the model for *D. platura* to determine if either prey or predator abundances were related. I included Hymenoptera and Staphylinidae emergence in models for each to account for potential competition between the two groups. There was a weak positive relationship between each natural enemy and *D. platura* emergence in ROSE 1.0 corn (Table 5.2) and for wasp emergence and *D. platura* emergence in the overall corn model combining ROSE 1.0 and 2.0 (Table 5.4). However, there was no relationship between either natural enemy
and *D. platura* emergence in the ROSE 2.0 corn model (Table 5.3). In ROSE 1.0, *D. platura* emergence had the strongest relationship of any factor with wasp emergence, though this relationship was weak (F = 0.5, P = 0.04; Table 5.2). Wasp emergence was positively related to rove beetle emergence in ROSE 2.0 (F = 0.86, P < 0.01; Fig. 5.3). This relationship also appeared in the rove beetle model, though it was weaker than in the wasp model. For ROSE 2.0, wasp emergence had a significant negative relationship with estimating *D. platura* to be in larval or pupal stage during trapping (Table 5.3). In ROSE 1.0, rove beetle emergence had the strongest relationship with estimated pupal *D. platura* during the trapping period in corn (F = -12.12; P < 0.001; Table 5.2) and second strongest relationship with *D. platura* estimated to be in larval stage during trapping in corn (F = -1.07; P < 0.001; Table 5.2).
Figure 5.1. The number of *Delia platura* that emerged per trap per day in corn (A) and soybean (D) in ROSE 1.0, which assessed the effect of planting date on arthropod emergence. The number of Hymenoptera in corn (B) and soybean (E) and Staphylinidae in corn (C) and soybean (F) that emerged per trap per day are shown in each year of the experiment. Letters represent means that are significantly different at the 0.05 level for that particular crop and year. Icon
Figure 5.2. The number of *Delia platura* (A), Hymenoptera (B), and Staphylinidae (C) that emerged per trap per day in corn in ROSE 2.0. Purple bars represent cropping systems preceded...
by hairy vetch and triticale (HV), red bars represent cropping systems preceded by red clover and timothy (RC), and crosshatched bars represent corn no-till planted into rolled cover crop residue (NT). Systems 2 and 4 are denoted with an I to represent interseeding that would occur in those systems later in the season. Letters represent means that are significantly different at the 0.05 level for that year. Icon credits: Diptera and Hymenoptera, G. Monger, K. S. Jaron from phylopic.org; Staphylinidae, N. Sloff.
Table 5.2. Relationship between *Delia platura*, Hymenoptera, and Staphylinidae with environmental variables in corn and soybean in ROSE 1.0.

<table>
<thead>
<tr>
<th>Factor</th>
<th><em>Delia platura</em></th>
<th><em>Hymenoptera</em></th>
<th><em>Staphylinidae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corn</td>
<td>Soy</td>
<td>Corn</td>
</tr>
<tr>
<td></td>
<td>Estimate Pr &lt; 0.05</td>
<td>Estimate Pr &lt; 0.05</td>
<td>Estimate Pr &lt; 0.05</td>
</tr>
<tr>
<td><em>D. platura</em></td>
<td>---</td>
<td>---</td>
<td>0.83 **</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td>0.07 **</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Staphylinidae</td>
<td>0.31 **</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Year</td>
<td>-0.34 ***</td>
<td>0.09 **</td>
<td>0.4 *</td>
</tr>
<tr>
<td>Planting date treatment</td>
<td>2.71 *</td>
<td>0.42 *</td>
<td>---</td>
</tr>
<tr>
<td>Julian planting date</td>
<td>---</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>Estimated <em>Delia</em> growth stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>egg</td>
<td>---</td>
<td>-0.21 *</td>
<td>---</td>
</tr>
<tr>
<td>larva</td>
<td>-0.44 *</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>pupa</td>
<td>0.43 *</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>adult</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Date trapping began</td>
<td>-0.29 *</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Accum. GDD during trapping</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Total vegetation</td>
<td>---</td>
<td>NS</td>
<td>---</td>
</tr>
<tr>
<td>Total weed biomass</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

* Significant at the 0.05 level, ** significant at the 0.01 level, *** significant at the 0.001 level

Factors that were removed from the stepwise model denoted with ---, NS indicates factors included in the model with P > 0.05
Table 5.3. Relationship between *Delia platura*, Hymenoptera, and Staphylinidae with environmental variables in corn in ROSE 2.0.

<table>
<thead>
<tr>
<th>Factor</th>
<th><em>Delia platura</em></th>
<th>Hymenoptera</th>
<th>Staphylinidae</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. platura</em></td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td>---</td>
<td>0.14 **</td>
<td>---</td>
</tr>
<tr>
<td>Staphylinidae</td>
<td>0.86 **</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Year</td>
<td>4.93 ***</td>
<td>0.58 **</td>
<td>---</td>
</tr>
<tr>
<td>Cropping system</td>
<td>---</td>
<td>-1.08 ***</td>
<td>---</td>
</tr>
<tr>
<td>Julian planting date</td>
<td>NS</td>
<td>0.14 ***</td>
<td>-0.08 **</td>
</tr>
</tbody>
</table>

Estimated *Delia* growth stage:

<table>
<thead>
<tr>
<th>Stage</th>
<th><em>Delia platura</em></th>
<th>Hymenoptera</th>
<th>Staphylinidae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Larva</td>
<td>NS</td>
<td>-1.11 ***</td>
<td>-0.08 *</td>
</tr>
<tr>
<td>Pupa</td>
<td>NS</td>
<td>-1.69 **</td>
<td>NS</td>
</tr>
<tr>
<td>Adult</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Cumulative SDR ~0 ** --- ---

Total vegetation --- -0 ** -0 *

Proportion legume in cover crop -3.24 *** NS 0.5 *

Cover crop biomass NS --- NS

Factors removed from the stepwise model denoted with ---, NS indicates factors included in the model with $P > 0.05$, * significant at the 0.05 level, ** significant at the 0.01 level, *** significant at the 0.001 level

---

Table 5.4. Relationship between *Delia platura*, Hymenoptera, and Staphylinidae with environmental variables in corn in both ROSE 1.0 and ROSE 2.0.

<table>
<thead>
<tr>
<th>Factor</th>
<th><em>Delia platura</em></th>
<th>Hymenoptera</th>
<th>Staphylinidae</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>D. platura</em></td>
<td>---</td>
<td>0.33 **</td>
<td>---</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td>0.27 **</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Staphylinidae</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Year</td>
<td>-3.22 ***</td>
<td>-2.22 **</td>
<td>-8.18 ***</td>
</tr>
<tr>
<td>Tillage</td>
<td>---</td>
<td>-1.09 ***</td>
<td>10.81 ***</td>
</tr>
<tr>
<td>Cover crop biculture</td>
<td>NS</td>
<td>---</td>
<td>0.28 **</td>
</tr>
<tr>
<td>Julian planting date</td>
<td>---</td>
<td>---</td>
<td>1.09 ***</td>
</tr>
</tbody>
</table>

Estimated *Delia* growth stage:

<table>
<thead>
<tr>
<th>Stage</th>
<th><em>Delia platura</em></th>
<th>Hymenoptera</th>
<th>Staphylinidae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg</td>
<td>---</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Larva</td>
<td>---</td>
<td>NS</td>
<td>-1.13 ***</td>
</tr>
<tr>
<td>Pupa</td>
<td>---</td>
<td>0.62 *</td>
<td>4.1 ***</td>
</tr>
<tr>
<td>Adult</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Date trapping began NS -0.02 ** -0.75 ***

Date trapping ended -0.1 * --- -0.63 ***

Accum. GDD during trapping --- -0 ** -0.39 ***

Cumulative SDR -0 * NS -0 *

Annual SDR -0.03 *** --- NS

# of soil disturbance events --- --- 1.88 ***

Cover crop biomass NS NS NS

Factors removed from the stepwise model denoted with ---, NS indicates factors included in the model with $P > 0.05$, * significant at the 0.05 level, ** significant at the 0.01 level, *** significant at the 0.001 level

186
Discussion

Organic producers have limited access to chemical control materials for management of pests, which can lead to challenges in managing insect pests, such as seedcorn maggot, *Delia platura* (Diptera: Anthomyiidae) (Hammond 1991, USDA NASS 2015). I investigated two cultural tactics, shifting planting date and the use of tillage to manage preceding cover crop residue, for their potential to influence *D. platura* emergence in corn and soybean, and the effect of these tactics on emergence of two groups containing potential parasitoids, Hymenoptera and Staphylinidae. I hypothesized that numbers of emerged *D. platura* would be lower as planting was delayed, greater as biomass of the preceding cover crop increased, greater where tillage was used prior to planting, and lower as potential natural enemy emergence increased. Additionally, I used multiple regressions to investigate relationships between arthropod emergence and growing degree days, cover crop species, and the estimated life stage of *D. platura*.

My hypothesis that fewer flies would emerge as planting date was delayed was supported by my results in corn. However, there was no effect of planting date on fly emergence in soybean. Variation in emergence was lower by year for soybean than corn. I captured fewer emerging flies in soybean overall than in corn, so the low emergence may have prevented me from capturing differences by date or by year for soy. Emergence was related to estimated life stage of *D. platura*. The traps I used are designed to catch adult flies as they complete pupation and emerge from the soil, so flies must be adults to be trapped (Hammond 1991). The relationship between emergence and estimated life stage of *D. platura* was negative for larvae and positive for pupae. Although sufficient GDD had accumulated over some of the trapping periods for flies to complete an entire pupal stage, differences in emergence associated with life stage may have been because flies already in the pupal stage were more likely to accumulate
sufficient GDD to complete pupation and emerge as adults during the trapping period compared to flies still in the larval stage (Cullen 2010, Funderburk et al. 1984, NEWA 2018). Additionally, planting dates differed between the two cash crops with soybean typically planted earlier, so the earlier date treatments in soybean had fewer trapping events that overlapped with adult fly emergence than corn (Fig. 5.1). This highlights the importance of considering growing degree days and the life stage of pests as planting decisions are made (Hammond 1991, Silver et al. 2018, Werling et al. 2006). I detected a much stronger effect of year and of planting date than any of the factors using Julian day or GDD. Delia flies prefer cool, moist soil conditions in the spring (Hesler et al. 2018). Across ROSE 1.0 and 2.0, year had a much stronger effect on fly emergence than any other aspect of crop management. As a variable, year may have captured effects of both time and environmental conditions within the field, including rainfall and soil moisture, in a way that GDD alone could not.

Emerging D. platura, D. radicum, and D. antiqua, use olfactory cues to locate hosts and D. platura use cues from decomposing plant residue (Lamb and Boivin 2018, Lamy et al. 2017). The species composition and amount of plant residue affects D. platura oviposition in soybean (Hammond 1991, Hammond 1993). I did not detect any strong relationships between fly emergence and the amount of plant biomass, either through cover crops or weeds. I did find that fewer flies emerged as the proportion of legume in each legume-grass biculture increased. Additionally, I observed greater emergence from corn, which is a grass, than from soybean, which is a legume. This is in contrast to a previous study that found greater emergence from treatments where alfalfa had been incorporated into the soil compared with incorporated cereal rye or corn residue (Hammond 1991). Although legumes are included in cover crop mixtures to provide nitrogen to the following crop, these residues take time to decompose (Wallace et al.
In humid temperate climates, seedcorn maggot is only problematic early in the season when manure is freshly applied but cover crops have only just begun to decompose (Hammond et al. 1993, Schmidt et al. 2017). The addition of liquid dairy manure in tilled corn plots may have influenced the olfactory cues available to adult flies, indicating more suitable oviposition sites (Bhattacharyya et al. 2007, Rengel et al. 1999). Alternatively, legumes may have better supported natural enemy populations, which could potentially reduce *D. platura* emergence (Rivers et al. 2018).

As hypothesized, tillage affected emergence of *D. platura* in corn, with fewer flies emerging from no-till planted corn compared to systems with corn planted with tillage. This result is consistent with long-term studies of tillage related to seedcorn maggot (Hammond 1997). However, this pattern was only evident in one year, in substantially more flies emerged than in any other year in the two experiments. The inconsistent effects of tillage may have been due to the fairly high seeding rates of cover crops used in both ROSE trials, resulting in greater biomass of rolled residue and providing greater sources of olfactory cues for oviposition than in a typical no-till rotation (Keene et al. 2017, Myer 2018). Additionally, the lower emergence than expected of seedcorn maggot in tilled systems could be due to the frequent number of disturbances in our tilled systems. Pest management in organic systems is strongly geared towards weed management and the multiple secondary tillage events required early in the growing season may have been more influential than the primary tillage event before planting (Cavigelli et al. 2017, Keene et al. 2017). Previous studies of the relationship between *Delia platura* and tillage have focused on incorporation of residues with tillage prior to cash crop planting, without considering secondary tillage events after oviposition (Hammond 1991,
Hammond 1993, Hammond 1997). The additional tillage events may have caused mortality of *D. platura* after oviposition in the ROSE trials.

Natural enemies, especially those associated with soil, can be supported by the presence of cover crops and reduction in tillage (Shearin et al. 2007, Woodcock et al. 2010, Kosewska et al. 2014, Rivers et al. 2016). However, they are also dependent upon access to adequate food resources for survival and reproduction (Letourneau et al. 2012). I observed a negative effect of tillage for natural enemies, with greater parasitoid abundance for both taxa in no-till corn than in tilled corn plots in most years. There was little overlap in factors that corresponded to the greatest *D. platura* emergence and factors that corresponded to the greatest natural enemy emergence. This could indicate that the natural enemies emerging from traps in these systems are not using *D. platura* as hosts or prey.

Planting date had less impact on parasitoid wasp and rove beetle emergence than fly emergence. Both wasp and rove beetle emergence were greater in no-till treatments than in tilled treatments, indicating differences in mortality or conditions conducive for these arthropod groups. Rove beetle emergence in cover crop mixtures was positively related with proportion of legume in the mixture. This could indicate that rove beetles effectively reduced fly emergence where the proportion of legume was greater. However, the relationship between parasitoid wasp and rove beetles and *D. platura*, was weakly positive or absent, rather than negative. This could indicate that emergence of either flies or natural enemies related more strongly to favorable habitat conditions than to the presence of each other, unlike my hypothesis of natural enemy emergence reducing *Delia platura* emergence. A previous study reported competition between two rove beetle species feeding on *D. radicum* (Bili et al. 2016). In ROSE, any relationship between the parasitoid wasps and rove beetles was weakly positive, indicating competition was
likely not a factor in their successful emergence. Another study reported reduced parasitism of *D. radicum* by Staphylinidae in canola intercropped with wheat but no effect on Hymenoptera. In ROSE, both groups appear to co-occur where tillage is reduced.

Overall, crop management appears to have strong influence on emergence of *Delia platura* in organic corn and soybean, as well as on the emergence of two parasitoid groups. Fly emergence was decreased with delay in planting date of corn. It is unclear from my results whether this translates to reduced damage or whether this benefit extends to soybean, as other studies have found (Hammond 1993). Fly emergence was not affected by the species composition of the cover crop biculture preceding corn or cover crop biomass in either corn or soybean, though emergence was lower as the proportion of legumes in each biculture increased. Systems in which corn was established with tillage experienced greater fly emergence than no-till corn, though this result was not consistent. Rove beetle emergence increased with the proportion of legume and both beetle and wasp emergence was consistently greater in no-till compared to tilled corn, indicating using cover crop mixtures containing legumes and reducing tillage can help support natural enemies while potentially reducing fly emergence. Based on my results, planting date, cover crop functional group composition, and tillage are all cultural practices that hold potential for effectively management seedcorn maggot emergence in organic corn and soybean.
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Chapter 6

Conclusion

Although all crop producers face potential trade-offs among practices to control pests, and manage crops and soil fertility, these trade-offs can be especially challenging to balance for organic producers. Reducing tillage in organic systems without compromising agricultural productivity has been an important and relevant research focus in the mid-Atlantic region and elsewhere to help growers save fuel and labor, improve soil health, and support soil biological activity (Keene et al. 2017, Mischler et al. 2010, Rivers 2016, Wallace et al. 2018). With limited access to insecticides for managing invertebrate pests, conservation of natural enemies and their services can be one of the most important pest management tactics that is available to organic producers (NOP 2005).

Reducing tillage in conventional systems with or without the addition of cover crops has been researched globally, including effects on arthropods (Brévault et al. 2007, Fernandez et al. 2008, Kosewska et al. 2014, Manetti et al. 2013). The use of these practices in organic systems is newer and has been of particular focus in the Mid-Atlantic region of the US (Jabbour et al. 2016, Mischler et al. 2010, Mirsky et al. 2013, Rivers 2016, Wallace et al. 2017). There is a growing body of research on these practices in the Mid-Atlantic and in other regions of the United States focused on agronomic production, cover crop performance and termination, and weed management (Reberg-Horton et al. 2012, Silva and Delate 2015), but effects of these combined practices on arthropods in organic systems are not as well understood outside of the Mid-Atlantic (Jabbour et al. 2016, Rivers 2016). Adoption of organic production methods, reduced-tillage, and winter cover crops can all influence pests and predators within agroecosystems. However, these management practices are often studied in isolation. In this dissertation, I address how
combinations of tillage and cover crops affect arthropods in organic production systems. Specifically, I investigated the response of arthropod pests and predators to four organic cropping systems incorporating cover crops and reducing tillage, as well as reviewing the overall effects of tillage on pests and predators across conventional and organic production systems.

The mixture of cover crops preceding corn had little effect on pests or predators. Ground beetles had greater activity density when trapped in standing hairy vetch and triticale than in red clover and timothy and there was a trend for more predators overall. Despite this, predation rate was not different between the two mixtures. I would encourage research into the effects of cover crop species or mixture choice on pests, predators, and biological control to continue. I only evaluated two cover crop mixtures that both consisted of one legume and one grass. Additionally, the timing of planting cover crops was different among the two mixtures to incorporate additional production benefits. We frost-seeded the red clover and timothy mixture the previous spring, allowing an extended period of time without tillage and forage production whereas we planted hairy vetch and triticale in early fall. Thus, if I had found more differences between these two mixtures on arthropods, it would have been difficult to separate preferences for particular plant species mixtures from effects of the time since they were established.

Although the cover crop species I evaluated are commonly used as cover crops, there are many other species commonly planted, as well. Hairy vetch, which was one of the species used before corn, provides high biomass and nitrogen but can be difficult to manage (Mischler et al. 2010, Wallace et al. 2018). Many no-till growers in Pennsylvania plant cereal rye instead, particularly conventional growers who have access to fertilizer sources that organic growers do not (Myer 2018). Planting mixtures, rather than choosing a single species, has been growing in popularity and can provide a range of benefits to producers (Murrell et al. 2017, Schipanski et al.
2017), but make it challenging to tease apart preferences among arthropods for particular plant species (Landis et al. 2000). Understanding whether the species of cover crop matters for generalist predators and for pests in these organic cropping systems will likely continue to be a relevant question as cover crop adoption grows.

My hypothesis that tillage would consistently have negative impacts on pests and predators was not supported by my results. In the meta-analysis of tillage, we demonstrated that tillage has no consistent effect on pests or foliar predators, though soil-dwelling predators were more abundant when tillage was reduced. The response of natural enemies and pests in my field research experiments were taxon- and context-specific. Of the most common generalist predators in the system, Araneae had lower activity density in response to tillage, while Carabidae and Opiliones were unaffected. Predation rate was also unaffected by tillage. Staphylinidae had variable responses to tillage with greater activity densities in pitfall traps in late June but lower emergence in emergence traps in early June, so the ability to detect Staphylinidae’s response to tillage seems to depend on sampling method and timing. The percentage of plants affected by slugs and pests feeding on the corn whorl early in the season was unaffected by tillage, though it was lower for chewing pests in tilled systems. The severity of early-season damage was unaffected by tillage, indicating that chewing damage on more corn plants will likely not reduce corn yield. As hypothesized, seedcorn maggot emergence was greater in tilled systems than no-till, indicating trade-offs in pest management for organic growers as they make tillage decisions for their production system.

My ability to detect impacts of tillage on predatory arthropods and early season pests may have been limited by experimental design. The ROSE project employed experimental plots as replicates for each cropping system at a single location in central Pennsylvania. The distribution
of pest populations within a given area can influence the accuracy of sampling. Arthropods are able to move between treatment plots or other areas within a field (Duffield and Aebischer 1994, Hinds 2017, Prasifka et al. 2005, Swezey et al. 2013). Ground dwelling predators including Carabidae, Staphylinidae, and some Araneae species can recolonize areas disturbed by insecticide application by first recolonizing field edges and moving inward (Duffield and Aebischer 1994). In a review of plot sizes used for sampling pests, Cantelo (1986) observed that the number of corn earworm (*Helicoverpa zea* Boddie) eggs detected in a plot changed substantially as plot size increased from 3 to 31 m² and larvae changed as plots increased from 3 to 14 m². Although the plots used in ROSE exceeded 31 m², their shape was long and narrow which may have provided more edge habitat than is ideal for accurate sampling. Corn earworm is not known to be concentrated in edge habitats (Cantelo 1986) but other species are (Duffield and Aebischer 1994). Thus, reducing the relative amount of edge habitat may have better enabled me to detect differences in predator activity density and pest damage between the different cropping systems, particularly between tilled and no-till corn plots. Additionally, although I sampled in two different cover crop bicultures, only one of these bicultures was evaluated using roller-crimping rather than moldboard plowing to terminate the cover crop and prepare the seedbed for corn planting. This prevented me from being able to test effects of the species of residue present, rather than the presence of residue alone.

In addition to limitations within the experimental site, I have interpretation limitations from the site itself. The single location of ROSE was at a university-managed research farm which is likely under different management, with greater crop diversity and with different surrounding landscape than a typical Pennsylvania agronomic crop farm (Myer 2018, Wallace et al. 2017, Wallace et al. 2018). I used a site that has been managed organically for 4 years prior to
the beginning of my data collection and under relatively similar management (Rivers 2016, Keene et al. 2017). However, effects of tillage for arthropods and other aspects of the field can take several years to be fully realized (Stinner et al. 1988). The benefits of organic management also tend to increase over time (Delate et. al. 2015, Jabbour et al. 2016, Rivers 2016). Both pest and predator populations vary by location, even within a similar region (Bohnenblust et al. 2014, Jeanneret et al. 2003, Lundgren et al. 2015, Wyckhuys and O’Neil 2006). It is unclear how my results may differ if evaluated in other regions, as arthropod communities, their response to soil management and vegetative resources, and the scale of production change. The design of the experiment as a systems experiment, rather than a factorial design where I could evaluate individual factors and their combinations, makes it challenging to tease apart whether individual components have an effect. For example, it is impossible to tease apart effects of rolled cover crop residue and lack of tillage in System 1 from potential effects of having manure applied in the fall, rather than spring.

Another factor that can influence the composition and activity of pest and predator communities is the surrounding plant diversity, either adjacent to the field or within the field through weeds, cash crops, and cover crops (Bickerton et al. 2015, Landis et al. 2000, Fahrig et al. 2015, Prasifka et al. 2006, Rivers et al. 2018). I evaluated within-field plants as factors through the use of interseeding to establish cover crops in-season as part of the four cropping systems, as well as by using the weed community in each system as a covariate. Weed diversity and biomass did not have strong effects on either pests or predators. The use of interseeding to establish cover crops following corn had little effect on predator activity-density and no effects on predator diversity. There were no consistent impacts of interseeding on either late-season pest damage or predation rate late in the season. Based on my results, interseeding appears to be a
viable option for overcoming short fall planting windows and establishing cover crops prior to corn grain harvest without risk of increasing pest damage.

Other research has detected benefits to pest management and plant productivity from the use of intercropping (Bickerton et al. 2012, Cook-Patton et al. 2011, Crutsinger et al. 2006). I did not detect any additional pest challenges from the use of interseeding to establish cover crops. However, I only tested effects of one interseeded plant mixture, focused all of my sampling in corn, and limited natural enemy sampling to those found on the soil surface. Many pests spend time in the corn canopy, especially late in the season. Although I did not detect clear benefits for natural enemies or predation, it is possible that there were benefits above-ground that I did not measure and sampling for effects on foliar predators and biological control would be a valuable next step.

This study is one of few that evaluate specific differences in management within established organic systems, instead of comparing arthropod communities in organic management to conventional management (Caprio et al. 2015, Eyre and Leifert 2011, Fusaro et al. 2016, Kirchmann et al. 2016) or tracking arthropod response during the transition to organic management (Jabbour et al. 2016, Rivers 2016). However, one other study evaluating different border strips in organic systems also found no effect on arthropod activity density, concluding that organic management provided enough habitat complexity and resources within the plot to support arthropods (Fox et al. 2015). I found less effect of management on arthropod pests and predators than I had hypothesized when I began this research and did not detect consistent effects of preceding cover crop, termination method, or the use of interseeding on pest damage, predator activity density, or predation rate.
This dissertation provides insight into how combinations of cover crops and tillage affect pest and predators in organic systems, relationships between predators and pests, and impacts of pest damage on corn yield. Although many research questions remain unanswered, I have two main conclusions based on my research. As I found consistently low severity of early season damage and no negative relationship detected between early or late season pests and corn yield, pest management appears to be effective in organic reduced-tillage systems regardless of the cover crops grown before or after corn. Additionally, it appears that interseeding could be adopted as a strategy for establishing cover crops after corn, without increased concern over pest damage late in the season.

References


Appendices

Appendix A

Supplemental Information for Included Chapters

Appendix A3.1

*Community Overlap Between Four Reduced-Tillage Cropping Systems in ROSE 2.0*

NMDS plots showing community overlap between cropping systems prior to cover crop termination (A), after corn emergence (B), and after cover crops were interseeded (C).
Appendix B
Additional Projects and Protocols

Appendix B-1. Project proposal for investigating contribution of common generalist predators to predation in organic reduced-tillage systems

Abstract

Biological control is an essential ecosystem service for organic producers who have few rescue treatments available for managing pests. However, studies of natural enemies in agriculture often correlate predators and predation without truly testing the contribution of predators to measurable ecosystems services, such as predation and pest management. Newer techniques, including video surveillance and molecular gut content analysis offer valuable tools to understand predator behavior in response to available prey and quantify relationships between the activity density of predators and the predation services they provide. This appendix chapter summarizes methods I used to investigate the contribution of Carabidae, Lycosidae, and other generalist predators to sentinel predation within a cover-crop based reduced-tillage systems experiment. These projects have not yet been complete, so data is not presented. However, I summarize a plan for completion of data analysis and publishing of these two projects, followed by written protocols I designed for completing this work in the field and lab. This work will provide valuable insight into the role of individual predator taxa to predation of caterpillar prey in cropping systems, as well as provide troubleshooting of methods for using ELISA to detect gut contents in generalist predators.
Introduction

With few cost-effective rescue treatments available to organic agronomic producers, there is heavy reliance on cultural and biological control of pests. Often, the cultural practices that kill pests are detrimental to natural enemies, however. Thus, understanding the effect of cultural practices that promote natural enemies and the ability to render control services is essential to ensuring productive and profitable field crop growth.

Often papers fall short of truly identifying the most relevant predators in a system. Frequently, practices that increase activity density of a few highly abundant groups of generalist predators are identified as being good practices to follow if you want increased pest suppression. Others link reduced pest populations to the abundance of certain predators or to certain practices without knowing if the benefits of pest suppression are a side effect of other elements within that cropping system or truly due to the predator increase. This paper aims to look at generalist predator communities in relation to predation services rendered in multiple ways to estimate the use of caterpillar prey by common predations within the system, as well as who the key predators are that actually contribute to consumption of caterpillar prey.

In recent years, greater attempts have been made to identify trophic linkages between predators and prey in ecosystems, including agricultural ecosystems. In some cases, this has confirmed that common predators, such as Araneae, do feed heavily on pests such as aphids (Romero and Harwood 2010). In other cases, it has revealed that a much greater proportion of Araneae diet may consist of non-pest items, such as Collembola (Romero and Harwood 2010, Chapman et al. 2013). Intraguild predation is another factor that may limit how informative common predator populations are in terms of estimating predation potential of a cropping
system. These could include direct consumption, which would reduce populations caught in traps, or indirect through fear response, which would likely not appear in trapping data.

This experiment aims to better understand exactly which predators consume prey and are contributing to control of caterpillar pests in organic maize. We will use innovations in the ability to affordably study predation including video surveillance and gut content analysis to assess the likelihood that common predators in maize fields feed on caterpillar prey (Hagler et al. 2015, Grieshop et al. 2012). Since carabids are both fairly common and have been well-cited as generalist predators, I expect them to be relatively important contributors, especially in no-till plots (Douglas et al. 2015, Tamburini et al. 2015). Based on the likelihood that Araneae will consume non-pest prey, I anticipate that they play a smaller role than their abundance would indicate but for them to be favored by no-till plots and by interseeding. Lastly, I expect Formicidae to play a relatively high role in predation of caterpillars, despite their incompatibility with common trapping methods which may be underestimating their actual populations in fields (Grieshop et al. 2012). I expect Formicidae to be higher contributors to predation in tilled plots since it enables them to move more freely than areas with high residue (Grieshop et al. 2012).

Methods

Field Experimental Design

All sampling occurred in corn plots (Zea mays) in a three-year rotation with soybean (Glycine max) and spelt (Triticum spelta). These crops comprised a full entry experiment with all crops present in all years. Crops were arranged in a randomized complete block, split-plot design with four replications. Within each block, the three crops comprised two main plots, separated by grass alleyways to allow field operations to occur, with two split-plots in each main plot (totaling
four split-plots per block). Each split-plot measured 9m by 49 m and was managed under one of four cropping systems (Fig A). Cropping systems differences included cover crop species, their planting date and termination method, manure management, and tillage intensity and frequency. Short planting windows between cash crop harvest in the fall and reduction in temperatures can make establishing cover crops challenging in the fall, especially when harvesting corn for grain rather than silage (Curran et al. 2018). The cover crop treatments in this experiment were chosen to overcome this short planting window in some cases, as well as to improve cover crop benefits to soil, weed management, and arthropod communities.

In all four systems, corn variety ‘MC4050’ was planted on 76-cm rows at 81,510 plt ha\(^{-1}\) using a four-row John Deere 7200 planter equipped with Dawn Biologic ZRX residue managers, typically in late May or early June, depending on the cropping system (Table 1). In System 1, liquid dairy manure was broadcast at 74,831 L ha\(^{-1}\) and incorporated via moldboard plow following spelt harvest. A winter cover crop mixture of hairy vetch (\textit{Vicia villosa Roth} ‘Groff’) plus triticale (\textit{Triticosecale} ‘815’) was planted at a seeding rate of 34 kg ha\(^{-1}\) each. Cover crops were terminated by rolling with a roller-crimper in the spring to form a mulch on the soil surface into which corn was no-till planted immediately after. The timing of rolling occurred at Mischler 7 growth stage to optimize weed suppression (Mischler et al. 2010). A second rolling pass a week after planting, as well as two passes with a John Deere 886 high-residue cultivator at four and five weeks after planting were employed to ensure cover crop termination and enhance weed control.

In system 2, no manure was applied in the fall and a chisel plow was used after spelt harvest to prepare the soil for cover crop planting. Hairy vetch and triticale were planted at the same rates as System 1. In Systems 3 and 4, a cover crop mixture of medium red clover
(Trifolium pratense L. ‘VNS’) and timothy (Phleum pratense L. ‘VNS’) was drilled into standing spelt at 12 and 4.5 kg ha\(^{-1}\), respectively in early spring. This allowed it to establish prior to spelt harvest, added economic benefit through a fall forage cutting, and allowed a longer growing season and period without tillage than the hairy vetch and triticale mixture in the other two systems. After spelt was harvested in early August and the cover crop was able to grow, the cover crop was mowed and prepared for use as forage to prevent it from producing seed. After continued growth through the fall and winter, this mixture, as well as the hairy vetch and triticale in system 2, were mowed and incorporated using a moldboard plow. For fertility needs of the corn, manure was broadcast at 74,831 L ha\(^{-1}\) prior to plowing. Corn was planted 7-10 days following tillage. A mix of blind cultivation and inter-row cultivation was used in these systems to control weeds early in the season. Although the frequency varied from year to year depending on weed severity and environmental conditions, all three systems received the same treatment as each other in a given year.

In systems 1 and 3, corn was harvested for silage production in September using a small silage harvester with a cover crop seeded afterward. In systems 2 and 4, cover crops were interseeded, or drilled between corn rows, in early July at the V6 stage of corn to allow us to harvest corn for grain in late fall without compromising cover crop establishment. Corn grain was harvested using a small plot combine. Comparison of the four cropping systems allows us to assess effects of the species and management of winter cover crops, timing of tillage, and mid-season interseeding of cover crops on the abundance and community composition of epigeal arthropod communities, relative levels of predation, and the prevalence of damage from arthropod pests of corn.
**Sentinel Predation Assays**

Within the reduced-tillage cropping systems experiment previously described, I assessed biological control potential in two ways in 2017. In both ways, I attached five fourth instar *Galleria mellonella* larvae to index cards to serve as sentinel prey (The Nature’s Way, Ross, OH). Baited index cards were surrounded by a 19 cm high by 14.5 cm diameter trap constructed from 19-gauge hardware cloth and covered with a 150 mm x 15 mm white plastic petri dish to allow entry by invertebrate predators but exclude vertebrates, such as mice, and rain and secured in place with a single carriage bolt (Rivers et al. 2016) (cloth: 840178, Lowe’s Companies, Inc., Mooresville, NC, lids: 25373-187, VWR, Radnor, PA, bolts: 812626, The Hillman Group, Cincinnati, OH). In all cases, cards were placed 3 crop rows in from the edge and randomly placed lengthwise along the plot. After 24 hours, I collected the cards and the percent of sentinel prey experiencing feeding damage was recorded.

**Video Surveillance of Predation**

To assess the relative contribution of different predator taxa to predation, I employed a surveillance system based on a system used by M. Grieshop (Grieshop et al. 2012). Each system consisted of four waterproof cameras with infrared lighting, a 4-channel DVR, and hard-drive. (BD-2404B1, EZVIZ, City of Industry, CA). I outfitted each camera with a 10x macro lens to improve video quality (VIV-CL-37, Vivitar Corp., Santa Monica, CA). I placed one camera above each of four sentinel bait cards per plot to record feeding events for the 24 hour-period that cards were in the field during the 2017 season. DVRs were powered off a 35AH deep-cycle marine battery (UB12350ALT573, Universal Power Group, Coppell, TX). To protect DVRs and batteries from rain, they were placed in 62.5 L plastic totes with holes drilled for ventilation.
Additionally, I filled two plastic cups with 1 oz silica powder each and placed them in each tote to suppress moisture within the tote (2610, Activa Products, Inc. Marshall, TX). Video footage will be replayed using VLC media player (VLC 2.2.6, VideoLan, Paris, France) to identify predators to order and record the amount of feeding by each predator, allowing us to determine frequency of predation by specific predators and which are likely to make the greatest potential contribution to suppression of pest populations.

**Mark-Recapture Based Gut Content Analysis**

To reinforce my understanding of predator activity within the field and connect it to the overall invertebrate community, I marked sentinel waxworms with a protein mark and tested predators for this mark using gut content analysis. Each sentinel caterpillar was marked with 20% liquid egg white using a spray bottle, then allowed to air dry for 20 minutes before being attached to cards (AllWhites® Egg Whites, Crystal Farms, Minnetonka, MN). In 2017, two of the four cards placed during each sampling period for video surveillance were marked with protein. The other two cards remained unmarked. I collected predators adjacent to marked prey using pitfall traps measuring 129 mm deep by 114 mm wide lined with an adhesive card cut to fit the bottom of the container (Olson Productions, Medina, OH). Adhesive cards and marked sentinel cards were collected and replaced after 12 hours in the field, then collected again after a 2nd 12 hour period to allow 24 hours of field exposure. After collection, I returned both sentinel cards and adhesive cards to lab and immediately frozen at -18°C. I identified Carabidae (Coleoptera) specimens to species, except for members of Amara, and Lysocidae (Araneae) and Amara spp. to genus. The species of Carabidae I used in this assay were Amara spp. (7-10 mm), Bembidion quadriraculatum (3-4 mm), Harpalus pensylvanicus (13-16 mm), and Pterostichus
melanarius (15-18 mm) to capture a range of small and large species common throughout the season. The two most common Lycosids I collected were *Pardosa* spp. (3-5 mm), and *Trochosa* spp. (7-9 mm) which provide a range of sizes throughout the season, as well. I ground and assayed all specimens via an indirect ELISA sensitive to egg antibodies using methods from Hagler et al. 2015. To reduce dilution of larger samples, I dissected the guts of all carabids measuring over 1 cm prior to being assayed. Individual specimens were stored and assayed in 1.7 mL microcentrifuge tubes (VWR, Radnor, PA).

In 2018, I assessed the gut retention rates of both egg white and chicken serum protein (Sigma Aldrich, St. Louis, MO) marks amongst common predators caught using sticky cards in 2017. I sampled using dry pitfall traps without sticky cards in early May 2018 for *Amara* spp, June 2018 for *Bembidion quandrimaculatum*, and in August 2018 for *Harpalus pensylvanicus* and *Pterostichus melanarius*. I collected *Pardosa* spp. and *Trochosa* spp. throughout the season but caught the majority of them during May. I included some grass clippings or other vegetation collected nearby in the bottom of each pitfall trap to limit the likelihood of intraguild predation (Blubaugh 2016). Once I returned to the lab with trap catch, I identified predators of interest and transferred them to a 4 oz plastic cup with a piece of a cotton ball that had been cut into 10 smaller pieces and moistened with water. Cups were labelled with the date of collection, taxonomic identification, and protein mark treatment. Time treatments were not assigned until after feeding.

To encourage feeding, I starved ground beetles for at least 24 hours prior to feeding and starved spiders for at least 72 hours prior to feeding. I marked waxworms using the same technique as for the previous summer’s field experiment, using 20% egg white solution and 50% chicken serum solution. Care was taken to avoid cross-contamination between protein
treatments. On days of feeding assays, I fed marked waxworms to predators of each taxon in the lab and observed them every 20 minutes for the first two hours, then hourly for the following four hours until feeding successful occurs (Hagler et al. 2015). When I observed feeding, the specimen was assigned to a time group of 0h, 3h, 6h, 12h, or 24h, held alive until the assigned time after feeding, then frozen at -18° C. Because predators took an extended period of time to feed, particularly for spiders, and I wanted to ensure enough protein had been gathered during feeding, I would start the time clock when they completed feeding rather than when they began. If feeding by spiders extended longer than 2 hours, I interrupted feeding and began their time clock. When feeding was complete or interrupted, I removed the waxworms from the container and transferred the predator to a new, clean container for the duration of time until they were frozen. I transported and assayed specimens in 1.7 mL microcentrifuge tubes (VWR, Radnor, PA), using indirect ELISA sensitive to egg white for the egg white treatment and sandwich ELISA sensitive to chicken antibodies for the chicken serum treatment (Hagler et al. 2015). All assays were done in collaboration with J. Hagler’s lab in Maricopa, AZ. This study allowed to determine whether my 12-hour sampling periods were too long to adequately capture positive marks within the guts of common predators in these cropping systems, as well as whether using chicken serum may have been longer lasting than the egg white I used in my field experiment.

In addition to assaying predators, I also assayed marked waxworms and unmarked control waxworms under different conditions to test the efficacy of my marking techniques. All egg white-marked worms from the field experiment in 2017 and the lab feeding trials that were exposed to predators were assayed using indirect ELISA. Chicken serum-marked worms were assayed using sandwich ELISA. In addition to all of these waxworms, which I considered to be “wet” after exposure to field conditions and to the cotton balls in predator cups, I also saved
marked waxworms that had never been exposed to predators and considered these to be a “dry” treatment. I used unmarked dry waxworms as negative controls in all assays.

**Current Status, Proposed Data Analysis, and Publishing Plan**

**Video Surveillance**

Replay of video footage is in progress, so analysis has not yet been performed. I am planning to assess whether there are treatment differences in the likelihood a common predator will visit a card and feed, as well as the overall visitation and feeding rate by card. I also plan to assess the handling time and predation success by common predators to better understand whether predators commonly collected in our field are actually contributing to predation. I am planning to calculate correlation coefficients for efficiency of predator at killing prey by the mean number of encounters a predator has with the waxworms to calculate a relative importance for each predator family (Brust and House 1990). After this review of footage is complete, the data will be combined with sentinel predation data covered in Chapter 3 of this dissertation and compared to predator communities to assess how the abundance of visits by predator compares to the activity density caught using pitfall traps.

**Mark-Recapture Based Gut Content Analysis**

At this point, all assays are complete but I have not begun analysis. I will plot the retention of the protein marks for each taxa to visualize the distribution of their ability to pick up and retain protein marks after feeding. For any taxa with sufficient sample size (>8), I will use ANOVA to test differences between the two protein marks and MANOVA to test differences in retention among taxa. I also plan to test for differences between the “wet” and “dry” waxworm
treatments, and between the egg white-marked waxworms held in field conditions versus lab feeding conditions to test the durability of the mark under these different conditions. I plan to publish this data as a methods paper to help future scientists wishing to use these techniques in humid field conditions, such as those that we have in Pennsylvania. Although J. Hagler has published a substantial body of work on these techniques, nearly all of it has occurred in arid conditions in the southwest US (Hagler 1997, Hagler 2011). Thus, these field assays contribute additional knowledge of protein mark success in other climates. Additionally, the comparison between egg white, which is a relatively ineffective but cheap protein mark, and chicken serum, which is a newer option for Hagler lab that is cheaper than traditional marks such as chicken IgG and rabbit IgG, will provide useful insight into choosing an effective protein mark for a range of available budgets.

ELISA methods manuscript co-authors: Karly H. Regan1, Christina A. Mullen1, James R. Hagler2, Scott A. Machtley2, and Mary E. Barbercheck1

1. Department of Entomology, Pennsylvania State University, University Park, PA
2. USDA-ARS Arid Land Agricultural Research Center, Maricopa, AZ

Protocol for surveillance of predation:
What: Determine the predators contributing to sentinel prey consumption in different cropping systems.
How: Waxworm larvae will be placed in cages to allow feeding by arthropods. Cameras will be placed by each cage to monitor feeding of waxworms by predators.
When: Three times per season: two weeks prior to cover crop termination to compare relative numbers and activity of predatory arthropods on the soil surface, two weeks after corn has emerged, and 4-5 weeks after interseeding occurs. This experiment will co-occur temporally with our standard sentinel assay.
Crops: corn
Sampling formula: 4 plots x 4 blocks x 4 traps/plot = 64 traps/cameras (16 4-channel DVR setups)
Where: one randomly at each corner of the plot, ~2ft between the pitfall trap and sentinel cage at each location
Reps/Plot: 4
Protocol: pg TBT (but see 18-26 for standard pitfall procedure and 27-31 for standard sentinel procedure)

Intro: This experiment will take advantage of the sentinel prey assays already performed by placing a DVR that can handle 4-channels of video in each plot. One camera will be placed by each sentinel cage. Two cages per plot are placed for standard sentinel prey assay, while two additional cages will be placed for protein-marked prey assay. Cameras will be placed by cages for both sentinel prey methods.

See sentinel card protocol for details on construction and placement of cages and cards.

1) Construction of camera-related equipment
This project makes use of 4-channel security cameras that have a DVR and hard drive. DVR’s are powered in the field by deep-cycle marine batteries. It is essential that DVRs and batteries remain protected from moisture. This protocol covers wiring of DVRs and batteries, construction of waterproof housing, and construction of camera stands.

A) Camera Mounting
Materials needed:
   a. 64 bullet security cameras (model #: Q-See QT228-8B5-5)
   b. 96’ of ½” Schedule 40 PVC pipe (for 16 plots with 4 cameras per plot)
   c. 64 T-shaped connectors for ½” Schedule 40 PVC pipe
   d. 128 small screws (each camera comes with a packet of 3)
   e. Drill
   f. Pencil
   g. Permanent marker
   h. Phillips head screwdriver
i. PVC hack saw or PVC cutter (available in ASI or Headhouse workshops with proper training; contact Scott DiLoreto, dsd134@psu.edu or David Despot, dad9@psu.edu for training)

Mounting Construction:
1. Cameras come with pre-drilled holes for mounting
2. If camera has been secured for upside down usage, use screwdriver to loosen screw and rotate camera until it is upright, then re-tighten
3. Choose the 2 holes closest to the front of the camera and use pencil to mark holes on T-shaped connector piece
4. Darken holes with marker for easier visibility
5. Drill holes on marks (it is important to be precise so the camera will line up with holes; use the smallest drill bit available to allow screw to fit tightly)
6. Line camera up over holes and use screwdriver and screws to attach camera to T-shaped connector
   a. Tighten screws until you can just barely see point of screw coming through pipe and no further (if you tighten further, PVC pipe will not fit into connector)
7. PVC pipe typically comes in 10’ sections
8. Use meter stick and marker to mark 6” lengths on pipe
9. Cut PVC pipe into sections
10. Place a 6” section of pipe into each hole of T-shaped connector to form tripod stand

B) Wiring of batteries
Materials needed:
   a. 16 35ah deep cycle marine batteries with mounting bolts (part #: UB12350ALT573)
   b. 16 deep cycle battery chargers (NOCO G750, part #: BC 90-8160)
   c. 32 male cords (model #: iMBA-CCTV-PGM-10)
   d. 32” of 1/8” heat shrink tubing (part#: HST-18BK-100)
   e. 16” of 1/4” heat shrink tubing (part#: MFG 3MHST14-1)
   f. Wiring toolkit (located in 108 Headhouse III; model #: 4910 / part #: PA4910)
   g. Battery boxes (part #: Camco 55362)

Wiring Procedure:
1. Each DVR setup has two power cords, one for the DVR and one for the cameras. These two cords must be spliced together to form 1 cord.
2. Peel ~1/2” of red and black coated wires apart on 2 male cord ends
3. Use wiring tools to strip ~1/4” of coating from wire
4. Braid red wires from each male cord together
5. Braid black wires from each male cord together
6. Coat red wires to secure together
7. Repeat with black wires
8. Use heat shrink tubing to secure splice against water damage
9. Take eyelet attachment from battery charger
10. Use wire cutters to remove power adapter on end from cords ~1” from adapter
11. Separate red and black coated wires ~1” in
12. Place 2” section of heat shrink tubing over cord for later
13. Splice red end to red end from spliced male cords  
14. Splice black end to black end from spliced male cords  
15. Once all cords are secure, shift heat shrink tubing over connection and apply heat to seal  
16. When cord is complete secure, use mounting bolts that came with batteries to attach cord setup to battery  
17. Make sure to attach red eyelet to red terminal and black eyelet to black terminal  
18. Place bolt through eyelight, then add 1 thin washer, 1 thick washer, and 1 knut over bolt end  
19. Use wrench or pliers to tighten  
20. Store in battery boxes at all times but remove lid to charge batteries  
21. Charge batteries with alligator clip attachments  
22. Clip alligator clips over metal eyelets on battery terminals  
23. Leave near open window or in other well-ventilated area until charged  
   a. If fully drained, it takes ~24 hours to charge  
   b. Consult manual for charger instructions; always use 12V for our batteries  
   c. Short description is that red pulse means <75% charged, green pulse means 75-99% charged, and solid green means 100% charged  

**IMPORTANT BATTERY SAFETY NOTES:**  
- Exercise great caution when charging batteries  
- ALWAYS attach red clip to red terminal first, black clip to black terminal second, and power cord to electrical outlet last  
- Though unlikely, there is a risk of sparking, explosion, or other hazards when charging batteries  
- Face away from battery when plugging charger into outlet  
- Conduct all charging in headhouse adjacent to open window to allow ventilation (or other well-ventilated area) of gasses produced through charging  
- If any odd odor (such as sulfur) is detected upon re-entry of headhouse or if battery has swelled in size, leave headhouse IMMEDIATELY and call 911. Explain that you think a lead-acid battery has been charged incorrectly and describe the odor and/or swelling so they can seek help from EH&S for disposing of the battery properly and timely.  

C) Waterproof Housing  
Materials needed:  
   a. 16 Clear plastic totes with lids (66 quart size)  
   b. Drill  
   c. Hole saw attachment for drill  
   d. 3/8” drill bit  

Construction:  
1. Use the hole saw attachment to drill 2 holes per tote, 1 on each side directly underneath the handle  
2. Use the drill bit to drill additional ventilation holes: 4 per short side and 9 per long side of each tote
2) Organization and Preparation of Equipment

Materials needed:

a. 16 ventilated totes
b. 16 DVRs
c. TV monitor
d. HDMI cable
e. Power cords for TV, DVR, and cameras
f. 64 bullet cameras
g. 64 coaxial cables
h. 16 4-channel power-splitter cords
i. Colored marking tape in at least 2 colors
j. Permanent marker
k. Electrical tape
l. 128 twist ties
m. 2 oz plastic SOLO cups with lids
n. Silica powder

Process:
1. Upon arrival from shipping, the cameras need to be programmed before they will record
2. Work 1 DVR at a time to complete all steps in this section of the protocol
3. Connect all cords from cameras to their DVRs
4. Plug in all sources of power
5. Attach DVR to TV using HDMI cable
6. When prompted by the screen, select English as the operating language and create a password (Karly has been using “pred2017”)
7. The process to reset a password is lengthy, so make sure to type correctly and keep password in a safe and reliable place
8. Check that recording schedule is set to continuous and that the time and date are correct
   a. Time and Date will be on home screen
   b. Click on “Record” tab on left of screen to check continuous recording
9. DVRs of this model come pre-named “My EZVIZ DVR”- Rename to “DVR #” using 1-16 for each DVR
   a. This is located on the home screen
   b. This will help keep files and notes straight
10. Select advanced options then select video only (default is set to “video and audio” but we don’t need audio)
11. One programming is complete, unplug cameras from DVR
12. Attach a piece of tape to DVR with that DVR’s # on it (1-16)
13. Using the same color tape, attach a piece on the back next to the coaxial ports indicating the arrangement of ports by letter (I am using A for Port 1, B for Port 2, C for Port 3, and D for Port 4)
14. Take a coaxial cord that is attached to a camera
15. At the end closest to where it will plug into the DVR, attach a piece of tape the same color as previously used to attach a label for # - letter (i.e. 1A for the A port of DVR 1)
16. At the opposite end prior to where it plugs into the camera, attach a label with a new color of tape to indicate the #-letter for that cord (so there should be a label at each end of the coaxial cable with the same label on it but in two different colors of tape)
17. On the short piece of cord attached to the camera, attach a label with the same #-letter combo using the same color of tape as what this cord plugs into (i.e. all labels near the DVR are purple and all labels near the camera are green)
18. To waterproof the connection for the camera, wrap electrical tape around the entire cord junction between camera and coaxial cable
19. Unravel the entire coaxial cord and coil it in a large loop (approx. length of forearm) that will be easy to undo
20. Use at least 2 twist ties to secure the coil
21. Repeat with next three cameras for that first DVR
22. Place DVR at base of tote
23. Neatly stack 4 cameras and their cords next to the DVR
24. Fill a 2oz SOLO cup to the brim (or nearly so) with silica powder
25. Cap and stack neatly in tote
26. Repeat steps 3-26 until all 16 DVRs and their components are contained within totes
27. To transport to field, it may be easier to stack 2 DVRs with 8 cameras in a single tote and stack the empty tote underneath so that only 8 totes must fit in the back of the truck
28. Have batteries prepared for the field but DO NOT transport in the totes since they are heavy and can be hard to lift safely (and also might damage the tote)

3) Field placement and collection
Materials needed:
16 prepared totes of DVR and camera (see Section 2)
16 fully charged batteries
16 battery boxes
Flagging tape
64 white flags
192 sod staples
192 6” pieces of PVC pipe

Placement:
1. Make note of where each sentinel cage in a plot is located
2. Enter corn plot with tote between rows 6 and 7
3. Use a flag to mark the end of the plot at which you enter
4. Walk until you have reached the approximate center of the plot
5. Place tote on ground
6. Place a white flag within the corn interrow about 6 inches away from the tote on either end to warn anyone walking through that row that the tote is there
7. Remove 1 camera from tote, remove twist ties, and unwind cord
8. Place end of cord through large hole on one side of tote (the side you will be heading in) and plug into the appropriate port on the DVR
9. Attach power cord from coaxial cord to one port of the 4-channel power cord but do not attach this to battery yet
10. Take camera, 3 pieces of PVC pipe, and 3 sod staples and move toward one sentinel cage
11. If you’re not sure where the cages are, located one first without cord to figure out the most efficient way of getting to it
12. Choose one side of the cage to place the camera on
13. If cover crops and weeds are thick, clear excess plant material from between camera and cage
14. Put PVC pipe pieces into camera mount and place tripod on ground
15. Make sure that camera is 8” away from edge of cage and is flat on the ground, level with the cage
16. Use sod staples to secure each arm of PVC to the ground
17. It is particularly essential to secure the back arm since the camera is heavy and will cause the tripod to roll forward
18. One camera is securely attached to ground, check once again that there are no plants nearby the camera
19. Plants may droop at night and block view, so we want the area clear
20. Follow cord back to DVR tote at center of plot gently pulling excess coaxial cord taut as you go
21. Use twist ties to secure any excess cord inside the tote away from potential rodent chewing damage
22. Take marking tape and walk along cord one more time, marking the cord within each corn row between the cage and the tote to minimize tripping hazards
23. Repeat steps 7-22 until all four cameras have been placed
24. Fetch one battery and box from truck and bring to center of plot where tote is located
25. Place battery in one side of the tote
26. Remove lid from battery box and place on bottom of other half of tote
27. Stack DVR on top of lid to elevate it off the bottom of the tote
28. Open cup of silica powder to allow it to minimize moisture within the tote
29. Repeat until this has been done for all 16 plots
30. Steps 1-24 can be done the day prior to sentinel assay if needed but will require a second cup of silica powder
31. Open one cup between setting totes and using them then open the second one while cameras are operating (i.e. one cup per day in the field)
32. Once sentinel cards have been deployed, return to each tote and plug in power cord for DVR and for cameras (using the 4-channel power-splitter cord)
33. Watch DVR for a few minutes to ensure that the power light (bottom) turns red and that the recording light (middle) starts to blink red
34. The power light should light up immediately, while the recording light might take a minute or so to begin blinking
35. If the power source doesn’t turn on, double-check that all cords have been attached correctly and that there is no damage to cords from rodents or other causes
36. If lights still don’t power on, the battery may be dead. Ask Karly or Christy what to do.

Pickup:
1. Once sentinel cards have been fetched for a plot, unplug camera setup from battery before leaving plot
2. Once time-sensitive sentinel procedure is entirely complete (all cards of worms are in bags and secured in the truck), begin disassembling camera setup
3. Remove sod staples and PVC from each camera and move camera back to tote
4. Re-coil cords and secure with twist ties
5. Place cap on silica powder to avoid spilling in the tote
6. Remove battery from tote for easier carrying and put lid back on box
7. Stack DVRs and cameras inside of tote for transport
8. Collect PVC and staples in bucket since these can be stored in the field
9. If cameras are clean (it hasn’t rained at all since placement), cameras can be stored in chicken coop
10. DVRs and batteries must be transported back to campus so batteries can be re-charged and data files can be retrieved from DVRs (see Section 4)
11. If cameras appear dirty, transport cameras back to lab too so they can be cleaned

4) Storage of video footage
Materials needed:
   a. Television
   b. HDMI cable
   c. Power cords for DVR
   d. USB Drive
   e. Computer
   f. External hard drive

1. Plug in all power cords and use HDMI cable to connect DVR to TV
2. Enter in password upon startup of DVR (password = pred2017)
3. Plug USB drive into back of DVR
4. From menu along top of screen, select Export
5. Select all four channels of camera and search
6. Select a file from most recent sampling date
7. Export to flash drive
8. Double-check that file name is correct for given date and DVR (should include date, time, DVR name and channel #; if not, take notes on name so it can be located and changed on USB drive)
9. Repeat for all files from that DVR and sampling date
10. Shut down DVR and extract flash drive when finished exporting
11. Plug USB drive into computer
12. Rename files so that each file has ROSE2_VIDEO_date#_plot#_rep#
13. Create folder on computer for sampling date (ROSE 2 Video_date#)
14. Transfer all files from that date into the folder
15. Remove USB drive from computer
16. Repeat steps 1-15 for all 16 DVRs
17. When all DVR files have been added to computer, plug External HD in
18. Back up this date’s folder on External HD

5) Processing of video files
The goal is this activity is to locate time-points within the video files where predators are visiting the waxworms and feeding.
Use VLC Media Player to watch footage (has an orange traffic cone logo)
I recommend opening 2 windows of video to watch at a time and watching at 2-4 times normal speed. If you’re finding it difficult to focus on both windows, try just one window or adjust each to a lower speed. Please don’t exceed 2 windows at 4 times normal speed without talking to Karly first.

To begin:
1. Go to the folder on the hard drive you’d like to watch, select the first file, right-click > “Open With” > VLC media player.
2. Once the window is open, go to “Media > Open Multiple Files > Add > select all within a folder of choice > Play. This will enable all the files from a given folder to play sequentially as a playlist.
3. Once you have one playlist opened and running, repeat Step 1 to get a second window of VLC open.
4. Once the second window is open, follow Step 2 to add the rest of the files from that folder to your playlist.
5. Once you have them open, go to View > Status Bar to bring the video speed and time-points up along the bottom of the window. Do this for each window you have open.
6. Next, go to Playback > Speed > Faster to adjust the speed of playback. It will go up 1 whole time (i.e. from real speed to 2 x real speed by hitting once) each time you hit faster. If you’d prefer to move by less than 1 whole time, you can hit Faster (fine) to adjust by 0.1 times real speed. Do this for each window you have open.
7. Open the Excel file on the hard drive that is labelled “ROSE2017_Video Work Log”
8. Enter your name, today’s date and time, and list the files you’re watching on the “Work Done” tab
9. On the “File Info” tab for the sampling date you’re working on, record the time of day listed on the top left corner of the video file at the very beginning of the video and also the file name
10. Whenever an arthropod that is NOT one of the 5 waxworms enters the index card area of the video, pause the video and record it in the “Event Log” tab for that sampling date
11. Record the sampling date, DVR #, channel #, and video # in the appropriate tabs
12. Record the time listed on the video’s playback bar for when the arthropod enters the index card as “Start Time” and the time that it leaves the index card as “End Time.”
Appendix B-3. Protocol for Using ELISA to Detect Egg and Chicken Proteins in a Predation Experiment
Marked Sentinel Trapping (MST) and Dry Pitfalling (DPF)
(adapted from methods used by Dr. James Hagler, USDA-ARS in Maricopa, AZ and by Jermaine Hinds, Barbercheck lab)

What: Determine the predation rate in the different cropping systems by predators of interest, such as carabid beetles and spiders.
How: Waxworm larvae will be marked with a protein and then immobilized by taping them into a piece of notecard. The notecard will be placed in an inclusion trap (wire mesh cage). The trap will allow the predators to enter and feed on the larvae, consuming the protein mark. Predators will be collected onto a sticky card inside of a pitfall trap and assessed for the protein mark.
When: Three times per season: two weeks prior to cover crop termination to compare relative numbers and activity of predatory arthropods on the soil surface, two weeks after corn has emerged, and 4-5 weeks after interseeding occurs. This experiment will co-occur temporally with our standard sentinel assay
Crops: corn
Sampling formula: 4 plots x 4 blocks x 2 traps/plot = 32 inclusion traps; 2 exclusion traps/ block (in addition to the 32 traps already being placed at same time for standard sentinel and pitfall trapping)
Where: one randomly at the top of the plot and one randomly at the bottom of the plot, ~2ft between the pitfall trap and sentinel cage
Reps/Plot: 2
Protocol: pg TBT (but see 18-26 for standard pitfall procedure and 27-31 for standard sentinel procedure)

Goal: This experiment will take advantage of the sentinel prey assays already performed by marking the waxworms with a protein and then placing pitfall traps nearby to catch predators. Predators will be preserved and later analyzed using enzyme-linked immunosorbent assays (ELISA) to see if they test positive for the protein mark, which would indicate they fed on the marked waxworms.
1) Cage building
Inclusion cage building:
   a. Cut the wire mesh (1.3-cm mesh, 19-gauge hardwire cloth) into a rectangle that is 9-cm wide and 49.5-cm long.

   9-cm

   49.5-

   b. Attach the ends of the rectangle together with three pieces of metal wire.
c. Cut a 15.5-cm x 15.5 cm square of hardware wire and attach it to the cage sides using four pieces of metal wire.

![Metal wire attachments](image)


d. Cover trap with a 150 x 15-mm petri dish lid that is spray painted white.

**Pitfall trap building**

a. rooves
b. funnels

2) Lab Preparation
   (card prep can be done weeks prior but marking should be done within a few hours of field placement)

A) Sentinel Cards

Materials needed:

a. 64 Index cards (7.6 cm by 6.3 cm)
b. Egg white
c. Water
d. Measuring implement (graduate cylinder)
e. Small spritz bottle (stored in 108 Headhouse III)
f. *Galleria mellonella* larvae (enough to supply 160 once bad ones are sorted out)
g. Sieve for sorting waxworms (stored to the right of the sink in ASI)
h. Brown trays (stored to the right of the sink in 524 ASI)
i. Latex or nitrile gloves
j. Hem tape, cut into 160 thin strips
k. Scotch tape (enough to tape 160 worms)

1. Assemble index cards by placing a thin strip of hem tape (0.7 cm wide) in each corner of index card and one in the middle of the card (5 strips total per index card)
2. Mark index cards with project name (ROSE 2), date, PROTEIN – time (a.m. or p.m), and plot # - replicate #
   a. example:
      
      ROSE 2
      5/5/2017
      PROTEIN a.m.
      132-3

*Note: We are using 3 and 4 for replicates, assuming that standard sentinel trapping and pitfall trapping will be occurring at the same time. In this case, we are using 1 and 2 for standard
sampling and 3 and 4 for this marked sampling. If this marked sampling is the only sampling you will be doing, feel free to use 1 and 2 as replicates instead.

3. Mix egg whites with water in a graduated cylinder to create a 15% egg white solution (start with 15 mL of egg white and 85 mL of water)
4. Pour solution into spritz bottle and set aside
5. Sort waxworms to discard any with black marks, bruising, or lethargy
   a. Dump container of waxworms into sieve placed over brown tray and examine worms
   b. Place usable worms in a dish and set bad ones side on tray to dispose of
6. Once you have sufficient supply of quality waxworms, spread them in a single layer on a clean brown tray
7. Spray egg solution onto waxworms until thoroughly covered with a thin layer
8. Allow to dry for 15-30 minutes in the incubator to slow movement then run gloved hand over waxworms to gently rotate them
9. Repeat spritzing process on opposite of worms (if possible) to increase coverage on each worm
10. Repeat drying process
11. Once worms have been coated and dried thoroughly, place a single waxworm ventral side up with head toward the top of the labeled card on a piece of hem tape (hands should be gloved)
12. Press gently down to secure then place a thin strip of scotch tape to secure the last 3 segments of the abdomen to the card
13. Repeat until card has 5 attached waxworms then place card inside Ziploc baggie
14. Clip all baggies for each plot together in order by plot and replicate (i.e. 131-3, 131-4, 132-3, etc.)

B. Pitfall trap liners
Materials needed:
   a. 32 yellow-sticky cards (Olson Productions, Medina, OH)
   b. Scissors
   c. Sharpie marker
   d. 132 oz translucent plastic deli container
   e. Exacto knife
   f. 64 twist ties

1. Cut each yellow sticky card in half
2. Trim edges to allow cards to fit at the bottom of plastic deli container
3. Use exacto knife to cut a 1 cm slit in the center of each card
4. Peel back liner of 1 side of card halfway to allow you to insert twist tie into the cut slit in card, bend tie to hold in place, and secure 1 cm of twist tie to adhesive on card, then replace liner

5. Use Sharpie to label underside of each card (the side the twist tie end is secured to, not the side that the rest of the twist tie extends out from) with project name, placement date, time (a.m. or p.m), and plot # - replicate #
   a. example:
      ROSE 2
      5/5/2017
      a.m.
      132-3

*Note: We are using 3 and 4 for replicates, assuming that standard sentinel trapping and pitfall trapping will be occurring at the same time. In this case, we are using 1 and 2 for standard sampling and 3 and 4 for this marked sampling. If this marked sampling is the only sampling you will be doing, feel free to use 1 and 2 as replicates instead.
2) Field Protocols:

A) Pitfall Digging and Sentinel Cage Placement:

When: Several days prior to opening the pitfall

Materials needed (for 16 plots, 2 traps/plot):
   a. 32 32-oz translucent deli containers with their lids
   b. 32 soda bottle funnels
   c. 64 purple flags
   d. Sharp shooters
   e. Hand trowels
   f. Blue totes (digging pitfalls, collecting trap materials)
   g. Site Map

Method:
1. Pitfalling will occur prior to cover crop termination, one week after cash crop emergence, and one month after interseeded cover crop termination.
2. There are two pitfalls per plot.
   a. If sampling in the cover crop, walk about 10 steps (~5 feet) into the plot from the drive row, then turn and walk about 10 steps into the plot.
   b. If sampling after corn emergence, randomly select a representative crop row that is in either rows 3, 4, 5, 8, 9, or 10.
   c. Flag the crop row that you are entering and walk in at least 10 steps (~5 feet)
3. Place one pitfall randomly towards the bottom of the plot and one randomly towards the top of the plot (see diagram below).
   a. If digging at the same time as standard pitfalling, place four traps randomly in each corner of plot rather than placing a dry pitfall near an antifreeze pitfall

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4. Randomly select a location in between the crop rows but no more than a hand away from the nearest crop row.
   a. Disregard if cover crop is thick or without clear rows
5. Dig a hole to fit the size of the 32-oz deli container in between the cover crop or corn row
6. Place the deli container w/ lid into the hole
7. Pack soil around the cup, flush to the top of the container. Make sure you pack the soil down well and that there is a smooth surface after you’re finished (there isn’t a mound or sink hole).
8. Place a flag near each pitfall to aid in pitfall opening/collection.
9. One inclusion trap will be placed 2 ft away from a pitfall trap
10. Place the inclusion cage at a 45° angle, in respect to Tadpole Rd., from the pitfall going towards Rt. 45 and Tadpole Rd.
   a. NOTE: We place the traps in a specific way to ensure that they are easily found in tall cover crops and that they are relatively near the pitfall that had either been opened prior to the sentinel trapping or will be opened when we pick up the cards. Please DO NOT deviate from this placement protocol!
11. Placement is as follows:
   a. Set the trap on the ground.
   b. Trace the outer edge of the trap with the hand trowel
   c. Remove the trap and excavate soil within the traced area
   d. Place the trap in the excavated area
   e. Cover the bottom of the trap with soil until both the wires are covered with soil and the soil inside the trap is at the same level as the soil outside the trap.
      1. We do not want to create a sink hole or mound within or around the trap. The re-filled area should look just like the surrounding field area.
      2. It is very important to cover the trap bottom with soil because smaller predators may have trouble walking over the metal wires.
   f. Place a metal bolt into one of the corners of the trap bottom to prevent trap movement.
12. Randomly select a second location that is at least 5 ft from the plot edges in the other half of the plot (length wise) and repeat steps c-g.

Safety
   a. Wear work gloves
   b. Lay shovel face down on the ground.
   c. Be mindful of holes and rocks in the fields.

Clean up
   a. Wash hands with soap and water.
   b. Put all equipment back neatly in the back of the truck and/or shed

B) Pitfall opening and card placement:
Materials needed (for 16 plots, 2 traps/plot):
   a. Yellow totes (opening and collecting pitfalls)
   b. 32 pitfall rooves
   c. 32 sticky cards (labeled on bottom with twist tie attached in center for easy removal)
   d. 32 sentinel cards with marked waxworms
   e. Headlamps, if sampling at night
   f. Data sheet
   g. Pencil
   h. Clipboard
   i. Site map

Method
   a. Locate the pitfall and sentinel traps.
   b. Make sure that you are placing the correct sticky trap and there is a twist tie attached
      a. Labelling will have the plot #, sampling date, and replicate number.
      b. Replicate 3 is always closest to tadpole and replicate 4 is up the hill.
      c. Make sure the specimen cup and sentinel card numbers match
   c. Completely fill out the data section on the data sheet for the sample you are working with. Depending on the timing of sentinel trapping, information that you may need to record include: time of placement, height of cover crop/ cash crop, and growth stage of cover crop/cash crop.
   d. Remove the lid and correct any holes in the soil made from your fingers.
   e. Make sure that there isn’t any soil in the bottom of the deli container
   f. Peel paper off top of card (unlabeled side) and place the sticky card inside of the 32-oz. container.
      a. Save paper for collection later!
      b. Make sure twist tie is sticking straight up and will be easy to grab
   g. Place a funnel made with the top of a 2 liter soda bottle that has had the top cut off into the 32-oz. cup.
      a. Make sure that the funnel is even with the top of the deli container and not higher (this will create a barrier for the insects.)
   h. Place a roof over the pitfall.
i. Gently place the sentinel card with the 5 larvae flat on the ground inside the sentinel cage, larvae side up, inside the trap.
j. Place two stones onto the notecard to prevent the wind from blowing onto the side of the cage.
k. Cover the trap with a 150 x 15-mm white plastic petri dish lid.
l. Secure the lid with the twist ties.
m. Go to the next pitfall in the plot/block.

Safety
a. Minimize contact between your hands and the adhesive to avoid sticky hands
b. Be mindful of holes and rocks in the fields.
c. If your hands end up very sticky, tell Karly or Christy so they can provide cleaning materials

Clean up
a. Wash hands with soap and water.
   a. Lysol wipes can aid with removal of adhesive residue
b. Put all equipment back neatly in the back of the truck and/or shed
c. Hand the data sheets to Karly or Christy. DO NOT leave on clipboards.

C) Collection:

Materials needed:
a. Forceps
b. Ziploc bags
c. Paper liners from placement of stick traps
d. Headlamp (if at night)
e. Yellow totes
f. Data sheet
g. Pencil
h. Clipboard
i. Site map

Method
a. Keep the pitfalls open for two 12-hour periods.
b. After each 12-hour period, the sticky card will be collected.
c. Note time of collection on data sheet for each plot and replicate.
d. Grab sticky card using the twist tie
e. Place any arthropods (particularly beetles and spiders) that are on the bottom of the deli container onto the sticky card using forceps (or hands)
f. Carefully place paper liner back over sticky trap (line up as best you can so that adhesive is covered and can’t stick to bag)
g. Make sure that you can read the plot and replicate numbers on the sticky card
   a. Relabel the sticky card & its bag if you can’t read the numbers
h. Place covered sticky card into Ziploc bag
i. Remove rocks to collect sentinel card and place inside Ziploc bag
j. Use yellow hand tote to keep bags and clipboard organized
k. If night collection, place new sticky card and new sentinel card in same manner as initial placement
l. If day collection, remove the deli container, funnel, roof, flag, sentinel cage, and bolt.
m. Collect materials in blue tote for transport to the shed
n. Fill in the hole left from the deli container.
o. Place sticky cards and sentinel cards in the freezer promptly upon returning to campus in labelled Ziploc bags for each sampling date (should include your name, your e-mail address and/or phone number, project name, and date)

Safety
a. Wear headlamp and exercise caution when sampling at night.
b. Fill in hole when finished.
c. Be mindful of holes and rocks in the fields.
d. Try to minimize contact between your hands and adhesive

Clean up
a. Wash deli containers and lids in dishwasher.
b. Wash hands with soap and water.
c. Put all equipment back neatly in the back of the truck and/or shed
d. Hand the data sheets to Karly or Christy. DO NOT leave on clipboards.

3) Processing of Samples
A) Sentinel card assessment:
   1. Remove bags from the freezer.
   2. Examine the larvae under a dissecting microscope.
   3. Categorize the larvae as either:
      a. Intact (no evidence of feeding)
      b. Chewed on (less than 50% of the insect material removed)
      c. Eaten/ mostly gone (more than 50% of the insect material removed/ cadaver absent but with some material remaining)
      d. Missing/ no insect material remaining (absent with no material remaining)
   4. Store cards in totes in walk in freezer until data has been analyzed.

B) Sticky card sorting:

Materials needed (32 samples):
   a. Sticky cards
   b. Sentinel cards
   c. Toothpicks
   d. Microcentrifuge tubes
   e. Gallon-sized Ziploc bags
   f. Permanent markers

Method:
   1. Store cards in the freezer until the samples can be filtered.
a. Make sure to label with lab name and sample date
2. Allow sticky card samples to thaw until arthropods can be easily pried off of adhesive since the adhesive is rather firm in the freezer
3. Since waxworms are so soft and the hem tape is less sticky than what is on the sticky cards, leave sentinel cards as frozen as possible when removing waxworms
   a. Karly recommends only pulling out two sentinel cards at a time
4. Use toothpick to loosen 1 arthropod from card and place into microcentrifuge tube
5. Place only 1 arthropod per tube
6. Use a clean toothpick for every arthropod and dispose of used toothpicks
7. Collect all microcentrifuge tubes in Ziploc bag for that plot and replicate
8. Promptly return samples to freezer when done

Appendix B-4. References
Karly Regan, Curriculum Vita

Education:
2015 – 2019: Ph.D. Department of Entomology, The Pennsylvania State University, University Park, PA. Dissertation Title: “Arthropod Communities within a Reduced-Tillage Organic Systems Experiment”
2012 – 2015: M.S. Department of Plant Science, South Dakota State University, Brookings, SD. Thesis Title: “Effect of neonicotinoid insecticides on spider mite ecology”
2008 – 2012: B.S. Major in Biology, Minor in Women, Gender, and Sexuality Studies, University of Massachusetts, Amherst, MA

Selected Grants and Awards:
Dec 2018-June 2019: Penn State College of Agricultural Sciences Competitive Grants Program “A Bug’s Eye View: Using cameras to study predator-prey interactions between arthropods in the field” K. Regan. ($3000)
2016-2018: Northeast Region, Sustainable Agriculture, Research and Education Graduate Student Grant “Impacts of cover crops and tillage on predator-prey interactions within organic cropping systems” K. Regan (Coordinator) and M. Barbercheck (PI). ($14,635)
2016-2017: Sigma Xi: Grants in Aid of Research “Hunter and Hunted: Predator-Prey Interactions in Disturbed Habitats” K. Regan. ($450)
November 2018: Graduate Student Travel Award ($500), College of Agricultural Sciences, Pennsylvania State University.
August 2018: Paul R. Heller Memorial Award ($504). College of Agricultural Sciences, Pennsylvania State University.
May 2017: William Yendol Memorial Research Fund Travel Award ($470). Department of Entomology, Pennsylvania State University.

Publications