ABSTRACT

Title of Thesis: EVALUATING THE POTENTIAL BENEFITS AND LONG-TERM SUSTAINABILITY OF NEONICOTINOID SEED TREATMENTS IN MID-ATLANTIC GRAIN CROP PRODUCTION

Aditi Dubey, Doctor of Philosophy, 2020

Thesis Directed By: Dr. Kelly Hamby, Department of Entomology

Neonicotinoid insecticide seed treatments (NSTs) are heavily used in US grain production; nearly all corn and over a third of soybeans grown are treated. However, NSTs primarily provide protection against occasional early-season soil and seedling pests and rarely improve yield. Additionally, the active ingredients from NSTs can spread and persist in the environment where they can impact various non-target organisms including beneficial arthropods and soil microorganisms. To determine the costs and benefits of NSTs in Maryland grain crops, I evaluated the impacts of two popular NSTs, imidacloprid and thiamethoxam, and their associated seed applied fungicides on insect pest suppression, yield, non-target arthropods, and soil health in a three-year rotation of full-season soybean, winter wheat, double-cropped soybean, and corn. Pest pressure was low throughout the study, as is typical for Maryland, and the NSTs did not provide any yield benefits. Treatments variably impacted non-target arthropods, reducing the abundance of some predators and parasitoids. Seed applied fungicides also impacted non-target arthropods. Because parasitoid wasps

were disrupted in winter wheat up to 32 weeks after planting, I conducted a laboratory study to better understand NST suppression of cereal aphids and the mechanisms by which they affect cereal aphid parasitoids. Neonicotinoid seed treatments may not be effective enough to maintain aphids below the economic threshold in winter wheat; thus, they may negatively impact parasitoids through contaminated hosts. In my study, NSTs did not detectably affect soil health or the soil microbial community; however, they have the potential to harm aquatic communities through leaching and runoff. Given the lack of pest pressure and yield benefits, as well as the potential for non-target impacts, my research suggests that the use of NSTs in Maryland grain crops is neither warranted nor sustainable. It also highlights the need for further evaluation of the non-target impacts of seed applied fungicides, and of the effects of NSTs on water bodies within the Chesapeake Bay watershed.

EVALUATING THE POTENTIAL BENEFITS AND LONG-TERM SUSTAINABILITY OF NEONICOTINOID SEED TREATMENTS IN MID-ATLANTIC GRAIN CROP PRODUCTION

By

Aditi Dubey

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2020

Advisory Committee:
Associate Professor, Dr. Kelly Hamby, Chair
Dr. Galen Dively
Dr. Daniel Gruner
Dr. Nicole Fiorellino
Dr. Stephanie Yarwood

Preface

This dissertation is composed in part of previously published work, included here as Chapter 2 with the recommendation of the dissertation director, dissertation committee members and department graduate director. The citation for this publication is as follows:

Dubey, A., Lewis, M.T., Dively, G.P., Hamby, K.A., 2020. Ecological impacts of pesticide seed treatments on arthropod communities in a grain crop rotation. J. Appl. Ecol. 57, 936–951. https://doi.org/10.1111/1365-2664.13595

As directed in the graduate catalog, I state that I was responsible for the inception of the manuscript and the majority of manuscript preparation. This publication was reformatted to meet university guidelines, minor typographical errors were corrected, and supplementary material was placed in appendices, but the publication has otherwise been reproduced exactly. The publication is cited throughout the dissertation, where appropriate. Please refer to Appendix G for a letter certifying that this previously published work has been included in the dissertation with the approval of the dissertation director, dissertation committee members and department graduate director.

Dedication

I dedicate this dissertation to my parents and brother, for their unending love and support, and to my husband, who has been my rock throughout my degree.

Acknowledgements

I would first and foremost like to thank my advisor, Kelly Hamby, who set an example of excellence as a researcher, instructor, mentor, and role model. I would like to thank my committee members, Galen Dively, Daniel Gruner, Robert Kratochvil, Nicole Fiorellino, and Stephanie Yarwood, for their guidance and support over the years. I would particularly like to thank Galen Dively, who was involved in my research every step of the way, even in retirement.

I would like to thank the many members of the Hamby and Dively labs who have assisted in my research (and provided moral support) over the years, in particular Maggie Lewis, Maria Cramer, Terry Patton and Matt Dimmock. I would also like to thank the members of the Yarwood Lab who assisted with Chapter 1 of my dissertation, as well as Jude Maul and Sarah Emche at the USDA ARS. I would like to thank Kevin Conover, John Draper, and the staff at the Central Maryland and Wye Research and Education Centers for planting and maintaining my study plots, and Sydney Wallace and Meghan Fisher Holbert for their patience and assistance in keeping my growth chambers up and running. I would also like to thank Josh Kiner, Pam Biery and the other UMD Department of Entomology staff for their support with all things administrative.

This project was supported by the Hatch/Multistate funds [project no. MD-ENTM-8887/project accession no. 1009567 and project no. MD-ENTO-9589/project accession no. 1012455] from the USDA National Institute of Food and Agriculture, by USDA NIFA award number 2015-38640-23777 through the

North East SARE program under sub-award number GNE16-11B-29994, and by the Maryland Grain Producers Utilization Board and the Maryland Soybean Board. Outreach efforts and publication costs associated with this project were supported by the Crop Protection and Pest Management Program [grant no. 2017 70006-27171/project accession no. 1013913] from the USDA National Institute of Food and Agriculture.

Finally, I would like to acknowledge the constant support of my family, and my friends at UMD and beyond.

Table of Contents

List of Figures

List of Tables

Introduction

Background

Neonicotinoid insecticides are the most used insecticide class in the world (Nauen et al., 2008); in 2014, they made up 25% of total global insecticide sales (Bass et al., 2015). However, research suggests that these insecticides are overused (Simon-Delso et al., 2015; Tooker et al., 2017). Neonicotinoids were first introduced in the 1990s, and rapidly gained popularity due to factors such as their low mammalian toxicity relative to other insecticide classes and their systemic nature, which allows a variety of application methods (Simon-Delso et al., 2015). The most popular amongst these are neonicotinoid seed treatments (NSTs), which have been widely adopted in agronomic crop production. By 2011, 79-100% of corn and 34-44% of soybean grown in the US was planted with NSTs, and they are also used in wheat and cotton to a lesser extent (Douglas and Tooker, 2015). Rather than replacing older insecticide classes, much of this NST usage is on acres that were previously untreated, and cannot be explained by a corresponding increase in pest pressure (Tooker et al., 2017). The per seed application rate on treated acres has also continued to rise (Tooker et al., 2017). A recent assessment of acute insecticide toxicity loading (AITL), a metric that incorporates the total mass of insecticides used, their persistence in the environment, and their toxicity to insects, found that the AITL for oral toxicity on US agricultural land increased 48-fold from 1992 to 2014, with neonicotinoids accounting for 92% of that increase (DiBartolomeis et al., 2019).

Potential benefits of NSTs

The widespread adoption of NSTs has diverged from the principles of Integrated Pest Management (IPM). IPM is a philosophy that involves minimizing the use of chemical pesticides through monitoring pest populations, using biological and cultural control methods when possible, and only using chemical pesticides when they are necessary (Goulson, 2013). By minimizing environmental and economic costs, IPM aims to increase the long-term sustainability of pest management (Castle and Naranjo, 2009). Because NSTs are applied at planting, they are used before the pest pressure for that season can be anticipated; thus, they are largely used prophylactically (Douglas and Tooker, 2015; Goulson, 2013). Given that the use of NSTs is not usually motivated by pressure from specific pests, it is incompatible with IPM (Goulson, 2013). Additionally, NSTs only provide protection against a narrow range of early season soil and seedling pests because they only remain active in plant tissue for a few weeks post planting (Papiernik et al., 2018). There are some cases where such pests pose a threat and NSTs provide consistent benefits, such as mid-South soybean production (North et al., 2016). NSTs can also be a valuable tool against soil pests such as wireworms (Coleoptera: Elateridae) and white grubs (Coleoptera: Scarabaeidae), which recur over multiple years, are difficult to scout for, and cannot be combatted using rescue treatments (Douglas and Tooker, 2015; Sappington et al., 2018). However, in most systems, the pests targeted by NSTs are sporadic or minor pests that only occasionally cause economic damage, and many of these pests can be managed through cultural control practices such as

crop rotation (Papiernik et al., 2018; Simon-Delso et al., 2015). Research has found that in areas without consistent pest pressure, NSTs provide inconsistent or no yield improvement (Bredeson and Lundgren, 2015; Cox et al., 2007; Hunger et al., 2000; Labrie et al., 2020; Magalhaes et al., 2009; Mourtzinis et al., 2019; Myers and Hill, 2014; Reisig et al., 2012; Royer et al., 2005; Seagraves and Lundgren, 2012; Wilde et al., 2007). The mid-Atlantic US does not usually experience high pressure from the pests targeted by NSTs, although they can be useful against soil pests. In addition, a 2014 EPA report identified the Northeast as a region where NSTs do no provide economic benefits in soybean production (Myers and Hill, 2014). However, NSTs are widely used in corn in this region, and are also used in soybean and wheat to a lesser extent. Therefore, one of the goals of this dissertation was to determine whether NSTs provide pest control and yield benefits, and whether their use is warranted, in Maryland grain production.

Non-target impacts of NSTs

The primary reason for the concern regarding the overuse of NSTs is the potential for non-target impacts. The active ingredients from NSTs pose a risk to a broad range of organisms because of their ability to spread and persist within the environment (Pisa et al., 2015). When neonicotinoids are applied as seed treatments, routes of exposure for non-target organisms include dust generated during drilling, treated plants themselves, and through treated soil, where the majority of active ingredient remains. In the soil, active ingredients have the potential to persist and accumulate, leach into groundwater or runoff into surrounding water bodies, or even be taken up by non-target plants (van der Sluijs

et al., 2015). NSTs also have the potential for transmission through food webs (Frank and Tooker, 2020); making entire communities within agroecosystems susceptible to NSTs. As communities with greater species diversity and community evenness are correlated with significantly lower pest populations (Lundgren and Fausti, 2015), community-wide impacts of NSTs have the potential to exacerbate pest problems. However, most prior research on non-target impacts of NSTs has focused on individual taxa rather than communities (Disque et al., 2018). One of the primary goals of this dissertation was to evaluate the impacts of NSTs on above and below ground communities in Maryland grain fields.

Below ground communities are especially susceptible to NSTs, because the majority of active ingredients remain in the soil (Alford and Krupke, 2017; Sur and Stork, 2003). Neonicotinoid persistence in soil varies greatly, with the calculated half-life of imidacloprid ranging an order of magnitude from 100 to 1230 days from application (Baskaran et al., 1999). Because NSTs may be used repeatedly over multiple growing seasons, active ingredients can accumulate in the soil over time (Bonmatin et al., 2015). This puts soil organisms at risk of long-term contact with neonicotinoids and the break down products of neonicotinoid degradation, which can be more toxic than the insecticides themselves (Hussain et al., 2016). Despite this high risk, organisms that contribute to soil functioning have been understudied with regards to the impacts of neonicotinoids (EASAC, 2015). For example, soil-dwelling arthropods such as predatory ground beetles (Coleoptera: Carabidae) may be exposed to neonicotinoids by feeding on plant

material, indirectly through prey, or through contact with contaminated soil (Douglas et al., 2015; Kunkel et al., 2001; Mullin et al., 2005; Peck, 2009a, 2009b). By disrupting the activity of these natural enemies, NSTs have the potential to cause secondary pest outbreaks and yield reductions (Douglas et al., 2015). The impacts of NSTs on other arthropod groups may be less clear; collembola, which play an important role in breaking down soil organic matter, can be positively or negatively impacted by neonicotinoids (de Lima e Silva et al., 2018, 2017; Disque et al., 2018; Peck, 2009b; van Gestel et al., 2017; Zaller et al., 2016). In addition to soil-dwelling arthropods, laboratory studies have found that acute doses of neonicotinoids are highly toxic to earthworms, while chronic exposure to lower levels causes physiological and behavioral changes (Capowiez et al., 2010; Capowiez and Bérard, 2006; Dittbrenner et al., 2010; Van Hoesel et al., 2017; Wang et al., 2012). Earthworms perform several important functions including decomposing organic matter, increasing soil porosity and aeration, and facilitating water and nutrient cycling (Beare et al., 1995; Edwards and Bohlen, 1996), and by harming them, NSTs have the potential to disrupt soil health. Neonicotinoids can also alter the activity, diversity, and structure of the soil microbial community (Cycoń et al., 2013; Cycoń and Piotrowska-Seget, 2015; Nettles et al., 2016; Wang et al., 2014; Zhang et al., 2018), which is integral to organic matter breakdown and nutrient cycling (Kibblewhite et al., 2008; Schloter et al., 2003). The impacts of neonicotinoids on soil microorganisms are highly variable and difficult to predict, depending on factors including soil type, weather conditions, agricultural practices, and the use of additional agricultural chemicals

such as seed-applied fungicides, and must be evaluated on a case-by-case basis. As part of this dissertation, I evaluated the impacts of NSTs on various soil quality parameters, the soil arthropod community, overall soil microbial activity, and the structure and diversity of the soil prokaryotic community within a Maryland grain crop rotation. My objective was to determine whether repeated use of NSTs could disrupt the ecosystem services provided by soil microorganisms and lead to a potential decline in soil health and quality.

In addition to soil organisms, above ground communities within agricultural systems, including beneficial arthropods such as pollinators, predators, and parasitoids, are also susceptible to NSTs. Arthropods may be exposed to the active ingredients from NSTs through physical contact with the pesticide dust generated during planting (Nuyttens et al., 2013); through consumption of or physical contact with contaminated plant material including foliage, nectar, pollen, and guttation fluid (Moscardini et al., 2014; Moser and Obrycki, 2009; Pisa et al., 2015; Prabhaker et al., 2011; Seagraves and Lundgren, 2012; Stapel et al., 2000; Tapparo et al., 2011; Van der Sluijs et al., 2013); through contaminated honeydew produced by phloem-feeding insects (Calvo-agudo et al., 2019); and in the case of predators and parasitoids, through contaminated prey or hosts (Bredeson et al., 2015; Papachristos and Milonas, 2008; Taylor et al., 2015). In addition to crops grown from treated seeds, other plants within and near fields can also take up neonicotinoids from the soil, creating a secondary source of contaminated plant material (Botias et al., 2016; Botías et al., 2015; Bredeson and Lundgren, 2019; Krupke et al., 2012). While the

widespread use of NSTs has primarily been scrutinized due to concerns about their relation to the decline of honeybees (*Apis mellifera* L.) (Godfray et al., 2014), their impacts on other pollinators and arthropod natural enemies requires greater attention (EASAC, 2015). Impacts of neonicotinoids on predators and parasitoids include reduced survival and reproduction, as well as physiological and behavioral changes that may disrupt the ecosystem services provided by these natural enemies (Chagnon et al., 2015; Pisa et al., 2015). A primary objective of this dissertation was to evaluate the impacts of NSTs on aerial and foliar arthropod communities in Maryland grain fields through various arthropod sampling methods. I also evaluated the uptake of neonicotinoids from the soil by weedy flowering plants within fields, as a potential route of exposure for pollinators and other non-target arthropods.

Overview of dissertation

The objectives discussed thus far, to determine the potential yield benefits of NSTs in Maryland grain production, as well as impacts on non-target arthropods and soil health, were carried out as part of a single field study evaluating the use of NSTs in a three-year rotation of full-season soybean, winter wheat, double-cropped soybean, and corn. The most noteworthy results from that field study were in winter wheat, where the activity density of aphelinid wasps, a group that contains several aphid parasitoids, was reduced throughout the spring, despite aphids only being controlled in the fall (Dubey et al., 2020; Pike et al., 1997). Cereal aphids are an important pest of wheat, as they vector barley yellow dwarf virus in addition to their direct feeding damage (Hesler et al., 2018).

Parasitoid wasps play a key role in natural control of cereal aphids (Schmidt et al., 2003), and by disrupting them, NSTs have the potential to cause secondary pest outbreaks, requiring further pesticide applications (Cloyd and Bethke, 2011). The duration of efficacy of NSTs in winter wheat can vary greatly (Kennedy and Connery, 2012; Kirkland et al., 2018; Royer et al., 2005; Zhang et al., 2016), and it is not clear whether the observed impact on aphelinid wasps was a result of continued neonicotinoid activity in wheat in the spring, or an effect that carried over from the fall. Thus, the final objective of this dissertation was to determine the duration of efficacy of NSTs against cereal aphids in Maryland winter wheat through laboratory studies and evaluate potential host-mediated impacts on aphid parasitoids over the course of the growing season.

Given that NSTs do not provide consistent yield benefits and can cause a variety of non-target impacts, their widespread use may not be warranted in Maryland. If NSTs do impact the ecosystem functions performed by beneficial arthropods, soil microbes and other organisms, they could damage agroecosystem health, and cause a decline in agricultural productivity over time (Chagnon et al., 2015). Due to the immense variability of factors such as soil, climate, agricultural practices, crop varieties, and biodiversity, costs and benefits of NSTs need to be determined separately for different crop production areas and systems. Much of the current research on neonicotinoids is from Europe, where NST use has been severely restricted since 2013 due to concerns related to pollinator health (Kathage et al., 2018). The findings of those studies may not be applicable when making decisions about NST usage in the US. The overall goals of this

dissertation were to determine whether the use of NSTs is warranted in mid-Atlantic grain production and to evaluate their impacts on this agroecosystem. By furthering our understanding of how NSTs can affect non-target organisms, including those that perform essential ecosystem functions, I hope to facilitate their effective and sustainable use.

Chapter 1: Evaluating impacts of insecticide seed treatments on the soil microbial community in a grain crop rotation in the Maryland Coastal Plains

Abstract

When neonicotinoid insecticides are applied as seed treatments (NSTs), the majority of active ingredients remain in the soil, where they may break down rapidly, persist for long periods, leach into ground water or runoff into nearby water bodies. When neonicotinoids persist in the soil, they can harm soil health by negatively impacting earthworms and the soil microbial community. We compared three seed treatments (fungicide products only; fungicide products + imidacloprid; and fungicide products + thiamethoxam) to an untreated control in a three-year rotation of full-season soybean, winter wheat, double-cropped soybean and corn in the Coastal Plains of Maryland. Specifically, we evaluated impacts on overall soil microbial activity measured through basal respiration, on the structure and composition of the prokaryotic community, and on various soil quality parameters such as wet aggregate stability and ammonium and nitrate ion concentration. None of the metrics quantified in this study were impacted by NSTs, suggesting that soil health is not threatened by the use of NSTs in Maryland Coastal Plains soils. These results may be due to a number of factors, including the low insecticide residue levels detected in the soil, our conservation tillage practices increasing the stress tolerance of soil microbes, and the legacy of two decades of NST use. Given the high NST adoption rates and frequent use; future field research should focus on potential impacts of NSTs on land without a

history of neonicotinoid use. Although NSTs did not affect soil health, they have the potential to harm aquatic communities through leaching and runoff. Their impacts on water bodies within the Chesapeake Bay watershed should be further evaluated.

Introduction

Over the last several decades, increasing agricultural production has resulted in expansion and intensification of land use (Foley et al., 2005). This includes expanded use of agrochemicals such as pesticides, leading to concerns about their effects on soil health through impacts on key soil organisms such as earthworms and soil microorganisms (Edwards, 2002; Foley et al., 2005). Earthworms are important ecosystem engineers that break down plant litter into organic matter, reduce soil compaction, increase porosity and aeration, and aid in transportation of nutrients and water (Beare et al., 1995, Edwards and Bohlen, 1996), while soil microorganisms play a crucial role in synthesis of organic material, nutrient cycling, and maintenance of soil structure (Kibblewhite et al., 2008; Schloter et al., 2003). Both groups are susceptible to the effects of pesticides. Earthworms can experience increased mortality, decreased growth and fecundity, and altered behavior (Pelosi et al., 2014). Impacts on soil microorganisms include changes in microbial activity, biomass, and community structure (Puglisi, 2012). These pesticide impacts have the potential to disrupt essential ecosystem services provided by these soil organisms and could lead to long-term impacts on soil health.

One class of pesticides that could seriously threaten soil health is neonicotinoid insecticides. Since their introduction in the 1990s, neonicotinoids have become the most used class of insecticides worldwide, gaining popularity due to attributes such as their low impact on vertebrates, systemic nature, and versatility of application methods (Nauen et al., 2008; Simon-Delso et al., 2015). They are especially common in the form of neonicotinoid seed treatments (NSTs), which are widely used in field crops such as corn, soybean, wheat and cotton. In 2011, 79-100% of corn and 34-44% of soybeans in the US were grown using NSTs (Douglas and Tooker, 2015).

When neonicotinoids are applied as seed treatments, less than 20% of the active ingredients are taken up by the plant, with the majority remaining in the soil, where they may break down rapidly, persist for long periods, leach into ground water, or runoff into surrounding water bodies (Bonmatin et al., 2015; Leiva et al., 2015; Li et al., 2018; Morrissey et al., 2015; Sur and Stork, 2003). While microbial breakdown plays the largest role in the degradation of neonicotinoids in soil, other biological and physical factors are also important, including soil moisture content, temperature, soil type (both texture and organic matter content), pH, precipitation, and ultraviolet radiation (Bonmatin et al., 2015; Gupta et al., 2008a, 2008b; Liu et al., 2011; Smalling et al., 2018; Zhang et al., 2018; Zhou et al., 2013). The half-life of neonicotinoids in soil varies greatly by region and soil type, and values calculated in laboratory studies may not correspond to those measured in the field. The calculated half-life of imidacloprid ranged an order of magnitude from 100 to 1230 days from application (Baskaran

et al., 1999) and that of clothianidin varies even more, from 148 to 7000 days (DeCant and Barrett, 2010). In one field study, the majority of residues in the soil broke down within 80 days of planting imidacloprid-treated corn, and by harvest time, residue levels were not significantly different from untreated fields (Donnarumma et al., 2011). Conversely, large-scale surveys in France and the UK found imidacloprid present in the majority of sampled soils, despite treatment occurring multiple years prior (Bonmatin et al., 2005; Botías et al., 2015). Because of the large variability in the fate of active ingredients from NSTs, the outcomes must be evaluated on a case by case basis.

Neonicotinoids in the soil have the potential to alter microbial community structure and activity (Zhang et al., 2018). Both imidacloprid and acetamiprid can reduce soil metabolic activity (Cycoń et al., 2013; Wang et al., 2014), while imidacloprid application may also have either a slight negative or positive effect on bacterial diversity in soils of low and high salinity, respectively (Zhang et al., 2015). Field rates of imidacloprid can also reduce substrate-induced respiration, bacterial abundance, as well as phosphatase and urease for a short period after application, with N_2-fixing and nitrifying bacteria displaying particular sensitivity to imidacloprid (Cycoń and Piotrowska-Seget, 2015). Yu et al. (2020) found that low levels of neonicotinoids increased soil microbial diversity while high doses decreased diversity. Neonicotinoids also changed the relative abundances of different phyla, increasing nitrogen metabolism and decreasing carbon metabolism (Yu et al., 2020). These studies demonstrate that neonicotinoid

insecticides can have a range of impacts on soil microorganisms, which could subsequently disrupt the ecosystem services they provide.

While these laboratory studies uncover valuable information, their results do not directly translate to neonicotinoid application in the field. This is due to the various external factors that alter the fate of neonicotinoids in soil and because laboratory studies generally use analytical grade neonicotinoids, while NSTs contain surfactants and other chemicals that change the behavior of the insecticides (Bonmatin et al., 2015). Additionally, commercial NSTs usually include multiple fungicide products, which also have the potential to alter the soil microbial community (Puglisi, 2012). Another difference between laboratory studies and field conditions is that laboratory studies evaluate single applications of neonicotinoids, but in the field NSTs are often used repeatedly, over multiple years. Depending on persistence, the active ingredients may accumulate in the soil, exposing soil organisms to higher insecticide concentrations over time (Goulson, 2013).

Only a few studies have evaluated how NSTs affect soil microorganisms in a field setting. A three-year study in Pennsylvania examined rhizosphere bacteria and fungi in corn treated with thiamethoxam rotated with soybean treated with a combination of thiamethoxam and imidacloprid. Microbial richness was not impacted, but community structure was altered in the fungal rhizosphere community in soybean and in both the fungal and bacterial communities in corn (Nettles et al., 2016). Another study in China evaluated the impacts of imidacloprid and clothianidin treated wheat on soil bacterial and fungal

communities, finding that bacterial and fungal richness and community structure were altered by the insecticides, but that the community recovered by the end of the growing season, with no apparent long term negative impacts (Yaofa Li et al., 2018). Both the ultimate fate of neonicotinoids in the soil and their impacts on soil microbes are highly variable and difficult to predict, and we do not understand the mechanisms that underpin community responses to neonicotinoids.

Microbial abundance and community structure may be impacted by exposure to neonicotinoids through multiple mechanisms. Microorganisms that can break down neonicotinoids and use them as a source of nutrients may outcompete others that cannot utilize the insecticides, altering community structure. The high toxicity of some neonicotinoid metabolites could also selectively impact certain taxa, further altering community composition and function (Hussain et al., 2016). Although soil microbial communities are thought to have a high degree of functional redundancy, a reduction in specialists such as nitrifying and N_2-fixing bacteria could negatively impact their ecosystem services (Chagnon et al., 2015; Cycoń et al., 2013; Philippot et al., 2013). Further research is required to evaluate the impact of NSTs on soil microorganisms in different regions and under different cropping systems, and to determine whether their repeated use is sustainable with regards to soil health.

The goal of this study was to evaluate the impacts of NSTs on soil health and the soil microbial community in grain crop production systems in Coastal Plains soils of Maryland. The study was part of a larger research project investigating the impacts of two popular NSTs, [Gaucho 600 (imidacloprid), and

Cruiser 5FS (thiamethoxam)], on arthropod communities during a three-year crop rotation of full-season soybean, winter wheat, double cropped soybean, and corn, which is commonly practiced in the mid-Atlantic United States. As part of that larger project, we determined that insecticide residue levels in the soil remained relatively low throughout the rotation, but that the highest levels were found in the final year, suggesting some accumulation (Dubey et al., 2020). For this study, we measured soil health parameters that included wet aggregate stability and nitrate and ammonium ion concentration at the beginning and end of the study. We hypothesized that these parameters could change gradually over time due to impacts on the ecosystem function performed by soil microorganisms (Kreutzweiser, 2008). We evaluated changes in the overall level of microbial activity using the Solvita® field test, a commercial test kit for measuring soil basal respiration (Doran et al., 1997; Haney et al., 2008). We hypothesized that there may be a reduction in overall microbial activity, especially shortly after planting, when neonicotinoid levels in the soil are at their highest. Treatment impacts on the soil prokaryotic community were evaluated through 16S rRNA sequencing. Given the geographic proximity and the similar no-till corn and soybean rotation, we hypothesized that the impacts on the bacterial community in our study might be similar to those identified by Nettles et al. (2016) in Pennsylvania, i.e. a change in the community structure but no impact on overall richness. We also anticipated that insecticide impacts on microbial activity and bacterial community structure could worsen over the course of the study, due to repeated NST use over multiple years.

Materials & Methods

Site Information

The study was conducted from 2015 to 2017 at two University of Maryland research farms: the Wye Research and Education Center in Queenstown, MD, USA (38°54'02.80" N 76°08'22.06" W) and the Central Maryland Research and Education Center in Beltsville, MD, USA (39°01'08.11" N 76°49'25.10" W).

The soils at both sites are Coastal Plain Typic Hapludults. The Beltsville site consists of Evesboro-Downer complex and Russett-Christiana complex soil map units, and soil texture analysis of topsoil samples from individual plots conducted by the Cornell Nutrient Analysis Laboratory (CNAL) at the beginning of the study identified the soil at Beltsville as either loamy sand or sandy loam. The Beltsville site was planted at the edge of a larger field, bordered by the field on two sides, with a dirt track and woody drainage ditch on the other two sides. The location is surrounded by a patchwork of woodlands and agricultural fields. The site was previously planted with neonicotinoid seed treated corn in 2012, and untreated winter wheat and double-cropped soybean in both 2013 and 2014. The field was not tilled during those years and was previously only tilled when required for specific experiments. The Queenstown site consists of Unicorn silt loam and Ingleside sandy loam soil map units and the soil was identified as sandy loam or loam. The Queenstown site was planted in the middle of a larger field, which was located on an island in the Wye River, a few miles before the river enters the Chesapeake Bay. The larger field is adjacent to the river on two sides,

separated by a narrow woodland border. The Queenstown site was planted with neonicotinoid seed treated corn in 2012 and 2014, and untreated full-season soybean in 2013. The field was not tilled during that time and was not regularly tilled in previous years.

Experimental Design

The study was conducted as part of a larger research project evaluating the costs and benefits of neonicotinoid seed treatments, and the same plots were used to collect pest suppression, arthropod community, crop growth, and yield data (Dubey et al., 2020). At both sites, four treatments were compared over a three-year rotation of full-season soybean, winter wheat, double-cropped soybean, and corn. The treatments were control or untreated seeds, fungicide seed treatment (products varied by crop and insecticide), fungicide + imidacloprid insecticide seed treatment (Gaucho 600; Bayer Crop Science, Monheim am Rhein, Germany), and fungicide + thiamethoxam insecticide seed treatment (Cruiser® 5FS; Syngenta AG, Basel, Switzerland). Full-season soybean was planted in May 2015, winter wheat in October 2015, double-cropped soybean in July 2016 and corn in May 2017. For detailed information about varieties, pesticide products and application rates, refer to Tables A1 and A2 in Appendix A. Due to variation in application and seeding rates, the quantities of active ingredients applied were similar in soybean and corn but were almost double that quantity in wheat.

Four replicate plots of the four treatments were planted at each site, arranged in a Latin Square (Appendix A, Fig. A1). Plots measured 9.1m x 15.2m; the rows of plots were separated by rows of untreated grain to provide space for

the planter to turn (measuring 9.1m x 12.2m at Queenstown and 9.1m x 15.2m at Beltsville); and columns of plots were separated by 9.1m bare strips to delineate plot boundaries. Treatments were planted into the same plots for all four crops, to evaluate potential cumulative impacts. Standard no-till agronomic practices and fertilization regimes for Maryland were followed, except that cover crops were not planted in between double-cropped soybean and corn. Foliar fungicides and insecticides were not applied during the study, except for application of the fungicide Caramba twice during the flowering stage of wheat at Queenstown to control fusarium head blight. To control weeds, herbicide products including Authority First DF (sulfentrazone, cloransulam-methyl; FMC Corporation, Philadelphia, PA, USA), GlyStar Plus (glyphosate, Albaugh LLC, Ankeny, IA, USA) and Makaze (glyphosate, Loveland Products, Loveland, CO, USA) were applied before planting and during early growth stages of the crop.

Soil Quality Parameters

To evaluate field uniformity at baseline, soil was collected in May 2015 before full-season soybean was planted. Soil was collected by taking 30 random soil cores from each plot, mixing them in a bucket, and taking a sample of the homogenized soil back to the lab. Soil probes had a diameter of 1.9 cm and cores were taken to a depth of approximately 12 cm. Soil was dried by spreading it out on paper plates and leaving it to air dry over one to two weeks, until it had a constant weight. Measurements were repeated at the end of the study after corn was harvested in October 2017.

The following parameters were tested:

Total percentage of carbon, hydrogen and nitrogen: Soil was analyzed using an elemental analyzer by the Analytical Lab at the University of Maryland (UMD) in 2015, and by CNAL in 2017.

Soluble salt concentration: The soil was analyzed by CNAL in 2015 and 2017 by extracting soluble salts from the soil with water and measuring the electrical conductivity of the soil using a conductivity meter.

pH: The soil was analyzed by CNAL in 2015 and 2017 by allowing a suspension of one part soil in two parts water to stand for one hour and then determining the pH using a LIGNIN robotic pH system.

Wet aggregate stability: Soil was analyzed by CNAL in 2015 and 2017 by using a rainfall simulator to steadily rain on a sieve containing a known weight of soil aggregates and measuring the fraction of soil remaining on the sieve.

Active Carbon: Soil was analyzed by the UMD Analytical Lab in 2015 and CNAL in 2017, by reacting soil with dilute potassium permanganate and using a colorimeter or spectrophotometer to measure loss of potassium permanganate color in the solution, which is correlated with presence of oxidizable carbon in the soil.

Available Nitrogen: Ammonium and nitrate ion concentration was measured by a research associate at UMD in 2015 and at CNAL in 2017. In 2015, soil for nitrate ion analysis was extracted using potassium sulfate and was measured in a flow-injection autoanalyzer, while soil for the ammonium ion analysis was extracted using potassium chloride and analyzed colorimetrically using a digital

spectrophotometer. In 2017, soil for both analyses was extracted using potassium chloride and measured in a flow-injection autoanalyzer.

Data was analyzed separately for 2015 and 2017 using analysis of variance (JMP Pro 13.2.1, SAS Institute Inc., Cary, NC, USA). Treatment, site and column (nested within site) were included as fixed effects due to known spatial variability between columns. The assumption of normality was tested using a Shapiro-Wilk test, and data was transformed as necessary. The assumption of homoscedasticity was tested using Levene's test and weighted least squares methods (Weighting factor: (residual variance)$^{-1}$ of the fixed effect that most deviated from homoscedasticity) were used, as necessary. When effect differences were statistically significant ($P<0.05$), means comparisons with Tukey's adjustment were used to compare treatment effects.

Soil Basal Respiration

Basal respiration was measured using the Solvita® Field Test (Woods End Laboratories Inc., Mt. Vernon, ME, USA), which measures the rate of CO_2 emission from the soil, providing a snapshot of soil microbial respiration (Doran et al., 1997). The test was conducted before full-season soybean was planted in 2015, three times during each growing season, and after corn was harvested in 2017. See Table A3 in Appendix A for the sampling timeline. Soil samples were collected using the method described in the previous section, with soil collected from within and between crop rows. In the laboratory, roots, invertebrates, and other debris were removed from the soil and 100g of soil was weighed out into

the plastic jars provided as part of the test kit. Gel probes were placed in each jar following the instructions provided and the samples were placed in a growth chamber at a constant temperature of 22°C. These probes follow the principle of the Beer-Lambert Law and change color in proportion to the concentration of CO_2. After 24 hours, the probes were removed from the soil and CO_2 emission was measured using the portable digital spectrometer included with the kit. The test was conducted on the same day that soil was collected.

Basal respiration data was analyzed separately for the 2015 pre-planting test, the 2017 post-harvest test, and for each crop, using analysis of variance as described previously. For each crop, the fixed effects were treatment, date, treatment*date, site, and column (nested within site). The treatment*date interaction was dropped as it was not significant in any case.

Illumina sequencing of the 16S rRNA gene

Soil for sequencing was collected during the VC-V2 growth stage of full-season soybean in 2015 (June 3rd at Beltsville and June 12th at Queenstown), at Feekes stage 11 of winter wheat in 2016 (May 26th at Beltsville and May 25th at Queenstown), and during the V3-V4 growth stage of corn in 2017 (May 30th at Beltsville and May 31st at Queenstown). The soybean and corn dates were chosen as they represent the period shortly after planting when the highest neonicotinoid concentrations can be expected in the soil. The winter wheat date was chosen to be temporally similar to the other two sampling dates, in order to generate comparable data from each year of the study.

Soil was collected using the same methods described previously and was stored at -80°C until DNA was extracted. DNA was extracted using the Qiagen DNeasy PowerLyzer PowerSoil kit (Qiagen, Hilden, Germany). The manufacturer's instructions were followed throughout, except that extractions were carried out using ~750 µl of a suspension of soil in phosphate buffered saline solution in a 2:1 ratio by volume, instead of using 0.25 g of dry soil. DNA samples were quantified using a Qubit 2.0 fluorometer (Invitrogen, Waltham, MA, USA) and then diluted to 5 ng/µl for PCR amplification and subsequent steps. Samples were stored at -20°C between steps. The primers 515F+adapter (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAG ACAGGTGCCAGCMGCCGCGGTAA-3') and 806R+adapter (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACVSGGGTA TCTAAT -3') were used to target the 16S region (Caporaso et al., 2012). The PCR reaction included 3.5uL of DNA, 17.5uL of Phusion™ Flash High-Fidelity PCR Mastermix (Thermo Fisher Scientific, Waltham, MA, USA), and 7uL of both primers (1ng/uL). The 16S Metagenomic Sequencing Library Preparation protocol (Part # 15044223 Rev. B, support.illumina.com) was used to process the PCR product for sequencing. The samples were cleaned up using AMPure XP beads (Beckman Coulter, Brea, CA, USA) and were indexed using the Nextera XT 96 index kit (Illumina, San Diego, CA, USA). Samples were pooled, and amplicon size of the library was checked using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). The library was quantified through qPCR was and the final library was diluted to 12 pM, spiked with 30% PhiX (Illumina,

San Diego, CA, USA), and sequenced on an Illumina MiSeq System using a 600-cycle v3 cartridge.

Sequencing data was processed and analyzed using the R statistical package (version 3.6.2). The DADA2 package (version 1.14.1) was used to filter and de-replicate sequences, infer samples, merge pair end reads, and check for chimeras (Callahan et al., 2016) by following the DADA2 pipeline tutorial (Callahan, n.d.). Taxonomic assignments were made by matching sequences with the SILVA database (version 138) (arb-silva.de). The resulting amplicon sequence variant table was analyzed using the Phyloseq package (version 1.30.0) (McMurdie and Holmes, 2013). Data for each crop was analyzed separately. Alpha diversity indices were calculated using the estimate_richness function, and treatment effects were evaluated through analysis of variance in JMP Pro, as described previously.

For the following analyses, amplicon sequence variants (ASVs) with a total abundance or number of reads less than 10 across all samples within a crop were removed and relative abundances were calculated. The analyses were attempted using relative abundance data and rarefied data, but there were no meaningful differences in results between the two methods, so only relative abundance data has been included. The tax_glom function was used to merge taxa to the class level, and the relative abundances of the 20 most abundant classes were graphed for each crop, with data combined across sites. Principal Coordinate Analysis (PCoA) using a Bray-Curtis dissimilarity matrix was used to visualize differences between treatments and sites, using the ordinate and plot_ordination

functions. Permutational Multivariate Analysis of Variance (PERMANOVA) using a Bray-Curtis dissimilarity matrix was used to compare communities between samples using the adonis function in the Vegan package (version 2.5.6) (Oksanen et al., 2019) with treatment, site and column (nested within site) as explanatory variables. The Bray-Curtis dissimilarity matrix is widely used in abundance-based data analysis as it is robust against taxonomy, enumeration and geography errors (Schroeder and Jenkins, 2018).

Results

Soil Quality Parameters

The soil parameters did not differ between treatments in baseline tests before the beginning of the study in spring 2015 (Table 1.1) or at the end of the study in fall 2017 (Table 1.2).

Table 1.1. The effect of seed treatments on soil quality parameters measured at the beginning of the study before soybean was planted in spring 2015. Analysis of variance was used with treatment, site and column (site) as fixed effects.

Metric	Treatment Mean + S.E.				Treatment F-value, P-value (df = 3, 21)
	Control	Fungicide	Imidacloprid	Thiamethoxam	
pH	6.14±0.10	6.27±0.09	6.07±0.07	5.91±0.12	1.18, 0.341
Wet aggregate stability (%)	48.98±1.59	46.18±0.91	46.30±2.39	54.68±3.17	2.09, 0.132
Soluble salt concentration (mmhos/cm)	0.068±0.004	0.066±0.005	0.073±0.004	0.074±0.005	0.09, 0.963
Nitrate concentration (mg/kg)	6.44±0.41	5.93±0.36	6.46±0.48	6.65±0.55	0.43, 0.729
Ammonium concentration (mg/kg)	5.20±0.23	5.01±0.24	4.70±0.17	5.13±0.28	0.15, 0.929
Active carbon (mg/kg)	372.9±14.6	375.8±11.9	367.6±18.7	369.3±21.8	0.82, 0.499
Total carbon (%)	1.246±0.131	1.256±0.076	1.278±0.091	1.233±0.114	0.26, 0.854
Total hydrogen (%)	0.301±0.010	0.314±0.008	0.329±0.018	0.316±0.015	0.82, 0.496
Total nitrogen (%)	0.099±0.007	0.110±0.005	0.110±0.006	0.101±0.007	0.88, 0.468

Table 1.2. The effect of seed treatments on soil quality parameters measured at the end of the study, after corn was harvested in fall 2017. Analysis of variance was used with treatment, site and column (site) as fixed effects. When effect differences were statistically significant ($P<0.05$), means comparisons with Tukey's adjustment were used to compare treatment effects. N.S. indicates that the means comparison test did not show differences between treatments. CON = Control; FUN = Fungicide; IMI = Imidacloprid; THI = Thiamethoxam.

Metric	Treatment Mean + Standard Error				Treatment F-value, P-value (df = 3,21)
	CON	FUN	IMI	THI	
pH	6.14±0.10	6.27±0.09	6.07±0.07	5.91±0.12	2.36, 0.101
Wet aggregate stability (%)	48.98±1.59	46.18±0.91	46.30±2.39	54.68±3.17	3.36, 0.038$^{N.S.}$
Soluble salt concentration (mmhos/cm)	0.068±0.004	0.066±0.005	0.073±0.004	0.074±0.005	0.65, 0.589
Nitrate ion concentration (mg/kg)	6.44±0.41	5.93±0.36	6.46±0.48	6.65±0.55	0.60, 0.625
Ammonium ion concentration (mg/kg)	5.20±0.23	5.01±0.24	4.70±0.17	5.13±0.28	0.84, 0.488
Active carbon (mg/kg)	372.9±14.6	375.8±11.9	367.6±18.7	369.3±21.8	0.1, 0.971
Total carbon (%)	1.246±0.131	1.256±0.076	1.278±0.091	1.233±0.114	0.1, 0.972
Total hydrogen (%)	0.301±0.010	0.314±0.008	0.329±0.018	0.316±0.015	0.70, 0.565
Total nitrogen (%)	0.099±0.007	0.110±0.005	0.110±0.006	0.101±0.007	1.13, 0.361

Soil Basal Respiration

Soil basal respiration, according to the Solvita® field test, was not significantly impacted by treatment at any time (Table 1.3).

Table 1.3. The effect of seed treatments on soil basal respiration measured using the Solvita® field test. Analysis of variance was used with treatment, date, treatment*date, site and column (site) as fixed effects. The treatment*date term was dropped as it was not significant in any case. CON = Control; FUN = Fungicide; IMI = Imidacloprid; THI = Thiamethoxam; FS = full-season; DC = double-cropped.

Crop	Mean CO_2 (lb/a) ± Standard Error				Treatment F-value, P-value (df = 3, 21)
	CON	FUN	IMI	THI	
2015 Pre-Planting	13.10±3.84	12.18±2.67	13.28±4.29	10.10±2.75	0.45, 0.723
2015 FS Soybean	26.25±2.72	32.41±3.91	36.03±4.90	29.45±3.10	2.36, 0.077
2015-2016 Winter Wheat	44.03±4.87	40.33±3.11	47.28±4.00	43.47±5.63	0.56, 0.642
2016 DC Soybean	35.95±4.98	37.75±5.06	39.85±5.14	33.23±3.59	0.44, 0.727
2017 Corn	31.93±3.92	35.10±6.63	27.38±2.51	36.53±5.63	0.76, 0.519
2017 Post-Harvest	26.23±4.20	25.09±4.52	20.25±1.38	19.34±1.97	1.44, 0.261

16S Illumina Sequencing

The 16S rRNA gene was sequenced using the Illumina Miseq System on one set of soil samples each from full-season soybean, winter wheat and corn. A total of 34,270 unique prokaryotic amplicon sequence variants (ASVs) were identified, with 20,085 ASVs in full-season soybean, 20,205 ASVs in winter wheat, and 20,025 ASVs in corn. The mean number of sequences per sample was 114,997 ± 11,283 for full-season soybean, 107,419 ± 10,437 for winter wheat and 101,669 ± 9,835 for corn.

Alpha diversity measured through the Shannon and Simpson diversity indices did not differ between treatments in any of the crops (Table 1.4).

Table 1.4. The effect of seed treatments on Shannon and Simpson diversity indices in full-season soybean, winter wheat, and corn. Analysis of variance was used with treatment, site and column (site) as fixed effects. CON = Control; FUN = Fungicide; IMI = Imidacloprid; THI = Thiamethoxam; FS = full-season; DC = double-cropped.

Crop	Diversity Index	Mean Index Value ± Standard Error				Treatment F-value, P-value (df = 3,21)
		CON	FUN	IMI	THI	
2015 FS Soybean	Shannon	6.852±0.233	7.091±0.120	6.849±0.342	6.713±0.374	0.26, 0.854
	Simpson	0.997±0.001	0.998±0.000	0.997±0.002	0.996±0.002	0.47, 0.705
2015-2016 Winter Wheat	Shannon	6.941±0.266	7.103±0.072	7.041±0.101	6.494±0.340	1.80, 0.178
	Simpson	0.997±0.001	0.998±0.000	0.998±0.000	0.991±0.007	1.26, 0.312
2017 Corn	Shannon	6.996±0.105	6.662±0.274	6.667±0.368	7.007±0.111	0.61, 0.617
	Simpson	0.998±0.000	0.997±0.001	0.995±0.003	0.998±0.000	0.58, 0.633

Relative abundance value ranges of the 20 most abundant taxonomic classes were visualized in full-season soybean (Fig. 1.1), winter wheat (Fig. 1.2) and corn (Fig. 1.3). For the mean relative abundance values, refer to Tables B1-B3 in Appendix B. *Acidobacteria* subgroup 6 was abundant in all three crops (corn 7.3%, soybean 7.3% and wheat 7.8%; all abundances listed are for the control), as was *Alphaproteobacteria* (corn 6.8%, soybean 8% and wheat 7.6%). Corn and wheat had high abundances of *Blastocatellia* subgroup 4 (corn 10.6%, wheat 9.2%), *Planktomycetacia* (corn 6.9%, wheat 7.4%) and *Verrucomicrobiae* (corn 6.8%, wheat 8.5%) while corn and soybean had high abundances of *Nitrosophaeria* (corn 9.2%, soybean 8%). While there was minor variation for some classes, community composition was largely similar between treatments in all three crops.

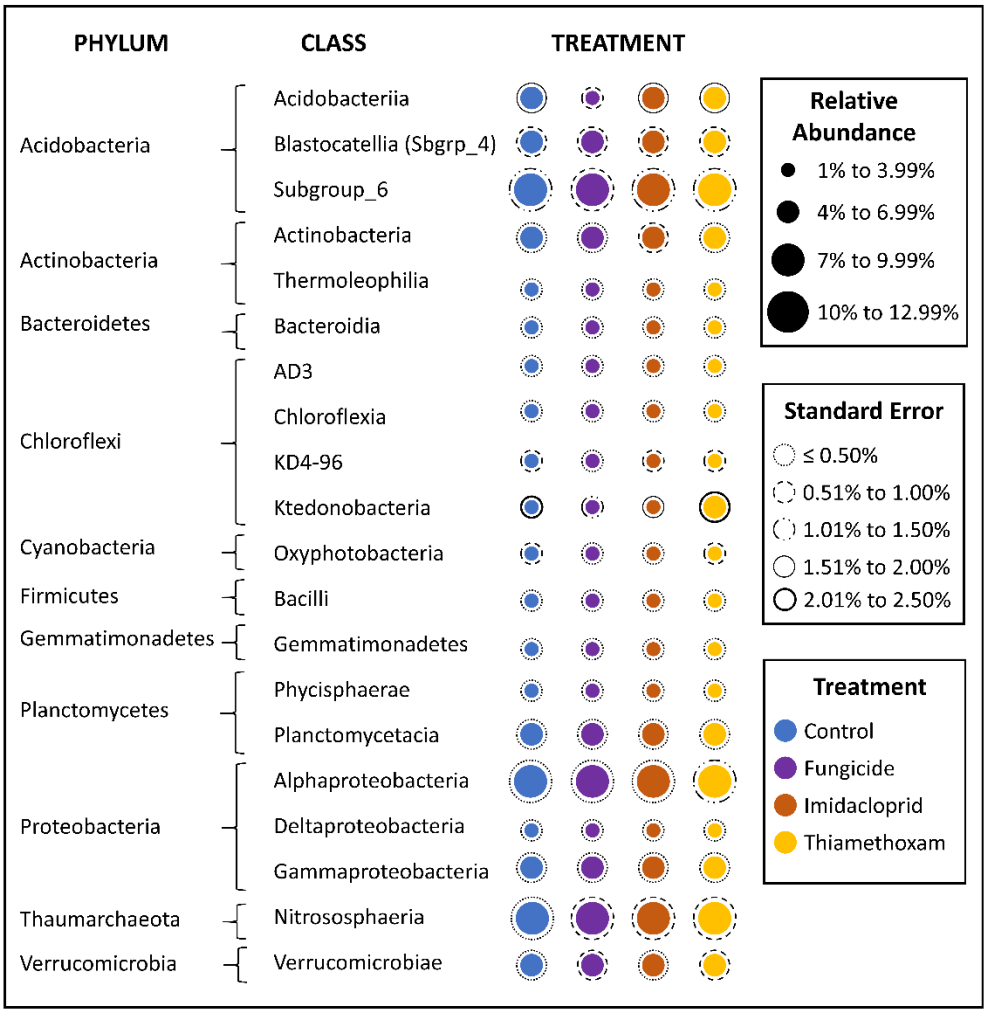

Fig. 1.1. Mean relative abundance of the 20 most abundant classes within the prokaryotic community in full-season soybean, combined across Beltsville and Queenstown sites. The range of values within which the mean and standard error fall are depicted for each class and treatment combination.

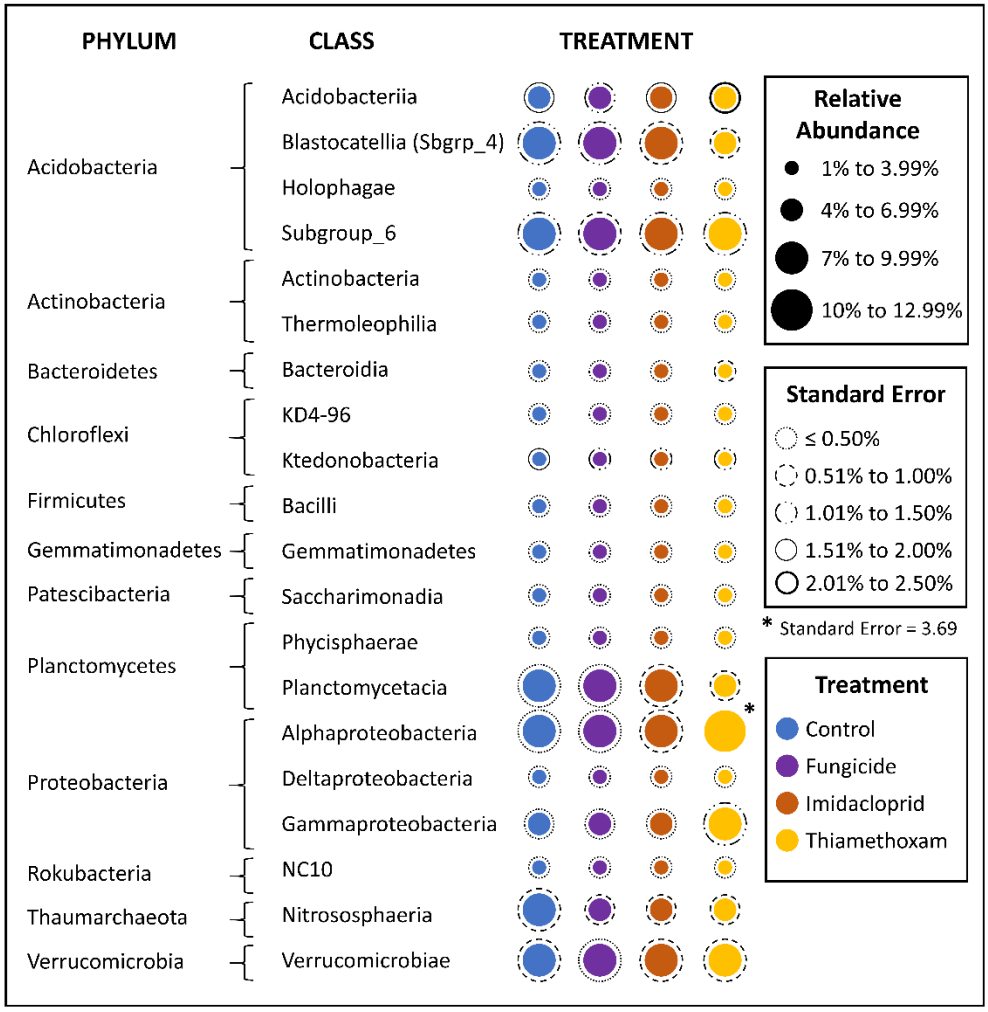

Fig. 1.2. Mean relative abundance of the 20 most abundant classes within the prokaryotic community in winter wheat, combined across Beltsville and Queenstown sites. The range of values within which the mean and standard error fall are depicted for each class and treatment combination.

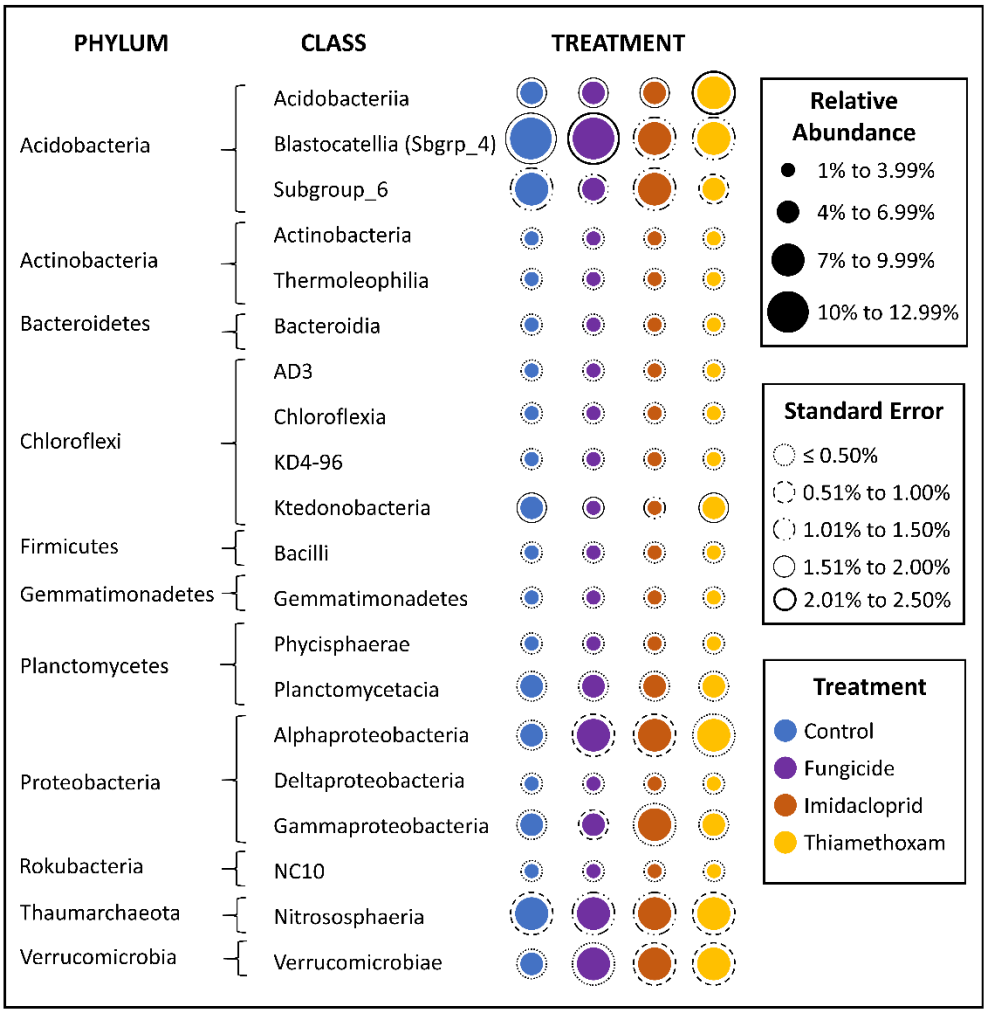

Fig. 1.3. Mean relative abundance of the 20 most abundant classes within the prokaryotic community in corn, combined across Beltsville and Queenstown sites. The range of values within which the mean and standard error fall are depicted for each class and treatment combination.

PERMANOVA analysis using a Bray-Curtis dissimilarity matrix indicated that prokaryotic communities varied by site but not by treatment in full-season soybean (treatment R^2=0.04, P=0.975; location R^2=0.26, P=0.001), winter wheat (treatment R^2=0.05, P=0.930; location R^2=0.24, P=0.001) and corn (treatment R^2=0.06, P=0.801; location R^2=0.26, P=0.001).

Principal Coordinate Analysis (PCoA) ordination was conducted using a Bray-Curtis dissimilarity matrix for full-season soybean, winter wheat and corn (Fig. 1.4). Communities differed between sites but not between treatments in all three crops, with the Queenstown samples clustered more closely together relative to the Beltsville samples.

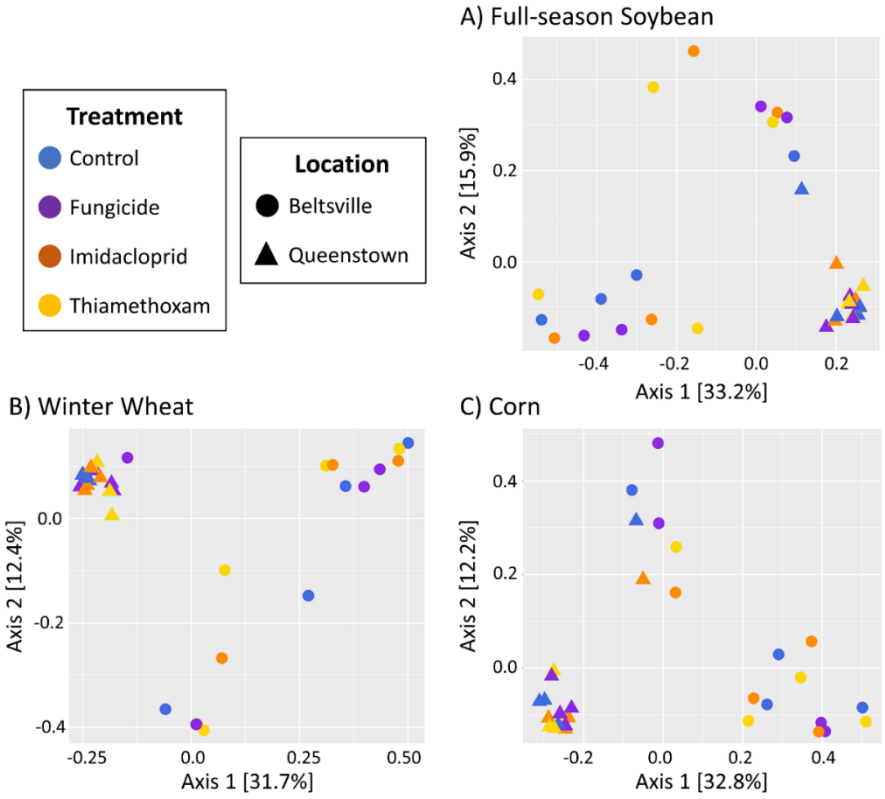

Fig. 1.4. Principal Coordinate Analysis (PCoA) ordination constructed using a Bray-Curtis dissimilarity matrix for A) full-season soybean, B) winter wheat and C) corn. Each point represents a sample with shape and color corresponding to site and treatment, respectively.

Discussion

We conducted a field study evaluating the impacts of neonicotinoid insecticide seed treatments (NSTs) on soil health in a three-year grain crop rotation in Maryland. Specifically, we evaluated treatment impacts on soil quality by measuring various soil quality parameters, overall microbial activity, and the structure and diversity of the prokaryotic community. We found that NSTs did not impact any of the tested parameters. These results did not support our hypothesis that NSTs would reduce overall microbial activity and alter the structure of the prokaryotic community, which was based on the results observed by Nettles et al. (2016). We anticipated similar results because their study was conducted in a no-till corn and soybean rotation, at a site in Pennsylvania that is geographically close to our sites in Maryland, with similar climate and agricultural practices, relative to studies conducted in other parts of the world. In that study, richness was not impacted by NSTs, but the fungal community was altered in soybean, and both the fungal and bacterial communities were altered in corn, compared to the untreated control. Although my results did not support my hypothesis, and differed from Nettles et al., they are consistent with the wider body of research on this topic.

A large-scale review of pesticide impacts on terrestrial microorganisms found that field rates of insecticides did not detectably alter microbial activity in ~40% of 85 studies and microbial community structure in ~15% of 18 studies (Puglisi, 2012). Similarly, field rates of fungicides did not affect microbial activity in ~50% of 253 studies and microbial community structure in ~15% of 28

studies. In particular, imidacloprid only impacted microbial activity in 3 out of 11 studies. In a greenhouse pot experiment using winter wheat, Van Hoesel et al. (2017) found that while imidacloprid and fungicide seed treatments impacted earthworm activity, they did not alter litter decomposition, soil basal respiration, microbial biomass, or specific respiration. Given the variability in the fate of neonicotinoids in the field, and in their impacts on soil microorganisms, there are several potential explanations for the lack of treatment effects observed in my study.

The first potential reason for the lack of impacts of NST is that insecticides were simply not present in the soil at high enough levels to impact soil organisms. As part of the larger study conducted using these experimental plots, soil residue analysis was conducted on samples collected on the same dates that soil was collected for 16S sequencing in 2015 soybean and 2017 corn, and ~ 7 weeks earlier in March, in 2016 winter wheat (Dubey et al., 2020). Insecticide levels in the soil were consistently low: ≤10 ppb of imidacloprid and ≤16ppb of thiamethoxam in 2015, ≤7ppb of imidacloprid in 2016, and ≤35ppb of imidacloprid, ≤17ppb of thiamethoxam, and ≤23ppb of clothianidin (a breakdown product of thiamethoxam that is also a commercial neonicotinoid product) in 2017 (Table 1, Dubey et al., 2020). These results show that only small amounts of neonicotinoid residues were present a few weeks after planting in 2015 and 2017, although we cannot be certain whether this was caused by leaching and runoff, or by rapid breakdown of insecticides in the soil. While Nettles et al. did not measure insecticide levels in the soil, the soil at their Pennsylvania site had higher

clay content, which is correlated with higher sorption, while our sites had higher sand content, which is linked to lower sorption and a higher likelihood of leaching, as well as faster neonicotinoid degradation (Bonmatin et al., 2015; Nettles et al., 2016). Therefore, the differences in results between the two studies may have been due to differences in neonicotinoid persistence in the soil. Although insecticide levels in the soil were low at our study sites, persistent effects on soil microbial communities can occur with relatively low levels of active ingredient. After 112 days, low dose (20 ppb) thiamethoxam-treated soil contained ~4 ppb thiamethoxam and exhibited a different bacterial community than the untreated control in a laboratory study (Yu et al., 2020). However, the lack of impacts observed from NSTs on the soil microbial community may also be due to crop production practices.

No-till agriculture is widespread in Maryland, and both my study sites were not tilled during the study and for at least two years beforehand. No-till shifts the microbial community towards stress-tolerators, which may be less disturbed by external stressors such as pesticides (Schmidt et al., 2018). Santos et al. (2006) found that herbicide use had fewer impacts on soil microbial activity in no-till systems compared to conventional-till systems. Difference in tillage practices could contribute to the difference between our results and those of Nettles et al. (2016), as their study site in Pennsylvania was tilled at the start of their study, which may have disrupted the microbial community, making it more susceptible to the impacts of NSTs. As Nettles et al. (2016) did not identify taxa through high throughput sequencing, we could not compare the microbial

communities between the two studies to determine whether the difference in tillage led to differences in community composition.

Given that NSTs have been widely used since the mid-2000s, it is also possible that NSTs altered the soil microbial community in the past and effects are no longer apparent. Microbial taxa may have adapted to utilize neonicotinoids or become resistant to them, or the community may have shifted towards taxa that can do so (Cycoń et al., 2013). While several bacterial strains have been identified as capable of breaking down neonicotinoids in the laboratory, little is known about degradation pathways in the soil (Hussain et al., 2016), making it difficult to correlate the fate of neonicotinoids with bacterial community structure in the soil. Ideally, future field research evaluating impacts of NSTs on the soil microbial community should be conducted in fields without a legacy of neonicotinoid use. Unfortunately, this is made difficult by the widespread use of NSTs, the drift of treated planting dust that can be detected beyond agricultural fields (Douglas and Tooker, 2015; Krupke et al., 2017), and foliar and soil application of neonicotinoids in other cropping systems (Simon-Delso et al., 2015).

Finally, it is possible that NSTs did in fact impact the soil microbial community in our study, but only in the soil nearest to the seed, which would not have been detectable in our study. Soil for our study was collected from both within and between rows of plants; this homogenization of soil from different areas may have diluted impacts present in the soil within the rows, which we would expect to have the highest neonicotinoid concentrations, and therefore the

strongest impacts (Pisa et al., 2015). Additionally, microbial communities within agricultural settings exhibit strong spatial heterogeneity, increasing the likelihood that neonicotinoids may not have consistent impacts in soil collected from different parts of the field and the difficulty of detecting impacts in homogenized samples (Franklin and Mills, 2003). Nettles et al. (2016) specifically collected soil samples from within the rhizosphere, which is another potential explanation for the varying results between the two studies. Another difference between the studies is that we only sequenced the prokaryotic community, while Nettles et al. (2016) evaluated and identified impacts on both the fungal and prokaryotic communities. Therefore, it is possible that NSTs did impact the fungal community in our study, but those impacts remained undetected.

Although NSTs did not appear to impact soil quality or the soil microbial community, given the high precipitation and sandy soil at our study sites, there is a high likelihood that the low residue levels in the soil correspond with neonicotinoids leaching and/or runoff into surrounding water bodies. Neonicotinoids are small, highly water soluble molecules, with high leaching potential (Bonmatin et al., 2015; Botías et al., 2015; Morrissey et al., 2015). Neonicotinoids have consistently been found in water bodies in agricultural regions, sometimes year-round (Hladik et al., 2018, 2014; Hladik and Kolpin, 2016). A survey of three creeks in the Chesapeake Bay watershed from April to June 2014 found a surge in neonicotinoid levels in runoff samples collected in mid-May, corresponding with the timing of corn and soybean planting (Hladik and Kolpin, 2016). When combined with the sandy soil and high precipitation at

our sites in Maryland, these findings support the idea that the active ingredients from the NSTs in our study may have runoff into surrounding water bodies within a few weeks of planting. Neonicotinoid concentrations in water are often sufficiently high for chronic and acute toxicity towards a number of aquatic invertebrates, especially insects from the orders Trichoptera, Ephemeroptera, and Diptera. This can disrupt aquatic food webs and cause cascading trophic effects, endangering aquatic ecosystems and negatively impacting fisheries (Miles et al., 2017; Morrissey et al., 2015; Starner and Goh, 2012; Yamamuro et al., 2019). Therefore, even in the absence of impacts on soil health, NSTs can pose a risk to aquatic communities.

Conclusions

We did not identify any effects of NSTs on microbial activity, diversity, and structure of the prokaryotic community in bulk soils. Our results are in contrast to other studies that measured rhizosphere soils and included fungal sequencing. Since we also did not detect high levels of NST residues in the bulk soils, it is possible that the sandy soils we studied favored faster decomposition or leaching or runoff. It is also possible that the no till system that we studied is better buffered against changes due to NST additions. Both the soil microbial community and the fate of neonicotinoids from NSTs are governed by a complicated set of factors, making it difficult to predict the outcomes in a specific region and cropping system. Although it is encouraging that NST additions may not impact soil health, NSTs active ingredients may runoff into surrounding water bodies, where they may disrupt aquatic ecosystems and harm fisheries. For these

reasons, NSTs should be used judiciously and the concentrations and potential impacts of neonicotinoids within water bodies in Maryland should be further investigated.

Chapter 2: Ecological impacts of pesticide seed treatments on arthropod communities in a grain crop rotation

Abstract

While many studies have investigated non-target impacts of neonicotinoid seed treatments (NSTs), they usually take place within a single crop and focus on specific pest or beneficial arthropod taxa. We compared the impacts of three seed treatments to an untreated control: imidacloprid + fungicide products, thiamethoxam + fungicide products, and fungicide products alone in a three-year crop rotation of full-season soybean, winter wheat, double-cropped soybean and corn. Specifically, we quantified neonicotinoid residues in the soil and in weedy winter annual flower buds and examined treatment impacts on soil and foliar arthropod communities as well as on plant growth and yield. Unquantifiably low amounts of insecticide were found in winter annual flowers of one species in one site year, which did not correspond with our treatments. Although low levels of insecticide residues were present in the soil, residues were not persistent. Residues were highest in the final year of the study, suggesting some accumulation. We observed variable impacts of NSTs on the arthropod community; principle response curve and redundancy analyses exhibited occasional treatment effects, with treatments impacting the abundance of various taxa, including predators and parasitoids. Overall, foliar taxa were impacted more than soil taxa, and the fungicides occasionally affected communities and individual taxa. Pest pressure was low throughout the study, and although pest

numbers were reduced by the insecticides, corresponding increases in yield were not observed. Pesticide seed treatments can impact arthropod taxa, including important natural enemies even when environmental persistence and active ingredient concentrations are low. The foliar community in winter wheat showed that in some cases, these impacts can last for several months after planting. Given the low pest pressure and lack of yield improvement in full-season soybean and double-cropped soybean, winter wheat, and corn, we did not observe benefits that could justify the risks associated with neonicotinoid seed treatment (NST) use. Our results suggest that NSTs are not warranted in Maryland grain production, outside of specific instances of high pest pressure.

Introduction

Declines in arthropod biomass have been documented at multiple locations and are likely linked to habitat loss, climate change, and agrochemical pollutants (Hallmann et al., 2014; Lister and Garcia, 2018). Since their introduction in the 1990s, neonicotinoid insecticides have become the most heavily used insecticide class worldwide, due to their low vertebrate toxicity, systemic nature and versatility of application methods (Nauen et al., 2008). Neonicotinoid seed treatments (NSTs) are especially popular; by 2011, NSTs were used in 79-100% of corn (*Zea mays* L.) and 34-44% of soybean (*Glycine max* L. Merr.) planted in the USA (Douglas and Tooker, 2015). When neonicotinoids are applied as NSTs, less than 20% of the active ingredients are taken up by the plant (Alford and Krupke, 2017; Sur and Stork, 2003), instead largely remaining in the soil, where their environmental fate is not fully

understood. The half-lives of neonicotinoids in soil vary considerably and they may persist and accumulate for multiple years post planting (Bonmatin et al., 2015). Due to their water solubility, neonicotinoids can also leach into groundwater and runoff into waterbodies; neonicotinoid residues are frequently detected at levels above ecological thresholds in waterbodies that are adjacent to or receive runoff from crop lands (Morrissey et al., 2015). In addition, neonicotinoids may contaminate non-crop plants. Several studies have found neonicotinoid residues in plants growing near treated fields, but it is difficult to determine whether the active ingredients were taken up from the soil or deposited aerially (Basley and Goulson, 2018; Botías et al., 2015; Pecenka and Lundgren, 2015; Stewart et al., 2014). Due to the widespread use, environmental persistence, and mobility of the active ingredients from NSTs, they are common pesticide pollutants.

NSTs pollution can negatively impact many non-target organisms. Although NSTs require relatively low active ingredient concentrations and can reduce non-target exposure due to pesticide drift, they have similar impacts on non-target arthropod abundance as soil and foliar pyrethroid applications (Douglas and Tooker, 2016). Beneficial natural enemies may be exposed to NST active ingredients indirectly by consuming herbivores or directly, either through physical contact or by feeding on plant material or nectar (Gontijo et al., 2015; Khani et al., 2012; Moscardini et al., 2014; Moser and Obrycki, 2009; Papachristos and Milonas, 2008; Seagraves and Lundgren, 2012). For example, the presence of neonicotinoids in the soil can suppress predatory ground beetles

(Coleoptera: Carabidae) through direct contact with active ingredients (Pisa et al., 2015; Simon-Delso et al., 2015), or by ingestion of contaminated prey (Douglas et al., 2015). Work characterizing the impact of neonicotinoids typically focuses on specific pest or beneficial taxa; however, the interconnected arthropod community should also be evaluated as a whole. Increased taxon diversity and evenness is associated with reduced pest pressure (Lundgren and Fausti, 2015); therefore, community-level impacts of NSTs could disrupt natural pest control. In corn, clothianidin treated seed altered the overall arthropod community after planting, with several beneficial predators decreasing in abundance (Disque et al., 2018). Neonicotinoids can also negatively impact pollinators which exhibit acute toxicity at high doses as well as sublethal impacts such as impaired memory, impaired foraging ability, and increased parasite loads (Decourtye et al., 2004; Godfray et al., 2014; Henry et al., 2012; Pettis et al., 2012; Rundlöf et al., 2015; Vidau et al., 2011; Whitehorn et al., 2012). Because pollinators often rely on non-crop floral resources, uptake by non-crop plants may be an important route of exposure (Basley and Goulson, 2018; Botias et al., 2016; Dively and Kamel, 2012). Given the risks associated with NST pollution, consideration must be given to their use in multiple crops, their potential long-term environmental persistence, and their effects on arthropod communities when evaluating non-target impacts.

In addition to the many risks associated with NSTs, they often provide limited benefits. Active ingredients from NSTs generally remain bioactive in plant tissue for three to four weeks post planting, so they only provide protection against early season soil and seedling pests (Alford and Krupke, 2017; Myers and

Hill, 2014). Additionally, many of the pests targeted by NSTs are sporadic pests that rarely cause economic losses (Papiernik et al., 2018). NSTs are frequently used prophylactically, and growers may not recoup the cost of treatment unless significant early season pest pressure occurs (Cox et al., 2007; Myers and Hill, 2014; Wilde et al., 2007). The economic benefits of NSTs vary greatly based on region and cropping system and must be evaluated on a case by case basis (Papiernik et al., 2018).

In this study, we evaluated the impacts of repeated use of two popular NSTs [Gaucho 600 (imidacloprid), and Cruiser 5FS (thiamethoxam)] during a three-year grain crop rotation common to the mid-Atlantic United States: full-season soybean, winter wheat, double-cropped soybean and corn. Given that NSTs are most commonly used in corn but are less widely used in soybean and wheat, this represents a worst-case scenario where NSTs are used repeatedly in all three crops. Because commercial NSTs always include fungicides in addition to insecticides, we included a fungicide only treatment as well as an untreated control in order to isolate the impacts of the fungicides from those of the insecticides. To the best of our knowledge, this is among the first studies to quantify the impacts of seed applied fungicides on the arthropod community. The location and concentration of pesticide active ingredients drive non-target effects; therefore, we quantified the persistence of neonicotinoids in the soil and determined whether weedy winter annual flowers uptake residues. We hypothesized that higher levels of neonicotinoid residues would be present in the soil later in the study due to accumulation from multiple crops. Our second

objective was to evaluate the impacts of pesticide seed treatments on the overall arthropod community and on individual arthropod taxa. We anticipated the strongest impacts on the soil community, given the potential soil persistence of active ingredients and the short activity period in plant tissue. We expected community disturbance early on with recovery during each cropping cycle as observed previously in corn (Disque et al., 2018), but hypothesized that disturbance in the soil community would increase over the course of the study due to potential cumulative impacts of repeated NST use. We also hypothesized that the fungicide only treatment could also impact the arthropod community, due to direct toxicity of seed-applied fungicides towards arthropods (Minnesota Department of Agriculture, 2012) or indirect alteration of crop fungal communities. Our final objective was to measure the economic value of the treatments in terms of plant growth metrics and yield, to determine whether the environmental risks of NSTs are justified by economic benefits in mid-Atlantic grain production. We did not expect the insecticide treatment to significantly improve yield because Maryland tends to have low pressure from pests targeted by NSTs; however; neonicotinoids may stimulate plant growth in the absence of pest pressure (Jeschke et al., 2011), which could improve growth parameters and yield.

Materials & Methods

The study was conducted at the Wye Research and Education Center in Queenstown, MD, USA (38°54’02.80” N 76°08’22.06” W) and the Central Maryland Research and Education Center in Beltsville, MD, USA (39°01’08.11”

N 76°49’25.10” W) and compared treatments over a three year rotation of four crops at each site. The four treatments were untreated seeds (control), fungicide products alone (varied by crop; Syngenta), fungicide products + imidacloprid insecticide (Gaucho 600; Bayer Crop Science), and fungicide products + thiamethoxam insecticide (Cruiser® 5FS; Syngenta). Full-season soybean was planted in spring 2015, winter wheat in autumn 2015, double-cropped soybean in summer 2016, and corn in spring 2017. At each site, four replicate plots of each treatment measuring 9.1m x 15.2m were arranged in a Latin square (Appendix A, Fig. A1). The plot rows were separated by rows of untreated grain that provided space for the planter to turn. Plot columns were separated by 0.91m bare strips to delimit plots and facilitate sampling. To determine cumulative effects of repeated treatments, each treatment replicate was planted in the same location for each crop in the rotation. Standard no-till agronomic practices for the region were followed throughout, except cover crops were not planted during the study, to promote the growth of winter annual plants within the plots. No foliar fungicides or insecticides were applied, with the exception of wheat, where the fungicide Caramba (metconazole, BASF Agricultural) was applied twice during the flowering stage at the Queenstown site to control fusarium head blight. Weeds were controlled through pre-plant and early season herbicide applications of products including Authority First DF (sulfentrazone, cloransulam-methyl; FMC Corporation), GlyStar Plus (glyphosate, Albaugh, Inc.) and Makaze (glyphosate, Loveland Products). The field at Beltsville was previously planted with untreated soybean, and at Queenstown with neonicotinoid seed treated corn. The seeding

rate, variety, and active ingredient rate for each treatment and crop are listed in Tables A1-A2 in Appendix A. Due to differences in seeding and application rates, the amount of active ingredient per acre varied slightly between soybean and corn, with wheat concentrations almost double that of the other crops.

Residue analysis

In spring 2016 and 2017, we collected flower buds from winter annual plants growing within the experimental plots for neonicotinoid residue analysis. Winter annual species were chosen based on abundance and attractiveness to pollinators. In 2016, common henbit (*Lamium amplexicaule* L.) was collected at Beltsville and common chickweed (*Stellaria media* L. Vill.) at Queenstown. In 2017, we collected common chickweed at Queenstown and both species at Beltsville. Soil was collected for residue analysis before and shortly after soybean and corn were planted in 2015 and 2017, and in March 2016, while wheat was dormant (see Table 1 for sampling dates). Further details about material collection are included in Appendix C.

Residue samples (3g per sample for flowers, ~100g per sample for soil) were sent to the USDA National Science Laboratory (Gastonia, NC, USA) for analysis, where they were tested for imidacloprid, thiamethoxam, and clothianidin, another popular neonicotinoid that is also a breakdown product of thiamethoxam (Simon-Delso et al. 2015). Briefly, neonicotinoid residues were extracted with a refined official pesticide extraction method [AOAC OMA 2007.0, the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe)], using an acetonitrile and water solution. Extraction was followed by

enhance matrix reduction (EMR) clean-up and analysis using certified standard reference materials and liquid chromatography coupled with tandem mass spectrometry detection (LC/MS/MS) utilizing the precursor and product ions of analytes of interest. The USDA National Science Laboratory reported detection levels were 1ppb for imidacloprid, 1ppb for thiamethoxam and 1ppb for clothianidin in flowers in 2016, and 10ppb for imidacloprid, 5ppb for thiamethoxam and 30ppb for clothianidin in flowers in 2017. In soil, the USDA National Science Laboratory detection level was 5ppb for imidacloprid, 10ppb for thiamethoxam and 15ppb for clothianidin.

Arthropod sampling

Throughout the study, the epigeal and soil invertebrate community was measured using pitfall traps (3 subsamples per plot) and surface litter extractions (4 subsamples pooled into two Berlese funnel extractions per plot). Samples were collected three times during each growing season. A small number of pitfall traps were lost due to animal activity in the field. However, we successfully collected at least one subsample per plot in each case. Activity density of aerial and foliar arthropods close to the ground was measured through sticky cards (3 subsamples per plot). In soybean, arthropod abundance in the plant canopy was measured by sweep netting, where 15 sweeps were taken in a straight line through the center of each plot once per season. Samples from one 2015 sweep net imidacloprid replicate at Beltsville and one 2016 sticky card double-cropped soybean sampling date at Queenstown were misplaced prior to processing. We also conducted visual inspections of plants to quantify pest pressure and beneficial arthropods in all

crops. Data from subsamples within replicates was averaged for analysis for all sample types. The sampling timeline can be found in Table A4-A7 in Appendix A and further details can be found in Appendix C.

Crop sampling

We measured the impact of NSTs on plant growth by recording stand density and plant height in all crops. In wheat, we also counted the number of tillers and measured the Normalized Difference Vegetative Index (NDVI), which can be used to indirectly measure crop biomass (Erdle et al., 2011). These metrics were included to test manufacturer claims that neonicotinoids can increase plant health and growth even in the absence of insect pests, and determine whether NSTs could be beneficial for Maryland farmers regardless of pest pressure (Jeschke et al., 2011). We also measured yield at the time of harvest. Details for each crop are included in Appendix C.

Statistical analysis

Arthropod data analysis

For arthropod sampling, taxa were identified to family in most cases, and adults and immatures were combined for all taxa. Insects from the following orders that could not be identified to family were excluded from all analyses: Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera. Ants (Hymenoptera: Formicidae) were excluded from Principal Response Curve (PRC) and individual taxon analyses for sticky cards, pitfall traps and litter due to their highly clumped distribution in the soil, which makes it difficult to correlate their abundance in the soil to treatment effects. However, they were included in the

Redundancy Analysis (RDA) for sweep net sampling which captures activity on the plant.

To characterize the impact of treatment over time, arthropod community composition was analysed in CANOCO 5 (Microcomputer Power, Ithaca, NY, USA) using Principal Response Curve Analysis (PRC) for pitfall traps, litter extraction and sticky card data for each crop, similar to previous studies (Disque et al. 2018). Briefly, PRC multivariate analysis is based on Redundancy Analysis (Van den Brink and Ter Braak J. F., 1999), with adjustments for the change in community response over time. In our study, total abundances for each taxon were averaged over subsamples within a replicate plot for each site prior to analysis. Taxa where the sum of individuals across sampling dates and sites for a crop was less than one were excluded from the PRC. For each crop and sample type, the date*treatment interaction term was used as an explanatory variable, and date and the site*column interaction were used as covariates to restrict data shuffling due to known spatial variability across columns. Canonical coefficients were generated for each date and plotted over time to evaluate the community response to the treatments relative to the untreated control; the control is plotted along the horizontal axis (representing time), and the magnitude (represented by canonical coefficients plotted on the vertical axis) and shape of curves represent the deviation of treatments from the control. The analysis also generates taxon-specific weights for the individual taxa that exhibit the strongest effects; taxa with high positive weights are more likely to follow the pattern depicted in the PRC, while taxa with high negative weights exhibit an opposite response. A Monte-

Carlo permutation procedure with N=499 was used to test the null hypothesis that the canonical coefficients of the treatment response equalled zero for all sampling times, and to calculate a Pseudo-F statistic, as performed in previous studies (Disque et al., 2018). Due to the sticky cards from the first sampling date at Queenstown being misplaced, only data from Beltsville was included for the first date for double-cropped soybean sticky card PRC. Because sweep net samples were conducted on a single date, captures were analyzed using RDA (Van den Brink and Ter Braak J. F., 1999).

PRC and RDA analyses were followed by analysis of variance of key arthropod taxa (JMP Pro 13.2.1, SAS Institute Inc., Cary, NC, USA) within crops and sample types, which were selected if they met all the following criteria: taxon weight >1 or <-1 in the PRC for at least one crop; total abundance ≥ 10 individuals across all treatments and sampling dates for that crop and sample type; mean abundance > 1 individual per treatment for at least one treatment within that crop and sample type. For each crop and sample type, treatment, site and column (nested within site) were included as fixed effects due to known spatial variability between columns. For pitfall trap, litter extraction, and sticky card data collected on multiple dates, mean abundances for each replicate plot from all three sampling dates were summed across dates for analysis. For visual counts, data from multiple sampling dates were summed for double-cropped soybean and wheat; however, in wheat data from the two winter sampling dates and the three spring/summer sampling dates was summed separately. Visual counts were only conducted once in full-season soybean, while in corn, each date was analyzed

separately due to variation in sampling methods. Sticky card data from the first sampling date for double-cropped soybean were excluded as samples from one site were misplaced before identification. The assumption of normality was tested using a Shapiro-Wilk test, and data was transformed as necessary. The assumption of homoscedasticity was tested using Levene's test and weighted least squares methods (Weighting factor: (residual variance)$^{-1}$ of the fixed effect that most deviated from homoscedasticity) were used when needed. To evaluate effect size, fungicide, imidacloprid and thiamethoxam treatments were compared to the control through a Hedge's g effect test using the cohen.d function (effsize package) (Torchiano, 2019) in the R statistical program (Version 3.5.1) (R Core Team, 2018). For pitfall trap, litter, sticky card and sweep net samples, if the ANOVA for a taxon was significant for any crop, effect sizes were calculated for all crops where that taxon was present, to allow for comparison between crops. This was not done for visual count data as sampling methods and collected taxa were not comparable across crops. Effect sizes were also calculated for collembola and soil mites in pitfall traps and litter data regardless of significance level, as they comprised up to 80% of the total soil arthropod abundance. When reporting data from ANOVAs, data are reported for the treatment effect unless the overall model was not significant, in which case model statistics are reported.

Crop data analysis

Plant height, stand count and yield data were analyzed with analysis of variance, using the model and methods described in the previous section for arthropod taxa. For NDVI and tiller counts, date and date*treatment were also included as fixed

effects, as data was collected on multiple sampling dates. The date*treatment was dropped from the model when not significant. When $P<0.05$ for the treatment effect, the fungicide, imidacloprid and thiamethoxam treatments were compared to the control through contrasts.

Results

Residual Analysis

Winter annual flowers

The USDA National Science Laboratory reported detection level was 1ppb for imidacloprid, 1ppb for thiamethoxam and 1ppb for clothianidin in flowers in 2016. In 2016, neonicotinoid residues were not found in any samples. In 2017, the reported detection level was 10ppb for imidacloprid, 5ppb for thiamethoxam and 30ppb for clothianidin in flowers. In 2017, unquantifiably low amounts (<10ppb) of imidacloprid were found in five of the chickweed samples from Beltsville, specifically two control samples and one from each of the other treatments. Detections did not exhibit a spatial relationship with the treatments.

Soil

In soil the reported detection level was 5ppb for imidacloprid, 10ppb for thiamethoxam and 15ppb for clothianidin. Before planting in 2015, low levels (≤10ppb) of imidacloprid were present in several replicates at Beltsville (Table 2.1). Similar levels of imidacloprid were detected after treated soybean was planted, and unquantifiably low amounts of thiamethoxam and clothianidin were found in one thiamethoxam and one imidacloprid treated replicate. At

Queenstown, no residues were detected prior to planting, and after planting only one thiamethoxam replicate and one imidacloprid replicate contained residues. In 2016, during wheat dormancy, unquantifiably low levels of imidacloprid were found in all plots at Beltsville, with higher amounts (7ppb) detected in the imidacloprid treated plots. In contrast, at Queenstown, unquantifiably low amounts of imidacloprid were detected only in the imidacloprid treated plots. Before corn was planted in 2017, low levels of imidacloprid were present in both imidacloprid sample replicates, and one control and thiamethoxam sample replicate at Beltsville. At Queenstown, no residues were detected prior to corn planting. After corn was planted, imidacloprid was detected across multiple treatments at Beltsville, and in the imidacloprid treated plots at Queenstown, with higher levels (≥10ppb) present in the imidacloprid treated plots at both sites. Thiamethoxam was detected in both thiamethoxam replicates (15-16ppb) at Queenstown, and thiamethoxam (17ppb) and clothianidin (23ppb) were found in one thiamethoxam sample replicate from Beltsville.

Table 2.1. Neonicotinoid residues in soil samples collected in 2015, 2016 and 2017. The detection level was 5ppb for imidacloprid, 10ppb for thiamethoxam and 15ppb for clothianidin. nd = not detected. Trace indicates that the insecticide was present but at levels below the quantification threshold. Pre-planting data from Queenstown is not included for 2015 soybean or 2017 corn as no insecticides were detected. For 2015 and 2017, the two values indicate data from the two pooled replicate samples, while in 2016, all the replicates were pooled into a single sample.

Site	Treatment	Insecticide Residue (ppb)		
		Imidacloprid	**Thiamethoxam**	**Clothianidin**
Full-season Soybean: Pre-plant - 5/12/2015 and 5/21/2015				
Beltsville	Control	8, trace	nd, nd	trace, nd
	Fungicide	6, 7	nd, nd	nd, nd
	Imidacloprid	trace, trace	nd, nd	nd, nd
	Thiamethoxam	7, 6	nd, nd	trace, nd
Full-season Soybean: Post-plant - 6/3/2015 and 6/12/2015				
Beltsville	Control	10, trace	nd, nd	trace, nd
	Fungicide	trace, 8	nd, nd	nd, nd
	Imidacloprid	8, trace	nd, trace	nd, nd
	Thiamethoxam	trace, 8	nd, trace	trace, nd
Queenstown	Control	nd, nd	nd, nd	nd, nd
	Fungicide	nd, nd	nd, nd	nd, nd
	Imidacloprid	trace, nd	nd, nd	nd, nd
	Thiamethoxam	nd, nd	16, nd	nd, nd
Winter Wheat: Dormancy - 3/2/2016 and 3/7/2016				
Beltsville	Control	trace	nd	nd
	Fungicide	trace	nd	nd
	Imidacloprid	7	nd	nd
	Thiamethoxam	trace	nd	nd
Queenstown	Control	nd	nd	nd
	Fungicide	nd	nd	nd
	Imidacloprid	trace	nd	nd
	Thiamethoxam	nd	nd	nd
Corn: Pre-plant - 4/10/2017 and 4/12/2017				
Beltsville	Control	7, nd	nd, nd	nd nd
	Fungicide	nd, nd	nd, nd	nd, nd
	Imidacloprid	8,9	nd, nd	nd, nd
	Thiamethoxam	trace, nd	nd, nd	nd, nd
Corn: Post-plant - 5/30/2017 and 5/31/2017				
Beltsville	Control	7, nd	nd, nd	nd, nd
	Fungicide	trace, nd	nd, nd	nd, nd
	Imidacloprid	11, 35	nd, nd	nd, nd
	Thiamethoxam	12, trace	17, nd	23, nd
Queenstown	Control	nd, nd	nd, nd	nd, nd
	Fungicide	nd, nd	nd, nd	nd, nd
	Imidacloprid	14, 26	nd, nd	nd, nd
	Thiamethoxam	nd, nd	15, 16	nd, nd

Arthropod sampling

Community impacts

2015 full-season soybean: In total, we analyzed 9,750 individuals from pitfall traps, 22,112 from litter extraction, 13,997 from sticky cards and 2,320 from sweep nets (Appendix D, Tables D1-D4). Arthropod communities did not respond to the pesticide treatments in pitfall trap (Pseudo-F=0.1, P=0.924) (Appendix D, Fig. D1), litter (Pseudo-F=0.2, P=0.946) (Appendix D, Fig. D2), or sticky card (Pseudo-F=0.2, P=0.356) PRC analyses (Fig. 2.1). Similarly, no treatment impacts on the arthropod community (First axis Pseudo-F=0.4, P=0.412) occurred in RDA analysis for sweep net data (Appendix D, Fig. D3).

2016 double-cropped soybean: We analyzed 24,760 individuals from pitfall traps, 23,135 from litter, 9,790 from sticky cards (excluding the first date at Queenstown, where the samples were misplaced) and 1,549 from sweep nets (Appendix D, Tables D1-D4). Pesticide treatments did not impact arthropod communities over the season for pitfall trap (Pseudo-F=0.2, P=0.814) (Appendix D, Fig. D1) or sticky card (Pseudo-F=0.4 P=0.198) (Fig. 1) PRC analyses. Litter data (Pseudo-F=0.3, P=0.064) revealed impacts during the early season for all three treatments, with an increase in the abundance of collembola and predatory mites (Mesostigmata) (Appendix D, Fig. D2). The insecticide treatments altered the arthropod community, reducing abundances of several taxa (First axis Psuedo-F=0.9 P=0.004) in RDA analysis of sweep net data (Appendix D, Fig. D3).

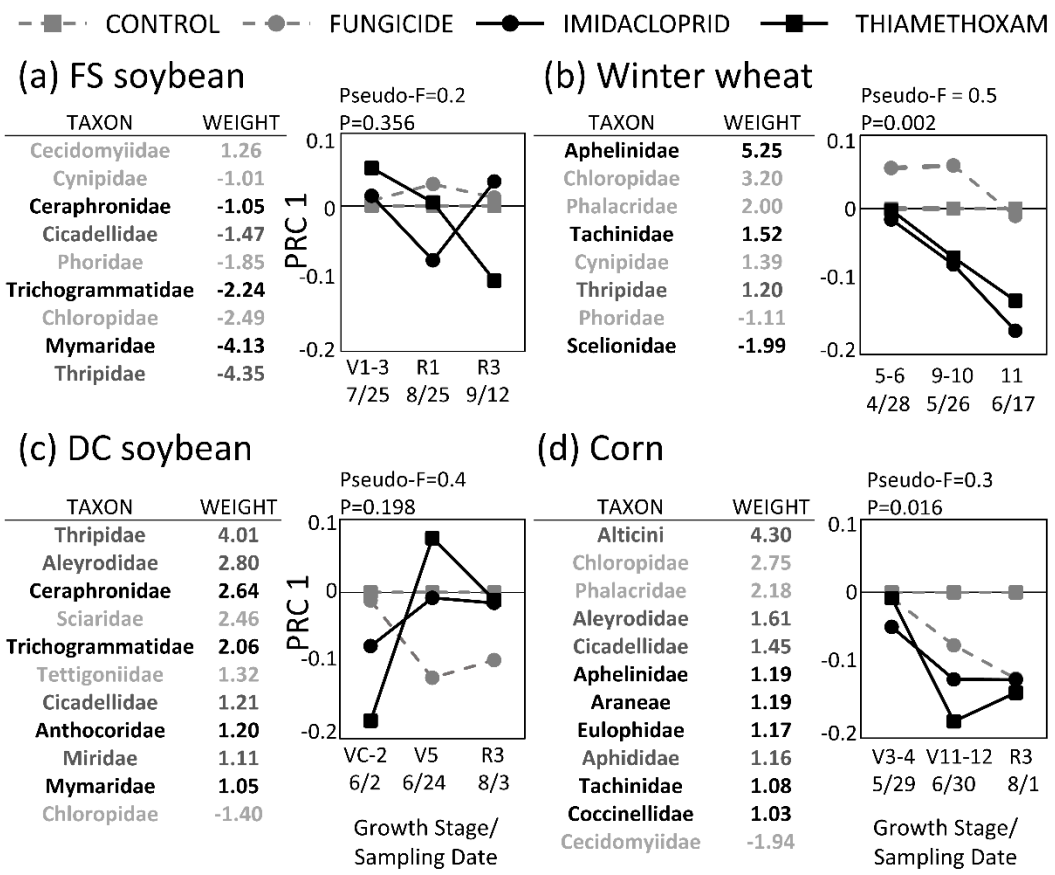

Fig. 2.1. Principal Response Curve analysis of sticky card data for all crops. Date*treatment served as the explanatory variable, with date and site*column used as covariates. Subsamples were averaged by taxa for each replicate, and only taxa with overall means greater than one were included. Ants (Formicidae) were also excluded due to their highly clumped distribution. A Monte-Carlo permutation procedure with N=499 was used to calculate the Pseudo-F statistic. Taxon weights indicate which groups most contributed to the observed community response. Higher positive weights indicate that taxon abundances in the treated plots followed the trend depicted by the response curve, whereas higher negative values indicate the opposite. Taxon weights between -1 and 1 were excluded due to weak response or lack of correlation with the trends shown. Beneficial groups are shown in black, herbivore pests in dark grey, and other groups in light grey. FS = full-season, DC = double-cropped.

2015-2016 winter wheat: We analyzed a total of 9,438 individuals from pitfall traps, 18,529 from litter extraction and 5,273 from sticky cards (Appendix D, Tables D1-D3). PRC analysis revealed no community responses to the pesticide treatments in pitfall trap (Pseudo F=0.2, P=0.712) (Appendix D, Fig. D1) or litter communities (Pseudo-F=0.2, P=0.976) (Appendix D, Fig. D2). However, the

sticky card community increasingly declined in response to insecticide treatments over the sampling dates (Pseudo-F=0.5, P=0.002) (Fig. 2.1).

2017 corn: In total, we analyzed 9,448 individuals from pitfall traps, 5,536 from litter extraction, and 5,247 from sticky cards (Appendix D, Tables D1-D3). Pesticide treatments did not impact arthropod communities over time in pitfall trap (Pseudo F=0.2, P=0.27) (Appendix D, Fig. D1) or litter extraction (Pseudo-F=0.5, P=0.198) PRC analyses (Appendix D, Fig. D2). All pesticide treatments caused increasing declines over time for sticky card taxa (Pseudo-F=0.3, P=0.016) (Fig. 2.1).

Effects of seed treatments on individual taxa within crops

2015 full-season soybean: Soil taxa – None of the measured taxa from pitfall traps (PT) or litter (LE) were significantly impacted by the treatments (Mesostigmata LE model $F_{10,21}$=1.63, P=0.167; Mesostigmata PT $F_{3,21}$=0.39, P=0.760; Staphylinidae LE $F_{3,21}$=0.54, P=0.662; Acari LE model $F_{10,21}$=1.68, P=0.152; Acari PT $F_{3,21}$=1.34, P=0.288; Collembola LE model $F_{10,21}$=1.00, P=0.473; Collembola PT model $F_{10,21}$=1.82, P=0.119) (Appendix D, Fig. D4).

Foliar taxa – The abundance of predatory thrips was reduced in both insecticide treatments compared to the control (Phlaeothripidae VC $F_{3,21}$=15.16, P<0.001) (Fig. 2.2). Planthoppers were suppressed by the thiamethoxam treatment (Cicadellidae VC $F_{3,21}$=6.79, P=0.002) while plant thrips were suppressed by both insecticide treatments (Thripidae VC $F_{3,21}$=51, P=0.006). Lady beetles (Coccinellidae SN model $F_{10,20}$=0.59, P=0.804), Aphelinidae (SC $F_{3,21}$=0.75, P=0.533), Chloropidae (SC model $F_{10,21}$=0.47, P=0.890), Phalacridae (SC model

$F_{10,21}$=0.31, P=0.969) and Sciaridae (SC model $F_{10,21}$=0.67, P=0.738) were not impacted.

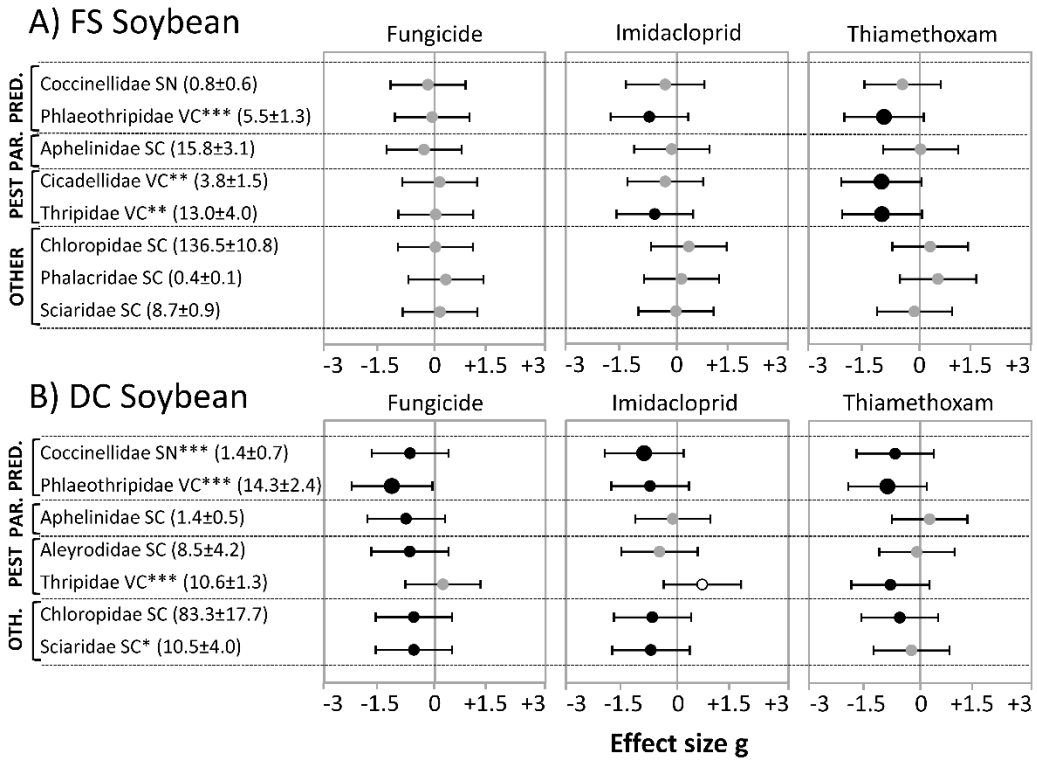

Fig. 2.2. Comparisons of arthropod abundances in the fungicide, imidacloprid and thiamethoxam treatments to the control through Analysis of Variance followed by Hedge's g effect test (±95% confidence intervals) for sweep net (SN), sticky card (SC), and visual count (VC) taxa in full-season (FS) and double-cropped (DC) soybean. The values in parentheses indicate mean taxon abundance ± standard error for the control. * indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001 for the ANOVA treatment effect. Small grey circles represent a negligible or small effect size (between -0.5 and 0.5), small and large black circles represent medium (between -0.5 and -0.8) and large (less than -0.8) negative effect sizes, respectively, while small and large white circles represent medium (between 0.5 and 0.8) and large (greater than 0.8) positive effect sizes, respectively.

2016 double-cropped soybean: Soil taxa - Pesticide treatments did not impact any pitfall trap or litter taxa in double-cropped soybean (Mesostigmata LE model $F_{10,21}$=1.87, P=0.108; Mesostigmata PT $F_{3,21}$=0.69, P=0.567; Staphylinidae LE $F_{3,21}$=2.78, P=0.066; Acari LE $F_{3,21}$=0.51, P=0.677; Acari PT model $F_{10,21}$=1.99, P=0.088; Collembola LE $F_{3,21}$=2.83, P=0.063; Collembola PT model $F_{10,21}$=1.47,

P=0.219) (Appendix D, Fig. D4).

Foliar taxa – Lady beetles (Coccinellidae SN $F_{3,21}$=5.06, P<0.001) and predatory thrips (Phlaeothripidae VC $F_{3,21}$=9.66, P<0.001) were reduced in in all three pesticide treatments. Plant thrips (Thripidae VC $F_{3,21}$=11.54, P<0.001) were suppressed in the thiamethoxam treatment but increased in the imidacloprid treatment, while dark winged fungus gnats were reduced somewhat in the fungicide and imidacloprid treatments (Sciaridae SC $F_{3,21}$=3.70, P=0.028) (Fig. 2.2). Sticky card collected Aphelinidae (SC model $F_{10,21}$=1.15, P=0.372), Aleyrodidae (model $F_{10,21}$=0.99, P=0.479) and Chloropidae (model $F_{10,21}$=0.87, P=0.573) were not impacted.

2015-2016 winter wheat: Soil taxa – The abundance of rove beetles from litter extraction was strongly reduced in both insecticide treatments (Staphylinidae LE $F_{3,21}$=6.36, P=0.003) (Fig. 2.3). No other taxa were impacted (Mesostigmata LE $F_{3,21}$=1.00, P=0.413; Mesostigmata PT model $F_{10,21}$=2.16, P=0.066; Acari LE model $F_{10,21}$=0.77, P=0.658; Acari PT model $F_{10,21}$=1.81, P=0.120; Collembola LE model $F_{10,21}$=1.56, P=0.186; Collembola PT $F_{3,21}$=0.38, P=0.771) (Fig. 2.3).

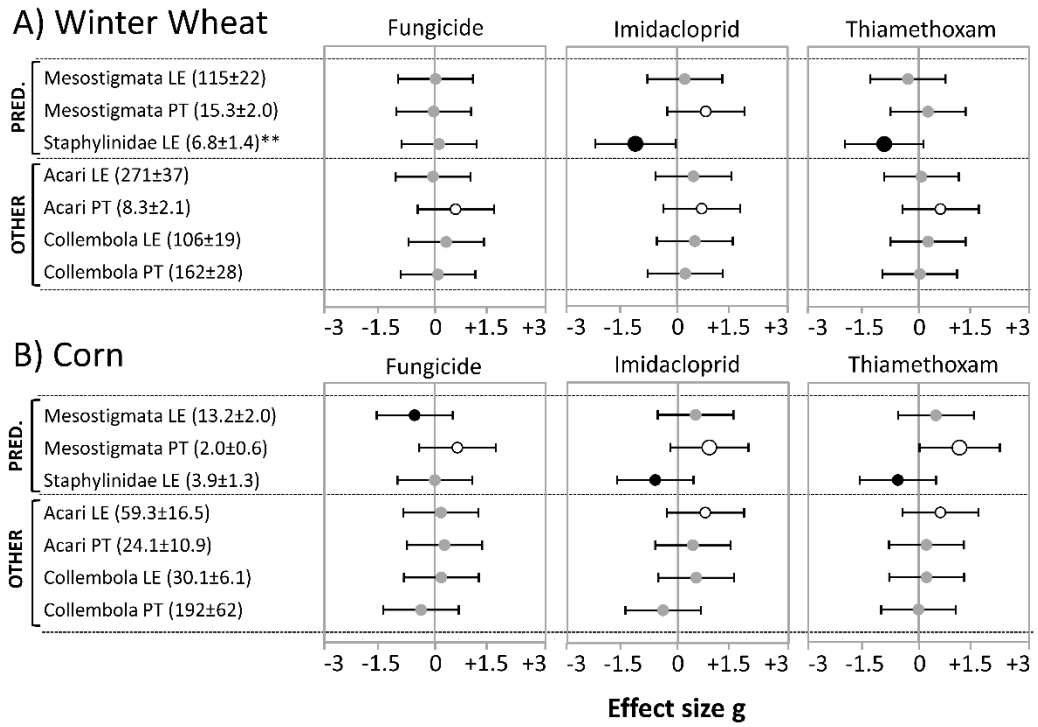

Fig. 2.3. Comparisons of arthropod abundances in the fungicide, imidacloprid and thiamethoxam treatments to the control through Analysis of Variance followed by Hedge's g effect test (±95% confidence intervals) for litter (LE) and pitfall trap (PT) taxa in winter wheat and corn. The values in parentheses indicate mean taxon abundance ± standard error for the control. * indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001 for the ANOVA treatment effect. Small grey circles represent a negligible or small effect size (between -0.5 and 0.5), small and large black circles represent medium (between -0.5 and -0.8) and large (less than -0.8) negative effect sizes, respectively, while small and large white circles represent medium (between 0.5 and 0.8) and large (greater than 0.8) positive effect sizes, respectively. Acari refers specifically to the mite order Oribatida and the family Tarsonemidae.

Foliar taxa – Sticky card collected aphelinid wasps ($F_{3,21}$=18.54, P<0.001) were strongly suppressed in both insecticide treatments (Fig. 2.4). In the winter, visually counted aphids (Aphididae) were strongly suppressed in both insecticide treatments ($F_{3,21}$=7.93, P=0.001), while in spring, they were suppressed in the imidacloprid treatment, but increased in the fungicide only treatment ($F_{3,21}$=4.55, P=0.013). Sticky card collected grass flies (Chloropidae) increased in the fungicide only treatment but were reduced in the imidacloprid treatment

($F_{3,21}$=6.41, P=0.003), while shining flower beetles (Phalacridae) increased in the fungicide only treatment and were reduced in the thiamethoxam treatment ($F_{3,21}$=8.59, P=0.001). Aleyrodidae (model $F_{10,21}$=1.04, P=0.446) and Sciaridae ($F_{3,21}$=1.50, P=0.208) collected using sticky cards were not impacted.

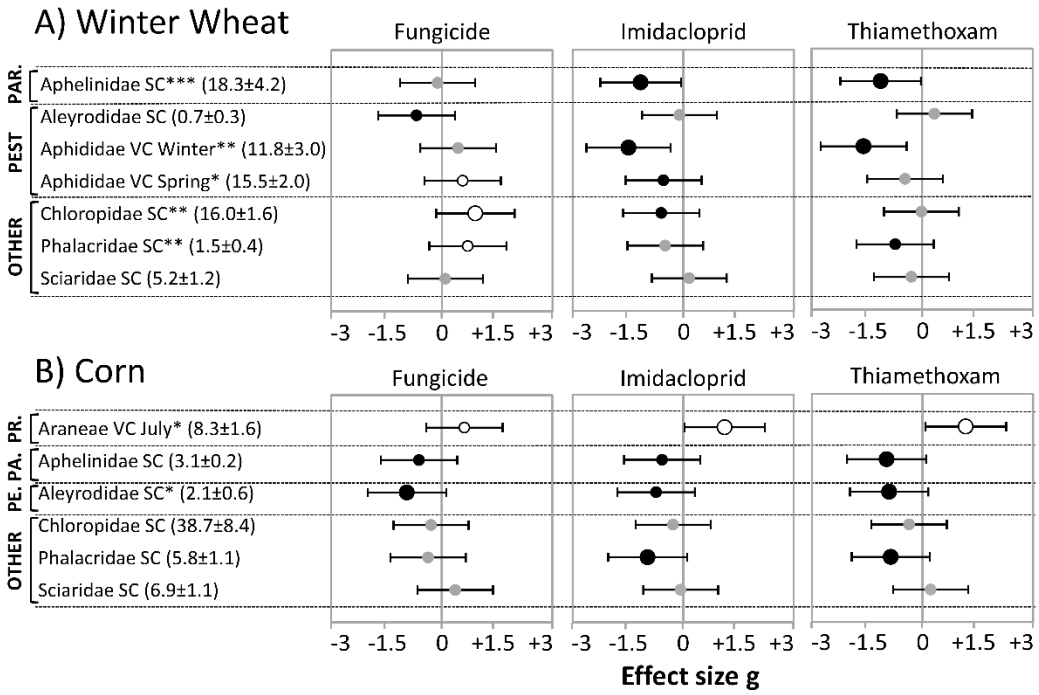

Fig. 2.4. Comparisons of arthropod abundances in the fungicide, imidacloprid and thiamethoxam treatments to the control for foliar taxa. Data was analyzed through Analysis of Variance followed by Hedge's g effect test (±95% confidence intervals) for sticky card (SC) and visual count (VC) taxa in winter wheat and corn. The values in parentheses indicate mean taxon abundance ± standard error for the control. * indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001 for the ANOVA for each taxon. Small grey circles represent a negligible or small effect size (between -0.5 and 0.5), small and large black circles represent medium (between -0.5 and -0.8) and large (less than -0.8) negative effect sizes, respectively, while small and large white circles represent medium (between 0.5 and 0.8) and large (greater than 0.8) positive effect sizes, respectively.

2017 corn: Soil taxa – Pesticide treatments did not impact any pitfall trap or litter taxa in corn (Mesostigmata LE model $F_{10,21}$=1.04, P=0.444; Mesostigmata PT $F_{10,21}$=1.16, P=0.347; Staphylinidae LE model $F_{10,21}$=0.56, P=0.824; Acari LE model $F_{10,21}$=1.08, P=0.420; Acari PT $F_{3,21}$=0.30, P=0.824; Collembola LE model $F_{10,21}$=1.28, P=0.304; Collembola PT Model $F_{10,21}$=0.46, P=0.711) (Fig. 2.3).

Foliar taxa – In July visual counts, spiders (Araneae) increased in abundance in all three pesticide treatments ($F_{3,21}$=4.77, P=0.011), while sticky card collected whiteflies (Aleyrodidae) decreased in all three treatments ($F_{3,21}$=3.73, P=0.027) (Fig. 2.4). None of the other sticky card taxa were impacted (Aphelinidae model $F_{10,21}$=1.32, P=0.281; Aphididae $F_{3,21}$=1.43, P=0.262; Chloropidae $F_{3,21}$=1.37, P=0.278; Phalacridae $F_{3,21}$=3.02, P=0.053; Sciaridae $F_{3,21}$=0.68, P=0.576).

Crop sampling

To evaluate treatment impacts on plant growth rates and health, plant height, stand count and yield were measured in all the crops (Table 2.2), with NDVI and the number of tillers also measured in wheat. Stand count was improved in imidacloprid treated plots compared to the control in full-season soybean ($F_{3,21}$=12.46, P<0.001) and in both insecticide treatments in corn ($F_{3,21}$=5.51, P=0.006), but not in wheat ($F_{3,21}$=0.39, P=0.760) or double-cropped soybean ($F_{3,21}$=1.21, P=0.331). The plant height was also greater in all three pesticide treatments compared to the control in corn ($F_{3,21}$=9.04, P<0.001), but not in full-season soybean (model $F_{10,21}$=0.80, P=0.628), double-cropped soybean (model $F_{10,21}$=1.31, P=0.290) or wheat ($F_{3,21}$=1.42, P=0.265). NDVI ($F_{3,114}$=0.06, P=0.983) and tiller counts (model $F_{11,52}$=1.24, P=0.286) were not impacted by the treatments in wheat. Yield benefits were not observed in full-season soybean ($F_{3,21}$=0.400, P=0.755), winter wheat (model $F_{10,21}$=1.48, P=0.215), double-cropped soybean (model $F_{10,21}$=1.76, P=0.132) or corn (model $F_{10,21}$=1.40, P=0.248).

Table 2.2. The effect of seed treatments on plant health parameters and yield for each crop. Analysis of variance was used with treatment, location and column (location) as fixed effects. For effect differences of P<0.05, contrasts were used to compare the fungicide (FUN), imidacloprid (IMI) and thiamethoxam (THI) treatments to the control (CON). * indicates P<0.05, ** indicates P<0.01, *** indicates P<0.001 and N.S. indicates not significant. Results where contrasts were performed are bolded. NA indicates that the overall ANOVA was not significant.

Metric	Treatment Mean ± S.E.				Treatment F-value, P-value
	CON	FUN	IMI	THI	
Stand Count (plants $2m^{-1}$ in corn and soybean, plants m^{-1} in wheat) (df=3,21)					
Full-season Soybean	**15.5±1.4**	**16.8±1.4** N.S.	**20.2±1.6*** **	**15.3±1.6** N.S.	**12.46, <0.001**
Winter Wheat	45.9±2.6	45.6±5.0	46.9±2.8	43.8±2.3	0.39, 0.760
Double-cropped Soybean	17.8±1.2	18.2±1.0	16.9±0.7	18.6±0.7	1.21, 0.331
Corn	**11.6±0.5**	**11.8±0.4** N.S.	**12.2±0.4****	**12.0±0.5****	**5.51, 0.006**
Plant Height (cm) (df=3,21)					
Full-season Soybean	26.4±1.0	26.7±0.9	27.8±1.2	27.6±0.8	NA
Winter Wheat	13.5±0.4	13.0±0.2	13.5±0.3	13.1±0.3	1.42, 0.265
Double-cropped Soybean	52.0±3.0	56.4±1.6	55.4±3.0	54.6±3.6	NA
Corn	**14.4±0.5**	**15.8±0.6****	**15.7±0.6*****	**15.3 ± 0.7****	**9.04, 0.001**
Yield ($kg\ ha^{-1}$) (df=3,21)					
Full-season Soybean	2973±465	3184±517	3159±486	3020±461	0.400, 0.755
Winter Wheat	2845±277	3373±201	3584±213	3383±389	NA
Double-cropped Soybean	3068±184	3165±179	3203±169	3148±178	NA
Corn	8850±485	9595±204	9583±711	9502±520	NA

Discussion

We conducted a three-year field study evaluating pesticide seed treatment impacts in a full-season soybean, winter wheat, double-cropped soybean and corn rotation. Our specific goals were to quantify neonicotinoid residues in the soil and in winter annual flowers, which underlies the magnitude of non-target impacts on the arthropod community. In addition to characterizing non-target impacts, we also quantified benefits to plant growth and yield to determine whether treatments were economically justified. Unquantifiably low amounts of insecticide were present in one winter annual species in one site year, which did not correspond

with our treatments. Low levels of insecticide residues were present in the soil, with the highest levels observed in the final year, suggesting some accumulation. Pesticide seed treatments variably impacted the arthropod community throughout the study. PRC and RDA analyses demonstrated occasional deviations from the control community of a relatively small magnitude, and pesticide seed treatments also impacted individual taxa. However, there was little consistency between crops and sampling methods. Overall, insecticide treatments had a stronger impact on foliar taxa than on soil taxa, and the fungicides also occasionally impacted arthropod communities. Pest pressure was very low throughout the study, and while the treatments occasionally improved early season plant growth, we did not observe yield differences in any crop.

Environmental persistence and routes of exposure to neonicotinoid residues

Uptake by plants

Neonicotinoid residues can be taken up from the soil by non-target plants, such as wildflowers and inter-seeded cover crops (Botías et al., 2015; Bredeson and Lundgren, 2019; Krupke et al., 2012; Pecenka and Lundgren, 2015); these are important resources for pollinators, and could be a source of neonicotinoid exposure (Bretagnolle and Gaba, 2015; Mandelik et al., 2016). Since these non-target plants were sampled during peak planting and crop production seasons, aerial deposition cannot be separated from uptake. To mitigate this issue, we sampled in late winter. Unquantifiably low levels of imidacloprid were present in *S. media* flower samples at Beltsville in 2017. Neonicotinoid levels were below the detection threshold for our analysis (5ppb) and did not correspond with our

treatments. Previous studies quantifying residues within non-target plants often detected levels of less than 5ppb (Bredeson and Lundgren, 2019; Pecenka and Lundgren, 2015); therefore, despite low soil residues, winter annual flowers may uptake small amounts of active ingredient.

Persistence in soil

In soil, the half-life of neonicotinoids can vary greatly, ranging from 28-1250 days for imidacloprid, and 7-353 days for thiamethoxam (Goulson, 2013), with temperature, sunlight, and soil texture, organic matter and moisture content impacting persistence (Bonmatin et al., 2015). Persistence in soil also varies by the amount of active ingredient used, which can differ greatly between crops due to different treatment and seeding rates. We did not detect high levels of neonicotinoid residues in the soil, but the highest levels of both insecticides were observed after 2017 corn planting, suggesting the possibility of some accumulation across crops, as hypothesized. This was further supported by higher imidacloprid levels in imidacloprid treated plots than surrounding plots prior to 2017 corn planting at Beltsville. Overall, imidacloprid was detected more often than thiamethoxam, with detections before the start of the study at Beltsville, even though imidacloprid was not used in that field the previous year. This difference in soil persistence is likely due to imidacloprid's longer half-life.

High moisture content, temperature and sunlight are all positively correlated with neonicotinoid breakdown, and thiamethoxam and imidacloprid also have high leaching potential (Smalling et al., 2018). Given the high summer temperatures and precipitation in Maryland, the low levels of neonicotinoid

residues in our plots could be caused by rapid microbial and photolytic breakdown of residues, or by leaching and runoff. Soil testing prior to the start of the study indicated that our plots had low organic matter content, which is correlated with reduced sorption of neonicotinoids, another potential cause for low residue levels (Smalling et al., 2018). We found relatively low residue levels compared to some other studies (Bonmatin et al., 2015), and the levels we found were below the known acute toxicity thresholds for various terrestrial arthropods (Douglas and Tooker, 2016; Pisa et al., 2015). However, chronic exposure to neonicotinoid residues in the soil at levels similar to those that we detected, including levels below our quantification thresholds, can impact development and survival in solitary ground-nesting bees (Anderson and Harmon-Threatt, 2019), and can lead to bioaccumulation and DNA damage in earthworms (Chevillot et al., 2017). Therefore, even these low residue levels could lead to non-target impacts over time.

Non-target impacts of pesticide seed treatments on arthropods

Our hypothesis that the soil community would experience the strongest impacts from pesticide seed treatments was not supported. We observed minimal impacts on soil community activity density as measured through pitfall traps and litter extraction; neither PRC nor individual taxon analyses exhibited responses to pesticides, except for a trend of increased mites and collembola in double-cropped soybean litter and increased mites in corn pitfall traps that was consistent across all pesticide treatments and a large reduction of rove beetles in the insecticide treated wheat. The lack of impact on soil taxa is consistent with the low levels of

insecticide residues we found in the soil, which were generally below the threshold for acute toxicity towards arthropods (Douglas and Tooker, 2016; Pisa et al., 2015). However, as mentioned earlier, chronic exposure to the low levels of insecticides that we detected could sub-lethally impact soil-dwelling organisms over time. Due to a much higher seeding rate, NST effects on rove beetles in wheat may result from the higher rate of active ingredient which was almost double the amount applied in soybean or corn.

Other studies have described variable NSTs impacts on soil taxa in corn and soybeans (Atwood et al., 2018; Disque et al., 2018). Clothianidin treated corn reduced the activity density of scelionid wasps, ants, carabid beetles and staphylinid beetles early in the season with effects diminishing over the course of the season in PRC analysis of pitfall data (Disque et al. 2018). In contrast, orbatid soil mites as well as isotomid and entomobryid collembola activity density increased relative to the control (Disque et al. 2018). In corn and soybean rotations, responses of arthropod communities extracted from soil cores and litter bags varied between crops, years, and functional guilds, with occasional positive responses to pesticide seed treatments (thiamethoxam and fungicide seed treatments) in detritivore and predator guilds, reduced predator richness and diversity in one year of the study, and no effect on herbivores in any year two weeks after planting (Atwood et al. 2018). Additionally, it has been suggested that individual studies of pesticide non-target impacts lack the power to detect effects due to relatively small sample size and high variability in arthropod community data sets (Douglas and Tooker 2016). A meta-analysis across 20 studies revealed

small negative effects [effect size d = -0.30 ± 0.10 (95% confidence interval)] on natural enemy abundance associated with NSTs, with a trend toward soil taxa being more impacted than foliar taxa (Douglas and Tooker 2016).

However, we detected stronger small, medium and large effect size positive and negative responses to pesticide seed treatments for foliar taxa as measured by sticky cards, sweep netting, and visual samples. Redundancy analysis of sweep net data demonstrated NST impacts on arthropod abundances in 2016 double-cropped soybean but not 2015 full-season soybean. Community impacts were driven by reductions in predatory taxa such as lady beetles, minute pirate bugs and predatory thrips, indicating that the insecticide treatments had strong negative impacts on natural enemies. Given the short period of neonicotinoid activity in crop plants (three to four weeks post planting in corn and soybean) (Alford and Krupke, 2017; Myers and Hill, 2014), we expected foliar communities to recover rapidly, as observed by Disque et al. (2018) in corn. In contrast, PRC analysis for sticky cards showed increasing deviations from the control community over time in insecticide treated winter wheat and for all pesticide treatments in corn, with no recovery over the sampling period. In corn, the group that contributed most to this deviation was flea beetles, suggesting that the community disturbance was driven by a reduction in pest abundance.

The results in wheat are more surprising, as wheat was sampled in April, May and June, 24 to 32 weeks post planting. The period of activity of NSTs in wheat is not as well defined as in corn and soybean; neonicotinoids could remain active for much longer in winter wheat, because of low temperatures and plant

dormancy during the winter and early spring. Unfortunately, we were unable to directly sample foliage for insecticide residues during our study. However, Zhang et al. (2016) found low levels (10 to 22ppb) of imidacloprid and clothianidin in seed treated winter wheat up to 28 weeks after planting and observed successful control of cereal aphids throughout the growing period. The presence of insecticide in plant tissue over a longer period could be a source of exposure for non-target beneficials such as lady beetles and minute pirate bugs that supplement their diet with plant material, or parasitoids that rely on nectar as a food source (Gontijo et al., 2015; Moscardini et al., 2014; Moser and Obrycki, 2009). In our study, the strongest drivers of the effects observed in the PRC analysis for wheat were aphelinid wasps, which were greatly reduced in both insecticide treatments. This family contains many important aphid parasitoids, which play a key role in controlling cereal aphids in wheat (Pike et al., 1997; Schmidt et al., 2003). Although the insecticide treatments reduced aphid abundance in the winter, this strong effect was no longer apparent in the spring, so prey scarcity does not explain impacts in the spring. It is possible that during the later sampling dates, insecticide residues were too low to control aphids but high enough to impact their parasitoids.

In foliar sweep net and visual samples from soybeans, we also observed reduced abundance or activity density of lady beetles (Coccinellidae), which are known to be impacted by neonicotinoids (Amjad et al., 2018; Disque et al., 2018; Zhang et al., 2016), as well as predatory thrips; some of these impacts occurred across all three pesticide treatments and thus may have been driven by the

fungicide treatments. In contrast, spider abundance was higher in the corn visual samples from the insecticide treated plots, and to a lesser extent the fungicide treated plots. Arachnids are less susceptible to neonicotinoids than insects (Douglas and Tooker 2016), and Easton and Goulson (2013) found that spiders were attracted to low doses of imidacloprid, which could explain the increased abundance of spiders. Another possibility is that sublethal impacts of the pesticides on insects made insect prey easier to capture (Main et al., 2018), thereby improving resource availability and increasing spider abundance.

Overall, we did not see any evidence of cumulative impacts over time in soil or foliar taxa. The taxa that were impacted varied from crop to crop, and no taxa were consistently impacted throughout the study. When possible, residue analysis of foliar tissue should be conducted to better understand the variation in pesticide seed treatment impacts between crops.

Impacts of fungicides on arthropods

In order to isolate effects of fungicides from those of insecticides, we examined fungicide seed treatments alone, which also impacted the arthropod community. In double-cropped soybean litter samples and corn sticky card samples, the impact of the fungicide only treatment on the community was similar to that of the imidacloprid treatment in PRC analyses. In addition, the fungicide treatment exhibited similar impacts as one or both insecticide treatments in double-cropped soybean individual taxa analyses, reducing abundance of predatory thrips, lady beetles and dark-winged fungus gnats as well as increasing the abundance of spiders in corn. In other studies, both fungicide and insecticide

seed treatments decreased earthworm surface activity and increased collembola surface activity in wheat (Van Hoesel et al., 2017; Zaller et al., 2016). In our study, there were also cases where only the fungicide treatment impacted certain taxa, such as increased abundance of aphids in wheat in the spring, along with increased activity density of grass flies and shining flower beetles in wheat individual taxa analyses.

To the best of our knowledge, few studies have evaluated the persistence of seed applied fungicides in agroecosystems, or their impact on the arthropod community, even though they can be moderately toxic to arthropods (Minnesota Department of Agriculture, 2012) and vary in their mobility as well as likelihood for leaching (Smalling et al., 2018). Given that the fungicide treatments consist of several active ingredients, those ingredients could interact synergistically with each other or with the insecticides to impact the arthropod community. The effects of fungicides on arthropod health have been investigated in pollinators; clothianidin can synergistically interact with the fungicide propiconazole increasing mortality in multiple bee species (Sgolastra et al., 2017). In addition, fungicides could alter arthropod abundance by interfering with entomopathogenic fungi, thereby altering disease pressure (Lagnaoui and Radcliffe, 2009). In our study, the soil community was dominated by fungivore taxa (mites and collembola). Therefore, fungicides could also affect arthropods through changes in fungal diversity and abundance, impacting resources available for fungivores. Regardless of the mechanism, our results clearly demonstrate that seed applied fungicides can disrupt arthropod communities in agroecosystems.

Economic impacts

Throughout the study, we did not experience pressure from any of the foliar pests for which NSTs are labelled, as exhibited in our visual scouting data. This is typical for Maryland; although NSTs suppressed thrips (Thripidae) and leafhoppers (Cicadellidae) in soybean, and aphids (Aphididae) in early season wheat, these pests were not present at economically damaging levels. Indeed, many of the pests for which NSTs are labelled are considered sporadic pests that most growers do not typically scout for or actively manage; for some of these pests, effective alternative management strategies such as early planting and crop rotation exist (Hesler et al., 2018; Papiernik et al., 2018; Sappington et al., 2018). However, soil pests such as wireworms (Elateridae) and white grubs (Scarabeidae), can require NST applications because they have multi-year life cycles and their damage cannot be mitigated with rescue treatments. In our case, scouting for grubs and wireworms before the start of the study in 2015 and shortly after planting corn in 2017 indicated very low soil pest pressure (<1 individual per plot). As we predicted, the insecticide seed treatments did not improve yield through pest suppression.

In some cases, NSTs improved early season stand density and plant height, supporting the claim that NSTs can stimulate growth and improve plant health even in the absence of pest pressure (Jeschke et al., 2011). All three pesticide seed treatments also increased plant height in corn. However, these early season agronomic benefits did not translate to yield increases. Our results are consistent with several previous findings that NSTs may not provide economic

benefits in the absence of early season pest pressure (Cox et al., 2007; Mourtzinis et al., 2019; Myers and Hill, 2014; Wilde et al., 2007). This suggests that the use of NSTs in Maryland grain production may not be warranted outside of specific instances of high pest pressure.

Conclusions

We found that NSTs can impact arthropod communities in Maryland grain systems, despite low levels of neonicotinoid residues in the agroecosystem. The communities occasionally were unable to recover by the end of the sampling period, which in wheat was 32 weeks after planting. We observed suppression of predators and parasitoids that play an important role in controlling insect pests, which could have harmful management consequences. Although the levels of insecticide residues found in the soil were low, chronic exposure to those levels of insecticides has the potential to negatively impact important organisms such as pollinators and earthworms. We also cannot discount the possibility of insecticide runoff into nearby waterways, where the toxicity towards aquatic arthropods can alter aquatic food webs and cause cascading trophic effects (Miles, Hua, Sepulveda, Krupke, & Hoverman, 2017; Morrissey et al., 2015; Yamamuro et al., 2019). Given the lack of economically damaging pests throughout our study, we did not observe any yield benefits that could justify the risks associated with NST use. Without a corresponding increase in pest pressure (Douglas and Tooker, 2015), NST treated corn and soybean acreage has increased, and many of these acres were previously untreated with insecticides. The Acute Insecticide Toxicity Loading on US agricultural lands has increased 48- and 4-fold for oral and contact

toxicity from 1992 and 2014, primarily due to the use of neonicotinoids in corn and soybean (DiBartolomeis et al., 2019). Between 2011 and 2014, the overall quantity of neonicotinoids applied to corn also doubled, indicating an increase in the rate of products used (Tooker et al., 2017). Despite minimal or no benefits in many cases, NST use has continued to grow. Unfortunately, there is little availability of corn without NSTs in the US, leaving farmers with limited choices (Alford and Krupke, 2017). Given the levels of NST contamination in the environment and the impacts on non-target arthropod communities, tactics must be developed to minimize overuse.

Chapter 3: Evaluating temporal variation in the impact of neonicotinoid seed treatments on bird cherry-oat aphid *Rhopalosiphum padi* in winter wheat

Abstract

Neonicotinoid seed treatments (NSTs) are recommended as a tool for controlling cereal aphids and barley yellow dwarf (BYD) virus in winter wheat. However, efficacy of NSTs against cereal aphids varies, lasting from a few weeks to several months, and NSTs do not always improve yield or provide economic benefits in wheat. In my previous field research, NSTs reduced aphid abundance in the fall but did not improve yield; they also reduced the activity density of aphid parasitoids throughout the spring. To better understand the impacts of NSTs on cereal aphids and their parasitoids in Maryland winter wheat, I conducted lab trials evaluating the efficacy of imidacloprid and thiamethoxam seed treatments against the bird cherry-oat aphid (*Rhopalosiphum padi*), how efficacy changes over time, and whether it is impacted by aphid density. Both NSTs reduced aphid survival in the weeks immediately after planting, but only thiamethoxam provided longer term control, up to 16 weeks post planting. The impact of aphid density on control was not clear, as density only impacted aphid control at a single time point. Although treatments did reduce aphid survival, they were not sufficient to maintain the population below economic thresholds. My results also suggest that neonicotinoid activity persisting in the spring and/or impacts carried over from the fall could explain parasitoid patterns observed in the field. Given the level of cereal aphid control and the potential for non-target impacts on natural enemies,

NSTs may not be an effective tool for controlling cereal aphids and BYD in Maryland winter wheat.

Introduction

Neonicotinoid insecticide seed treatments (NSTs) are a popular pest control tool in U.S. grain production. Similar to their rise in popularity in other grain crops, by 2012, neonicotinoid seed treatments (NSTs) comprised 25-29% of the pesticides applied to wheat in the U.S., and wheat acreage planted with neonicotinoid treated wheat may continue to increase (Douglas and Tooker, 2015; Hesler et al., 2018). In the U.S., NSTs target three important early-season pests of wheat, Hessian fly [*Mayetiola destructor* Say (Diptera: Cecidomyiidae)], wireworms (Coleoptera: Elateridae), and cereal aphids (Hemiptera: Aphididae) (Hesler et al., 2018; Papiernik et al., 2018). Cereal aphids are a multispecies complex of aphids that infest wheat and other small grains that can cause direct yield losses due to feeding damage; in addition, several cereal aphid species also vector diseases (Hesler et al., 2018; Kieckhefer and Gellner, 1992). This includes the most widespread and economically important disease of cereals worldwide, barley yellow dwarf (BYD), which is caused by a complex of 10 virus species within the family Luteoviridae (Irwin and Thresh, 1990; Walls et al., 2019). The primary aphid vectors of BYDV are bird cherry-oat aphid (*Rhopalosiphum padi* L.), greenbug aphid (*Schizaphis graminum* Rondani), English grain aphid (*Sitobion avenae* F.), and corn leaf aphid (*Rhopalosiphum maidis* Fitch) (Hesler et al., 2018). Amongst these, *R. padi* is considered the most important vector because it is widespread throughout the U.S. and transmits the highest number of

BYD variants (Chapin et al., 2001; Hesler et al., 2018; Irwin and Thresh, 1990; Zwiener et al., 2005). In areas prone to infestations, BYD can cause an average of 11-33% and in some cases up to 80% yield loss in wheat fields, making BYD management via cereal aphid control a major driver of insecticide use in wheat (Hesler et al., 2018; Walls et al., 2019).

Although NSTs are recommended for cereal aphid and BYD control, results vary. While NSTs consistently reduce aphid abundance, this does not always translate to lower BYD rates, higher yields, or economic benefits (Gourmet et al., 1996; Hunger et al., 2000; Kennedy and Connery, 2012; Pike et al., 1997; Royer et al., 2005; Zwiener et al., 2005). One potential reason is that NSTs may only be active for a relatively short period, and most wheat grown in the USA is winter wheat, which is susceptible to cereal aphids and BYD in both the fall and the spring ("2019 Agricultural Statistics Annual," 2019; Walls et al., 2019). While NSTs only provide protection from foliar pests for four to six weeks post planting in corn and soybean (Alford and Krupke, 2017; Mccornack and Ragsdale, 2006), their persistence in wheat is less well understood. Winter wheat goes through a period of dormancy and vernalization before entering its reproductive phase in the spring (Porter et al., 1987). Low winter temperatures and wheat dormancy likely impact the persistence of neonicotinoids in the plant tissue. When imidacloprid and clothianidin treated winter wheat was planted in China, insecticides were still present in plant tissue up to 200 days after planting and continued to control aphids (Zhang et al., 2016). However, other studies have found that NSTs provide shorter windows of aphid control (Dubey et al., 2020;

Kennedy and Connery, 2012; Kirkland et al., 2018; Royer et al., 2005). Factors such as winter temperatures, planting date, and treatment rate may all contribute to this variation, and so the efficacy of NSTs in winter wheat must be evaluated separately in different wheat-growing regions.

Winter wheat is an important crop in the mid-Atlantic; in 2019, 165,000 acres of wheat were harvested in Maryland ("2019 State Agriculture Overview Maryland," n.d.). While NSTs have not yet been widely adopted in Maryland wheat, they are recommended as a tool for reducing aphid populations and controlling the spread of BYD. In my field study investigating the use of NSTs in Maryland grain crop production, NSTs reduced cereal aphid abundance in winter wheat in the fall and winter, but not during the spring (Dubey et al., 2020). Aphid abundance remained below the economic threshold throughout the study, and NSTs did not impact yield. However, NSTs strongly reduced the activity density of aphelinid wasps, several of which are important aphid parasitoids, throughout the spring (Dubey et al., 2020; Pike et al., 1997). Parasitoid wasps play a key role controlling cereal aphids, and a reduction in aphelinid wasp populations has the potential to disrupt biocontrol and lead to secondary pest outbreaks (Johnson and Tabashnik, 1999; Schmidt et al., 2003). Secondary outbreaks may require additional application of insecticides, further disrupting the ecosystem and placing an additional economic burden on farmers (Cloyd and Bethke, 2011). Therefore, both the efficacy and the potential non-target impacts of NSTs must be considered when deciding whether to use them in winter wheat.

To better understand the impacts of NSTs on cereal aphids and their parasitoids in Maryland winter wheat production, I designed a series of laboratory experiments to investigate the potential mechanisms driving the results observed in the field study. Because aphid abundance was not impacted by treatment in the field study, prey availability did not seem to underlie aphelinid responses. However, the clumped distribution of aphids combined with the low aphid densities at our sites may have masked the effect of prey availability (Dubey et al., 2020; Fievet et al., 2007; Winder et al., 2001, 1999). To determine whether NSTs continue to control cereal aphids in the spring, I conducted an experiment evaluating temporal variation in the impact of NSTs on *R. padi* populations throughout the growing season. To obtain field-relevant results, plants were grown using temperature and light settings designed to approximate Maryland growing conditions. Another potential explanation for the observed impact on Aphelinid wasps is that insecticides were still present in the plants at levels too low to control aphids but sufficiently high for toxicity towards the smaller-bodied parasitoids. To determine whether NSTs can cause host-mediated toxicity in aphid parasitoids, I investigated sub-lethal impacts on *Aphidius colemani* Viereck, a parasitoid of *R. padi*, over the course of the growing season.

Finally, the continued suppression of aphelinid wasps throughout the spring may have been a consequence of early season responses from which the population did not recover. Aphelinid species vary in their overwintering strategies, with some species entering diapause as adults and others diapausing as larvae or pupae within their hosts (Tatsumi and Takada, 2006). NSTs could

potentially disrupt overwintering by reducing the aphid population to such an extent that hosts are no longer available, or by impacting survival and fitness of wasps overwintering within contaminated hosts. In my field study, aphid numbers were very low in the winter (Dubey et al., 2020), and aphid abundance may impact neonicotinoid residues in plant tissue. Shortly after planting lower aphid abundance corresponded to higher neonicotinoid levels within winter wheat tissue (Bredeson et al., 2015). This variation could impact aphid suppression and prey abundance in addition to potentially exposing aphids to lower, sublethal levels of insecticide and providing contaminated hosts for overwintering wasps. While such an effect on parasitoids would be difficult to measure directly, I conducted a final experiment to determine NST effects on aphid populations of different sizes in the weeks immediately post planting, characterizing aphid population dynamics that could potentially impact parasitoids. Evaluating long- and short-term impacts of NSTs on cereal aphid populations and potential host-mediated impacts on aphid parasitoids enables us to better understand the benefits and costs of NSTs in Maryland winter wheat. A thorough understanding of the impact of NSTs on cereal aphids and their natural enemies will facilitate economically and environmentally sound pest management decisions. Unfortunately, the experiment evaluating host-mediated impacts on *A. colemani* had to be terminated before completion due to COVID-19 related campus closure. The methods and preliminary data from that experiment are included in Appendix E.

Materials & Methods

Aphid colony and wheat variety

Rhopalosiphum padi L. (Hemiptera: Aphididae) were collected from wheat fields in Maryland and Delaware in the spring and summer of 2018 and raised on untreated soft red winter wheat plants (*Triticum aestivum* L.) at 22°C with a 16:8 light: dark photoperiod. The colony was maintained for over a year before starting experiments. There was no indication that BYD was vectored within the colony. Aphid identity was confirmed by Dr. Gary Miller from the USDA Systematic Entomology Laboratory.

Soft red winter wheat (variety Branson, Brevant Seeds, Wilmington, DE, USA) was grown individually in planting cones (Ray Leach Cone-tainer cells, SC10 model, Tangent, OR, USA, 97389) filled with ~ 150 ml of Sun Gro Professional Growing Mix (Sun Gro Horticulture, Agawam, MA, USA) for experiments. The bottoms of the cones were plugged with cotton wool, which was removed two weeks after planting. Plants were fertilized at planting using ~0.5 g of Osmocote Plus Smart-Release Plant Food (Scotts Miracle-Gro, Marysville, OH, USA) per cone.

Seasonal conditions

A temperature and light regime designed to approximate Maryland growing conditions was used to grow wheat plants in the laboratory for our experiments. Growth chamber temperature programs were designed using NOAA weather data collected at Easton, MD (station WBAN:03756) over a five-year

period from 2012-2017. First, the 1-3 data points collected per hour were averaged to obtain a single mean temperature value per hour. Then, the hourly temperature for each date-hour combination was averaged across the 5 years to get a single set of 5-year average hourly temperatures from mid-October to the end of May. Finally, the hourly data from the first and second halves of the months was averaged to get two sets of 15-day average hourly temperatures per month. For example, the mean temperatures for 5AM from November 1st to November 15th were averaged to get a single 'Early November' temperature for 5AM, and mean temperatures from November 16th to November 30th were averaged to get a single 'Late November' temperature for 5AM. This was done for every hour to get a single set of 24-hour values for the first half of each month and another set of 24-hour values for the second half of each month. Temperature cycles were further simplified to accommodate the limitations of the growth chambers. As day length does not vary greatly from year to year, a single set of daily values for 2017 were obtained from NOAA ESRL data, and values from the first and second halves of each month were averaged to obtain an 'Early' and 'Late' day length value for each month. The set of 24-hour temperature values and the day length representing the 'Early' or 'Late' part of each month was repeated daily in the growth chambers for a 14-16 day period before being updated to the next set of values.

The wheat growing cycle was divided into three periods for plant care:

Pre-vernalization: This consisted of the temperatures from planting at mid-October to mid-December. Plants received 20 ml of water three times a week.

Plants were grown in two 3.72 m^2 Conviron CMP4030 walk-in growth chambers (Control Environments Limited, Winnipeg, Canada), and an equal number of plants from each chamber were used for experiments.

Vernalization: Temperatures representing the coldest part of the year, from mid-December to mid-March, were outside the growth chambers' capacity, and so the plants were moved to a single 8.36 m^2 walk-in cold chamber (Harford Duracool, Manitowic, WI, USA) set to 4°C and a 12:12 light: dark photoperiod for vernalization. For the first four weeks, plants received 20 ml of water twice a week; after that they received 20 ml of water once a week until the end of the vernalization period.

Post-vernalization: This consisted of temperatures from mid-March to the mid-May. Temperature and light settings were updated every 14-16 days. After vernalization, wheat was replanted into 0.5-gallon pots. Each plant was placed in a pot along with all the soil from the cone and was filled with an additional ~1.2 L of soil. Plants were fertilized during replanting with ~4.5 g of Osmocote Plus per pot. Plants from the same treatments were placed on trays in groups of six. Plants were watered three times per week; for the first five weeks, they received 150 ml of water, after which it was increased to 250 ml. Plants were grown in the two chambers used during pre-vernalization, and an equal number of plants from each chamber were used for experiments.

See Tables 3.1 and 3.2 for the temperature settings during pre- and post-vernalization periods, Table 3.3 for the photoperiod, and Tables F1-F4 in Appendix F for the actual temperature values recorded in the growth chambers.

Table 3.1. The growth chamber temperature settings used to approximate Maryland temperatures during the pre- and post-vernalization phases of the wheat growing season. Hourly temperatures from a five-year period were averaged for each day, and then the hourly temperatures were averaged over the first (Early) and last (Late) 15 days of each month to obtain two sets of hourly data per month. During vernalization, a constant 4°C temperature was used.

Hour (24:00)	Temperature (°C)			
	Late October	Early November	Late November	Early December
0:00	12	9	6	6
1:00	11	9	6	6
2:00	11	9	6	6
3:00	11	8	5	6
4:00	11	8	5	6
5:00	11	8	5	5
6:00	11	8	7	5
7:00	13	10	9	7
8:00	15	11	10	9
9:00	16	11	10	10
10:00	17	13	10	10
11:00	18	14	10	10
12:00	19	15	11	10
13:00	19	15	11	10
14:00	18	15	11	10
15:00	18	14	11	10
16:00	17	13	10	9
17:00	16	11	8	7
18:00	15	10	8	6
19:00	14	10	7	6
20:00	14	9	7	6
21:00	14	9	7	6
22:00	13	9	7	6
23:00	12	9	6	6

Table 3.2. The growth chamber temperature settings used to approximate Maryland temperatures during the post-vernalization phases of the wheat growing season. Hourly temperatures from a five-year period were averaged for each day, and then the hourly temperatures were averaged over the first (Early) and last (Late) 15 days of each month to obtain two sets of hourly data per month.

Hour (24:00)	Temperature (°C)			
	Late March	Early April	Late April	Early May
0:00	5	11	12	14
1:00	4	11	12	14
2:00	4	10	11	14
3:00	4	10	11	13
4:00	4	10	10	13
5:00	4	9	10	13
6:00	6	10	12	15
7:00	8	13	15	16
8:00	10	15	15	17
9:00	10	15	16	19
10:00	10	17	16	19
11:00	10	17	18	21
12:00	10	18	19	21
13:00	10	18	19	22
14:00	11	18	19	22
15:00	11	18	19	22
16:00	10	18	18	21
17:00	10	17	18	21
18:00	10	15	16	19
19:00	8	14	15	17
20:00	7	14	15	16
21:00	6	12	13	16
22:00	6	12	13	16
23:00	5	12	13	16

Table 3.3. The growth chamber light settings used to approximate day length in Maryland during the pre- and post-vernalization phases of the wheat growing season. During vernalization, a 12:12 light: dark photoperiod was used. Day length refers to the period of time during which lights were turned on.

Phase	**Pre-Vernalization**				**Post-Vernalization**			
Time Period	Late October	Early November	Late November	Early December	Late March	Early April	Late April	Early May
Day length (hh:mm)	10:50	10:17	9:50	9:33	12:19	12:58	13:33	14:06

Evaluating temporal variation in NST efficacy against R. padi over the growing season

Because commercial NSTs usually include fungicides in addition to insecticides, we included a fungicide only treatment as well as an untreated control to differentiate between the impacts of insecticides and fungicides for a total of four pesticide treatments: control or untreated; fungicide only (Vibrance Extreme; Syngenta AG, Basel, Switzerland); fungicide + imidacloprid (Vibrance Extreme + Gaucho 600; Bayer Crop Science, Monheim am Rhein, Germany); and fungicide + thiamethoxam (Vibrance Extreme + Cruiser 5FS; Syngenta AG). Commercially, each insecticide product would be paired with different fungicide products, but we used the same fungicide product across treatments for consistency. Soft red winter wheat (variety Branson, Brevant Seeds, Wilmington, DE, USA) were treated by the Syngenta Seedcare Institute (Syngenta AG) with a medium rate of neonicotinoid active ingredients; the active ingredients and treatment rates per seed for each product are presented in Table 3.4.

Table 3.4. The active ingredients and applications rates for the pesticide products used to treat wheat seeds. Vibrance Extreme was applied at the same rate for the fungicide, imidacloprid and thiamethoxam treatments.

Product	Active Ingredient (ai)	Mg ai per seed
Vibrance Extreme	Sedaxane	0.0013
	Difenoconazole	0.0063
	Mefenoxam	0.0016
Gaucho 600	Imidacloprid	0.0217
Cruiser 5FS	Thiamethoxam	0.0143

NST activity against aphids was measured at six time points in the growing cycle (Table 3.5). The larger gap between the vernalization and post-vernalization time points was because the plants required several weeks to establish and resume growth after replanting. At each time point, 20 mid- to late-stage aphid nymphs (as categorized below) were added individually to four replicate plants from each treatment by placing the nymphs in the soil near the base of the plant. After adding aphids, each plant was caged using a mesh sleeve supported by wooden dowels. The plants with aphids were kept for two weeks in a growth chamber at 22°C with a 16:8 light: dark photoperiod. At the end of two weeks, the aphids within each cage were counted by destructively sampling the plant. Aphid life stages were categorized as follows:

Early-stage nymph: approximately ≤ 1 mm in length; light green coloration; narrow body.

Mid- to late-stage nymph: approximately 1 to 1.5 mm in length; light green coloration; narrow body.

Apterous (wingless) adult: approximately ≥ 1.3 mm in length, dark green coloration with end of the abdomen reddish-brown; wide, rounded body.

Alate (winged) adult: approximately ≥ 1.3 mm in length; wings present.

Table 3.5. The timeline of experimental time points over the wheat growing season.

Phase	**Pre-Vernalization**		**Vernalization**		**Post-Vernalization**	
Time Point (weeks post planting)	4	8	12	16	24	28
Growth Stage (Feekes Scale)	2	2	3	3	9-10	10

Data for each time point was analyzed separately through one-way analysis of variance (JMP Pro 13.2.1, SAS Institute Inc., Cary, NC, USA) with total number of aphids as the response variable and pesticide treatment as the explanatory variable. The assumption of normality was tested using a Shapiro-Wilk test, and data was transformed as necessary. The assumption of homoscedasticity was tested using Levene's test and weighted least squares methods (Weighting factor: $(\text{residual variance})^{-1}$ of the fixed effect that most deviated from homoscedasticity) were used when needed. When effect differences were statistically significant ($P<0.05$), means comparisons with Tukey's adjustment were used to compare treatment effects. The percentage of aphids per life stage was graphed for each pesticide treatment and time point to visualize differences in aphid population structure.

Evaluating temporal variation and role of aphid abundance in NST efficacy against R. padi immediately post planting

Post planting aphid density experiments compared three pesticide treatments: control (untreated); fungicide + imidacloprid (Vibrance Extreme + Gaucho 600; and fungicide + thiamethoxam (Vibrance Extreme + Cruiser 5FS). Seeds were treated at the same rates as for the full season experiment (Table 3). The experiment was carried out at three time points: 2 weeks post planting (wpp) (Feekes stage 1), 4 wpp (Feekes stage 1), and 5 wpp (Feekes stage 2). At each time point, mid- to late-stage aphid nymphs (as categorized above) were added to plants from each pesticide treatment at three aphid densities: 5, 10 and 20 aphids per plant. Four replicate plants were set up with each pesticide treatment and aphid density combination, using the same methods as above. The total number of aphids per plant was counted by destructively sampling the plants 96 hours after adding the aphids.

To evaluate the effectiveness of NSTs in controlling aphids, I calculated % survivorship for each replicate plant within the control and the two insecticide treatments using the following formula:

$$\frac{Final\ number\ of\ aphids}{Initial\ numbers\ of\ aphids} \times 100 = \%\ survivorship$$

To evaluate the impact of aphid density on effectiveness of NSTs, I calculated % control with Abbott's correction (Abbott, 1925) for each of the insecticide treatments using the following formula:

$$\frac{C - I}{C} \times 100 = \%\ control$$

Where C is the % survivorship for the control and I is the % survivorship for each insecticide treatment. A single C value was calculated for each aphid density treatment by averaging the % survivorship of the four replicate control plants within that density treatment while I was the % survivorship for each individual replicate plant.

Percent survivorship and % control for each time point were analyzed in JMP Pro through analysis of variance with % survivorship or % control as the response variable and pesticide treatment, aphid density and the pesticide treatment*aphid density interaction term as the explanatory variables. ANOVA assumptions were evaluated and mitigated as discussed previously. When effect differences were statistically significant ($P<0.05$), treatment effects were compared through means comparisons with Tukey's adjustment in all cases except for the pesticide treatment for % control, where a t-test was used because there were only two treatments.

Results

Evaluating temporal variation in NST efficacy against R. padi over the growing season

The total number of aphids was impacted by pesticide treatment at the 4 WPP (weeks post planting) ($F_{3,12} = 26.61$, $P < 0.001$), 8 WPP ($F_{3,12} = 33.80$, $P < 0.001$), 12 WPP ($F_{3,12} = 5.56$, $P = 0.014$) and 16 WPP ($F_{3,12} = 7.45$, $P = 0.004$) time points, but not at 24 WPP ($F_{3,12} = 3.45$, $P = 0.052$) or 28 WPP ($F_{3,12} = 0.48$, P

= 0.699) (Fig. 3.1). At 4, 8, 12 and 16 WPP, the thiamethoxam treatments reduced total number of aphids compared to the control, but the fungicide and imidacloprid treatments did not. Fig. 3.2 shows the percentages of early-stage nymphs, mid- to late-stage nymphs, apterous adults and alate adults for the different treatments at each time point.

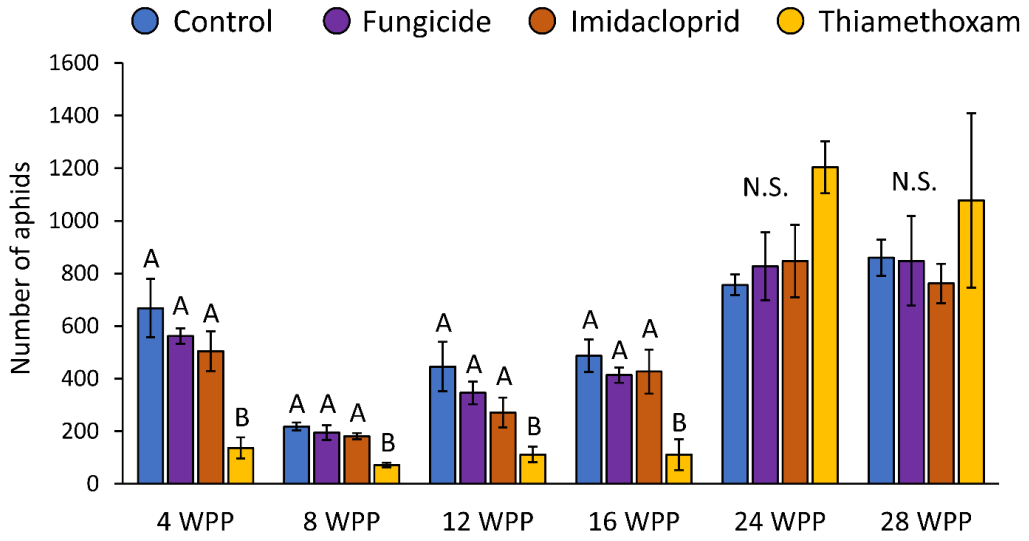

Fig. 3.1. Impact of seed treatments on the total number of aphids at 4 ($F_{3,12} = 26.61$, $P < 0.001$), 8 ($F_{3,12} = 33.80$, $P < 0.001$), 12 ($F_{3,12} = 5.56$, $P = 0.014$), 16 ($F_{3,12} = 7.45$, $P = 0.004$), 24 ($F_{3,12} = 3.45$, $P = 0.052$) and 28 ($F_{3,12} = 0.48$, $P = 0.699$) weeks post planting (WPP). The experiment started with 20 mid-late stage aphids per plant (n = 4) and ran for two weeks at each time point. Data for each time point was analyzed separately through analysis of variance. Significant differences within each time point are indicated by letters; N.S.= no significance; error bars depict standard error.

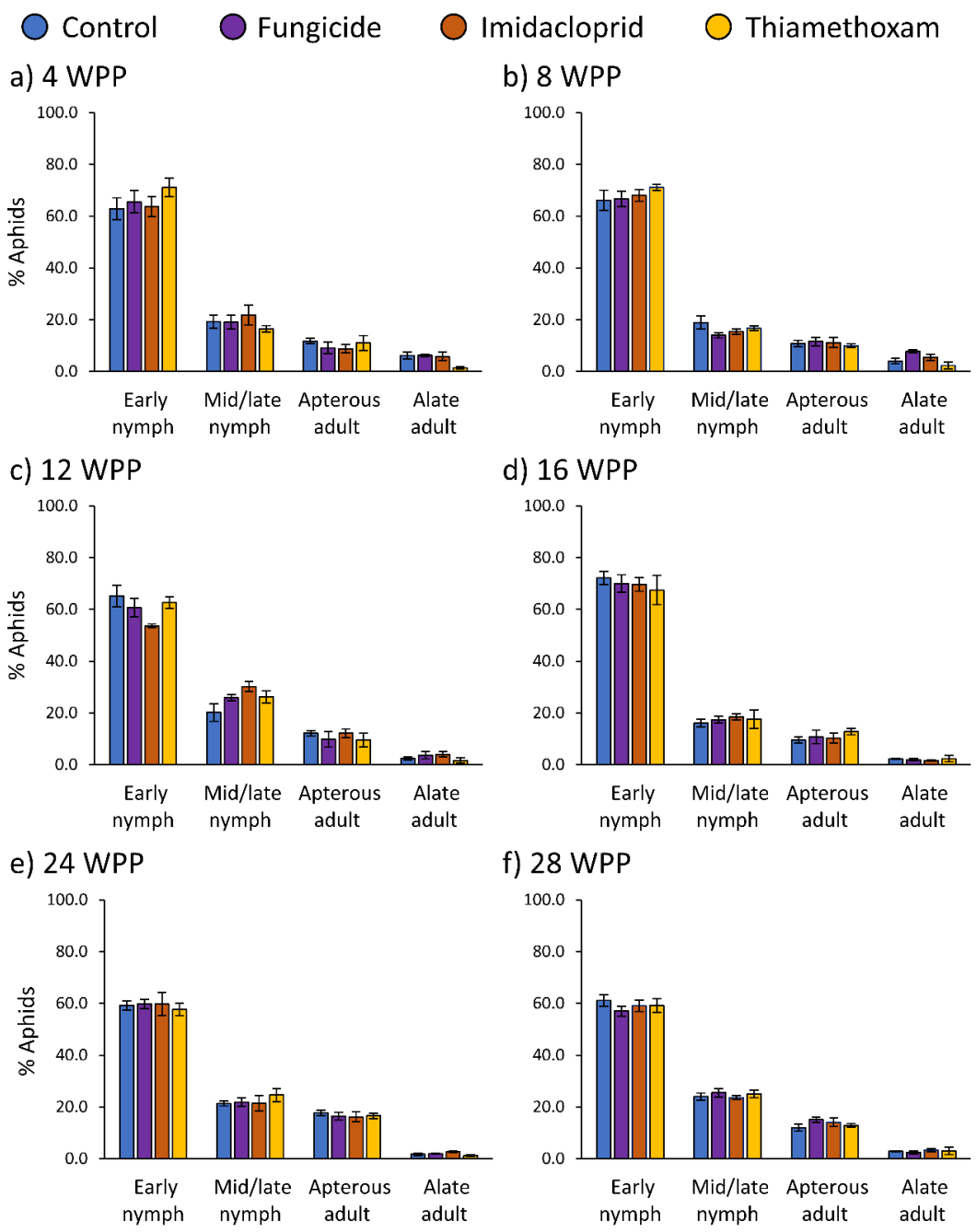

Fig. 3.2. Aphid population structure as represented by the percent of early-stage nymphs, mid- to late-stage nymphs, apterous adults and alate adults at 4, 8, 12, 16, 24 and 28 weeks post planting (WPP) for each treatment. The experiment started with 20 aphids per plant and ran for two weeks at each time point. Error bars depict the standard error.

Evaluating temporal variation and role of aphid abundance in NST efficacy against R. padi immediately post planting

Aphid Survivorship: Survivorship was not impacted by the pesticide treatment*aphid density interaction (2 WPP $F_{4,27} = 0.83$, $P = 0.516$; 3 WPP $F_{4,27} = 1.26$, $P = 0.311$; 4 WPP $F_{4,27} = 0.46$, $P = 0.765$) or aphid density (2 WPP $F_{2,27} = 0.90$, $P = 0.418$; 3 WPP $F_{2,27} = 2.43$, $P = 0.108$; 4 WPP $F_{2,27} = 1.89$, $P = 0.171$) at any time point. Pesticide treatment significantly impacted survivorship at all three time points (2 WPP $F_{2,27} = 83.24$, $P < 0.001$; 3 WPP $F_{2,27} = 9.35$, $P < 0.001$; 4 WPP $F_{2,27} = 20.19$, $P < 0.001$) (Fig. 3.3).

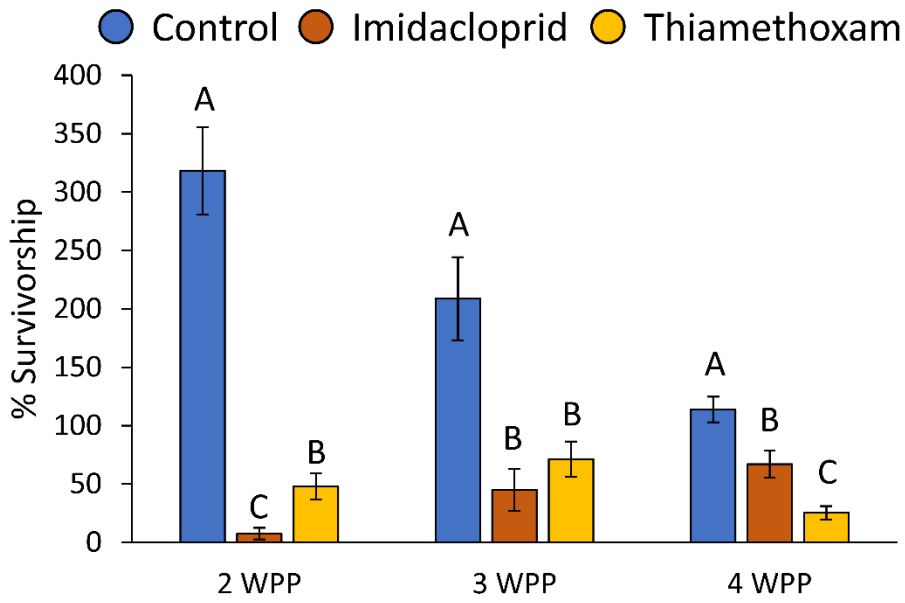

Fig. 3.3. Impact of seed treatments on aphid survivorship at 2 ($F_{2,27} = 83.24$, $P < 0.001$), 3 ($F_{2,27} = 9.35$, $P < 0.001$) and 4 ($F_{2,27} = 20.19$, $P < 0.001$) weeks post planting (WPP). The experiment started with 5, 10 or 20 mid-late stage aphids per plant and ran for 96 hours at each time point. Data shown here is averaged across aphid densities (n=12). Survivorship of over 100% can be attributed to aphid reproduction during the 96 hour period when aphids were left on the plants. Significant differences within each time point are indicated by letters; N.S. indicates no significance; error bars depict standard error.

Aphid Control: The pesticide treatment*aphid density interaction did not impact control at any time point (2 WPP $F_{2,18} = 0.67$, P = 0.523; 3 WPP $F_{2,18} = 2.27$, P = 0.133; 4 WPP $F_{2,18} = 0.27$, P = 0.767). Control was impacted by pesticide treatment at 2 and 4 WPP (2 WPP $F_{1,18} = 11.63$, P = 0.003; 3 WPP $F_{1,18} = 2.31$, P = 0.146; 4 WPP $F_{2,18} = 8.14$, P = 0.011) and by aphid density at 3 WPP (2 WPP $F_{2,18} = 1.49$, P = 0.253; 3 WPP $F_{2,18} = 4.81$, P = 0.021; 4 WPP $F_{2,18} = 0.03$, P = 0.974) (Fig. 3.4).

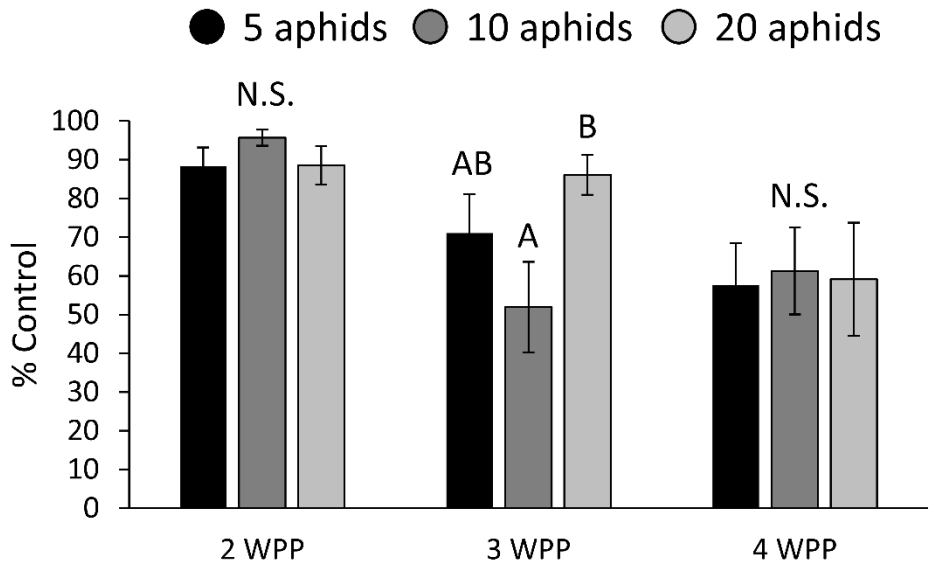

Fig. 3.4. Impact of aphid density on control of aphids by imidacloprid and thiamethoxam seed treatments at 2 ($F_{2,18} = 1.49$, P = 0.253), 3 ($F_{2,18} = 4.81$, P = 0.021) and 4 ($F_{2,18} = 0.03$, P = 0.974) weeks post planting (WPP). The experiment started with 5, 10 or 20 mid-late stage aphids per plant (n = 4) and ran for 96 hours at each time point. Data shown here is averaged across insecticide treatments. Significant differences within each time point are indicated by letters; N.S. indicates no significance; error bars depict standard error.

Discussion

I conducted a series of laboratory trials investigating the impacts of neonicotinoid seed treatments (NSTs) on the cereal aphid *Rhopalosiphim padi* and its parasitoids in Maryland winter wheat. Specifically, I evaluated how the efficacy of NSTs against *R. padi* changes over the course of the growing season, and the impact of aphid density on efficacy immediately post planting. I also designed an experiment to evaluate host-mediated sublethal impacts of NSTs on the aphid parasitoid *Aphidius colemani* but could not complete that experiment due to campus closure. Both insecticide treatments reduced aphid survivorship at all three time points in the post-planting trial, but while thiamethoxam reduced aphid population size up to 16 weeks post planting (WPP) in the longer-term trial, imidacloprid had no impact. Finally, aphid density affected aphid control by the NSTs at 3 WPP but not at 2 or 4 WPP.

The difference in the duration of control provided by imidacloprid and thiamethoxam does not reflect my field study, where results were similar for both NSTs (Dubey et al., 2020). In the lab, imidacloprid was more effective than thiamethoxam at controlling aphids at 2 WPP in the post-planting study, but was less effective than thiamethoxam at 4 WPP (Fig. 3.3), and had no impact on aphid population size (starting at 4 WPP) in the longer term study. Imidacloprid has shown lower adsorption and slower degradation in soil compared to thiamethoxam, making it more likely to move downward through the soil in response to rainfall or irrigation (Zhang et al., 2018). In the field study, the wheat received 153 mm of rain over the two month period post planting (data retrieved

from NOAA; weather station USC00180700 at Beltsville), which when adjusted for area, is equivalent to a cone receiving ~155 ml of water. In contrast, the plants for the lab trials received ~500 ml of water per cone over the same period. This may have caused the insecticide coating in the imidacloprid treatment to wash off the seeds and out of the soil within a few weeks of planting, while the thiamethoxam coating continued to be absorbed by and translocated through the plant (Simon-Delso et al., 2015). A semi-field study in Australia conducted with potted wheat plants and similar insecticide treatment rates found that imidacloprid and thiamethoxam seed treatments had efficacy against *R. padi* for 10 and 7 weeks, respectively (Kirkland et al., 2018). However, in that study, plants were watered minimally as needed to maintain plant health, to reduce leaching, supporting the conclusion that the lower efficacy period of imidacloprid relative to thiamethoxam in my study may have been due to the frequency of watering. While this difference between treatments was not apparent in my field study, thiamethoxam seed treatments may have a longer efficacy period than imidacloprid in some areas that experience higher precipitation early in the wheat growing season. Thiamethoxam has also been found to persist longer than imidacloprid in soybean leaves, and provide more consistent control of the soybean aphid *Aphis glycines* Matsumura (Magalhaes et al., 2009).

Although the efficacy period of imidacloprid was lower than expected, the results for thiamethoxam were in keeping with my field study; in the lab, thiamethoxam had efficacy against aphids up to 16 WPP, while in the field, it had efficacy in the fall (up to 7 WPP) but not in the spring (24 WPP onwards) (Dubey

et al., 2020). NSTs can have a wide range of efficacy periods in winter wheat, with some studies obtaining results similar to ours (Kennedy and Connery, 2012; Kirkland et al., 2018; Royer et al., 2005), and others finding that NSTs maintain efficacy against cereal aphids throughout the wheat growing season (Li et al., 2018; Zhang et al., 2016). However, those studies were conducted in China, with low and high treatment rates that were four and six times higher, respectively, than the medium US label rate used in my study. The large disparity in treatment rates between countries may account for the differences in efficacy period between studies. While the treatments did not significantly impact aphid population at 24 and 28 WAP, there was a trend of higher aphid numbers in the thiamethoxam treatment relative to the control. Previous laboratory studies have found that exposure to sublethal doses of neonicotinoid insecticides can have a hormetic effect and result in increased fecundity in multiple aphid species (Qu et al., 2015; Sial et al., 2018; Ullah et al., 2019; Wang et al., 2017; Yu et al., 2010), including *R. padi* (Deng et al., 2019). Although my results were not significant, they suggest that lower levels of thiamethoxam present in the wheat tissue during the later sampling dates may have had a similar hormetic effect. If NSTs have the potential to increase cereal aphid population during the later stages of winter wheat, this could have implications for pest management.

In addition to potential aphid population increase later in the growing season, the level of aphid control provided by NSTs in the weeks after planting may not be sufficient to prevent yield losses. NSTs reduced aphid population size in my field study; however, the population was well below the economic

threshold throughout, making it difficult to extrapolate the results to economically damaging infestations (Dubey et al., 2020). In this study, we evaluated how NST efficacy changes over time, and also evaluated whether aphid density altered efficacy, which had previously been observed (Bredeson et al., 2015). Density did not impact aphid control at 2 or 4 WPP, but at 3 WPP, control was higher in the 20 aphid treatment compared to the 10 aphid treatment. This suggests that efficacy may vary with aphid density; however, conclusions cannot be drawn based on a single time point. Regardless of density, the seed treatments did not cause 100% aphid mortality in any of the trials, even at 2 WPP. At 4, 8 and 12 WPP, the thiamethoxam treatment had a mean aphid abundance of 137, 72 and 112 aphids per plant respectively, two weeks after adding 20 aphids per plant. The population structure was also similar across treatments; early-stage nymphs constituted the majority of the population, suggesting that the population would continue to grow at a similar rate. However, the proportion of winged aphids was somewhat lower than the control, suggesting that the treatment could reduce dispersion and the spread of barley yellow dwarf (BYD) (van Toor et al., 2016). In the mid-Atlantic region, the fall economic threshold for cereal aphids in winter wheat is 150 aphids per row foot (Taylor and Laub, 2020; Whalen et al., n.d.). Based on the stand density measured in my field study, one row foot includes 6 or 7 wheat plants (Dubey et al., 2020). At the aphid densities observed in the lab, aphid populations exceeded the economic threshold (approximately 21-25 aphids per plant), in spite of the control provided by the thiamethoxam treatment. Previous studies have found that NSTs do not always control cereal aphids and

the spread of BYD, and one or two foliar pyrethroid applications at threshold are more effective (Kennedy and Connery, 2012; Mckirdy and Jones, 1996; Zwiener et al., 2005). Our results suggest that this may also be the case in Maryland.

In addition to evaluating NST efficacy against cereal aphids, another goal of this study was to better understand potential mechanisms for the observed non-target impacts of NSTs on aphelinid parasitoids in the field (Dubey et al., 2020). I hypothesized that in the fall, NSTs could reduce aphid populations to such an extent that wasps do not have sufficient overwintering hosts; conversely, if high levels of aphids survived the NSTs, they could provide contaminated overwinter hosts, disrupting emergence, survival, and fitness of wasps in the spring (Teder and Knapp, 2019). In the post planting trial, neither insecticide treatment completely controlled the aphid population, with up to 67 and 71% survivorship in the imidacloprid and thiamethoxam treatments, respectively. This suggests that treatments are unlikely to impact parasitoids by severely reducing host availability. However, the high survivorship supports the hypothesis that parasitoids could overwinter in contaminated hosts. It is also possible that NSTs could disrupt parasitoid population by lowering parasitism rates in contaminated hosts, as host contamination by neonicotinoids reduced parasitism rates in Aphelinid parasitoids of the whitefly *Bemisia tabai* (Gennadius) (Naveed et al., 2010) and the soybean aphid (Frewin et al., 2014). In the longer term trial, the thiamethoxam treatment no longer controlled the aphid population at the spring time points (24 and 28 WPP), suggesting the lower parasitoid activity density in the spring was not simply a response to lower host availability. However,

insecticides could still be present at sufficiently high levels to impact parasitoid populations through reduced parasitism, host mediated effects, or consumption of contaminated aphid honeydew (Calvo-agudo et al., 2019; Frewin et al., 2014; Naveed et al., 2010; Taylor et al., 2015). The results of the two trials suggest that multiple mechanisms could have contributed to the reduced aphid activity-density observed in the spring, either individually, or in combination.

Conclusions

Both imidacloprid and thiamethoxam treatments controlled *R. padi* in the weeks immediately post planting, but only thiamethoxam provided longer term control up to 16 WPP. This difference between insecticides was likely due to imidacloprid moving out of the soil rapidly in response to frequent watering, and was not reflected in my previous field study, where both NSTs had similar periods of efficacy. However, these results suggest that thiamethoxam could have longer efficacy than imidacloprid in areas with high levels of precipitation shortly after planting. While the insecticides did reduce aphid population size, the level of control observed may not be sufficient to maintain aphids below the fall economic threshold for mid-Atlantic winter wheat and to control the spread of BYD, and one or two foliar pyrethroid applications as needed may be a better solution. Finally, continued neonicotinoid activity in the spring and effects carried over from the fall are both possible explanations for the impacts observed on aphid parasitoid activity density in the field. Given the level of aphid control and the potential for non-target impacts on natural enemies, NSTs may not be an effective tool for managing cereal aphids and BYD in Maryland winter wheat.

Conclusions

Considering the extent of neonicotinoid seed treatment (NST) adoption in the US and elsewhere, there is a surprising lack of knowledge about many aspects of their use. This lack of knowledge is exacerbated by the extreme variability in the activity and impacts of NSTs, as highlighted throughout this dissertation. The movement of active ingredients through the environment is governed by a complex interaction between factors including application rate, soil type, temperature, precipitation, and agronomic practices, making it difficult to extrapolate findings from one geographic region and cropping system to another. In light of this variability, the costs and benefits of NSTs must be evaluated on a case by case basis. Such research has not previously been undertaken in Maryland, and the overall goal of this dissertation was to evaluate the costs and benefits of NSTs in Maryland grain production. My specific objectives were to determine pest control and yield benefits and evaluate the potential for negative impacts on non-target arthropods and soil health in Maryland corn, soybean, and wheat systems.

Previous research has shown that NSTs do not generally provide yield benefits in the absence of sustained early season pressure from soil and seedling pests (Myers and Hill, 2014; Tooker et al., 2017). Given that most pests targeted by NSTs are occasional pests that rarely reach economically damaging levels in Maryland, I did not anticipate yield improvements as a result of NST use (Papiernik et al., 2018). This was indeed the case, as the pests that were present,

such as cereal aphids in winter wheat, remained well below the economic threshold, and the NSTs did not impact yield in corn, soybean, or wheat. In fact, my follow up lab study suggested that NSTs may not have the efficacy needed to maintain cereal aphid populations below economic thresholds, even when pest pressure is high. Previous research has shown that one or two foliar pyrethroid application can be more effective than NSTs in controlling cereal aphid populations and the spread of barley yellow dwarf virus (Kennedy and Connery, 2012; Zwiener et al., 2005). Foliar pyrethroids and NSTs have similar non-target impacts (Douglas and Tooker, 2016) but pyrethroids can be used in response to pest pressure rather than prophylactically. Therefore, the use of foliar pyrethroids is more in keeping with the principles of integrated pest management (IPM) than NSTs and they may be a better tool against occasional pests (Douglas et al., 2015). However, there are situations where NSTs can be the best treatment option, such as fields with white grub or wireworm infestations, as these soil pests usually recur over multiple years and their damage cannot be controlled through rescue treatments (Douglas and Tooker, 2015; Sappington et al., 2018). Future research on the potential benefits of NSTs in Maryland should focus on fields with soil pest problems, to determine where NSTs are effective in those cases. Economic analysis should also be considered, as pest suppression does not always translate to economic benefits (Royer et al., 2005).

To make optimized sustainable pest management decisions, we also need to understand how management choices impact non-target organisms. My large-scale analysis of the foliar and soil arthropod communities had mixed results, with

some impacts on the foliar arthropod community, and on specific beneficial taxa such as rove beetles (Staphylinidae) and aphelinid wasps. The reduction in the activity density of aphelinid wasps in winter wheat was the most interesting result, as this effect continued up to 32 weeks post planting. This highlights the need for further research on the translocation and persistence of neonicotinoids in winter wheat, similar to Alford and Krupke's work in corn (Alford and Krupke, 2017). I identified multiple potential mechanisms for this effect on aphelinid wasps but could not fully investigate them as planned due to the COVID-19 pandemic. My laboratory studies show that multiple mechanisms are feasible, but the effects of NSTs on this important natural enemy group deserve further investigation. While my research clearly demonstrates that NSTs do have impacts on non-target arthropods, linking those impacts to tangible ecosystem services can be challenging (Douglas and Tooker, 2016). However, the impacts of NSTs can be far reaching; for example, the reduction in arthropod abundance caused by NSTs has been linked to declines in insectivorous birds (Hallmann et al., 2014). To fully understand the risks posed by NSTs, future research should not only focus on the non-target impacts of NSTs, but also try to explore their larger consequences.

A unique aspect of my research evaluating the impacts of NSTs on non-target arthropods is that it separated out the impacts of seed applied fungicides that are included in commercial NST packages. Like NSTs, seed applied fungicides are often used prophylactically, and the benefits associated with their use are often unclear (Lamichhane et al., 2020). Fungicide seed treatments include

multiple chemicals with different modes of action and target organisms, increasing the likelihood of impacts on arthropods and other non-target organisms through various mechanisms (Lamichhane et al., 2020). However, the impacts of seed applied fungicides on arthropods are largely unknown (Lamichhane et al., 2020). In my research, I found that seed applied fungicides can impact the larger arthropod community as well as specific beneficial taxa in the absence of neonicotinoids. Most lab studies evaluating impacts of neonicotinoids use the insecticide alone, while field studies include the combination of neonicotinoids and fungicides that is typically used by farmers. The results of my research show that this approach does not provide a complete picture, as synergistic interactions between neonicotinoids and fungicides may remain undetected, while some impacts of fungicides could be misattributed to neonicotinoids. The role of seed applied fungicides in the non-target impacts of NSTs must be investigated further.

In addition to non-target arthropods, I also evaluated impacts of NSTs on soil health. My results did not show any effect of the treatments on soil health parameters, overall microbial activity, or the soil prokaryotic community. Given the low residue levels detected in the soil, it is possible that NSTs simply did not impact soil health. However, the experimental design may have obscured some effects, as I combined soil from different parts of the field and did not sequence the fungal community. The lack of impacts may also be a legacy of NST usage over the previous decade. To fully understand the long-term effects of NSTs on soil health, field studies need to be conducted on land without a history of neonicotinoid use. Although the widespread adoption of NSTs and other types of

neonicotinoid applications makes this difficult, there may be some alternatives, such as land previously used for organic production.

Although the low levels of neonicotinoids in the soil have positive implications for soil health and arthropod communities, they raise concerns about the fate of the active ingredients from NSTs. While low residue levels could be caused by rapid breakdown of neonicotinoids in the soil, the sandy soil and high precipitation at my field sites makes it likely that neonicotinoids are leaching and/or running-off into water bodies. Previous research has shown increased levels of neonicotinoids in streams within the Chesapeake Bay watershed during the period when corn is planted (Hladik and Kolpin, 2016). The toxicity of neonicotinoids towards aquatic arthropods can disrupt aquatic food webs and cause cascading trophic effects, resulting in ecological and economic damage (Miles et al., 2017; Morrissey et al., 2015; Starner and Goh, 2012; Yamamuro et al., 2019). In 2019, 460,000 acres of corn were harvested in Maryland ("2019 State Agriculture Overview Maryland," n.d.). Given the close to 100% adoption of NSTs in US corn (Douglas and Tooker, 2015), and Maryland's location within the Chesapeake Bay watershed, there is an urgent need to investigate the potential movement of neonicotinoids from the soil into aquatic systems and their impacts therein.

Having considered both the costs and benefits of NSTs, I return now to my initial question; should NSTs be used in Maryland? Although they may be beneficial in specific cases, the absence of pest pressure combined with the potential for non-target impacts suggests that NSTs should not be used regularly

in Maryland. Instead of using prophylactic treatments against occasional pests, farmers should address pest problems within the framework of IPM, whenever possible. Unfortunately, while farmers can opt to purchase untreated soybean and wheat seed, they do not have that choice for corn. This reflects a larger national issue; untreated corn, and in some cases soybean seed, is simply not available (Tooker et al., 2017). Farmers do not always know that they are using neonicotinoid-treated seeds, or that treatment rates have increased over time, as the insecticides are a part of the standard treatment package sold by seed distributors (Tooker et al., 2017). Additionally, as it is in agrochemical companies' interest to maximize product sales, they create educational materials overstating the benefits of NSTs (Tooker et al., 2017). Farmers who choose to use untreated seed are faced with additional challenges; a Maryland farmer who decided to stop using NSTs due to concerns about soil health told me that he has to order untreated seed several months in advance and cannot choose his preferred seed variety. The results of my research can be used to educate Maryland farmers about the lack of benefits and the potential negative impacts of NSTs, and to encourage them to use untreated soybean and wheat seed unless NSTs are warranted by specific pest pressure. Additionally, if more farmers are inspired to order untreated corn seed, seed distributors may be compelled to make untreated seed more readily available over time. However, these issues make it is clear that the onus to reduce the use of NSTs cannot entirely be placed on farmers.

Recent estimates suggest that using NSTs within an IPM framework and eliminating their unwarranted application in large-acreage crops would reduce

environmental loading by over 2.8 million kilograms (Frank and Tooker, 2020). The only way to affect change on this staggering scale is through increased regulation. I do not think that neonicotinoids need to be banned outright; as discussed previously, NSTs can be a valuable tool in certain cases, and neonicotinoids also have many other applications, such as in tree injections to stop the destruction of our forests by devastating invasive pests such as the hemlock woolly adelgid (Frank and Tooker, 2020). However, the need for better regulation of NSTs is apparent. This could include mandating the availability of untreated seeds of all major varieties in combination with farmer education about why it is preferable; ensuring that using seed treatments is not a requirement for claiming crop insurance; or restricting NST sales in regions with infrequent pressure from targeted pests. I believe that in order to successfully advocate for regulatory change, we need to build a comprehensive body of research that fills the gaps in our knowledge about neonicotinoids and NSTs. I hope that by contributing to that body of work and highlighting areas for further study, my dissertation research will bring us one step closer to solving the problem of neonicotinoid seed treatments.

Appendix A: Plot map, seed treatment information, and sampling timelines for Chapters 1 & 2

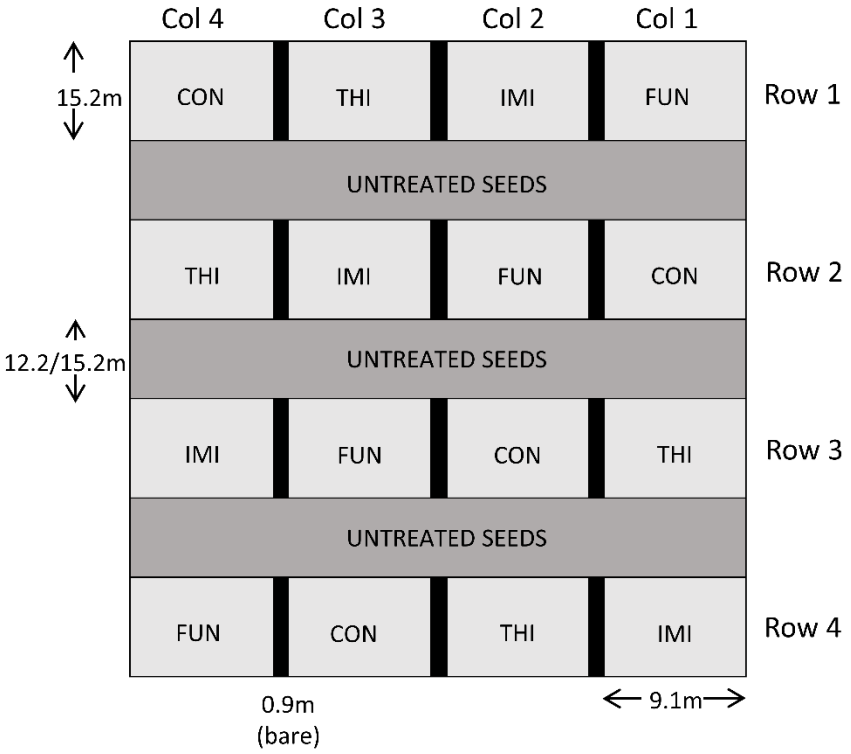

Fig. A1. Plot map showing the Latin square arrangement of four replicates of each treatment [control (CON), fungicide only (FUN), imidacloprid + fungicide (IMI), thiamethoxam + fungicide (THI)]. Rows were separated by turn rows planted with untreated grain (12.2 m at Queenstown, 15.2 m at Beltsville), and columns were separated by 0.91m bare strips.

Table A1. Seed treatment (Trt) active ingredients (ai) used in 2015 full-season (FS) soybean and 2015-2016 winter wheat. Soybean variety P93Y84 (Pioneer) was treated at a low rate, with a seeding rate of 383,013 seeds per hectare at Beltsville (BV) and 370,658 seeds per hectare at Queenstown (QT). For wheat, variety MBX14K297 (Mercer) was treated at a medium rate, which was chosen because NSTs are not widely used in Maryland wheat. The same seeding rate was used at both sites (4.32 million seeds per hectare).

Crop	Trt	Product	Active ingredient (ai)	mg ai seed^{-1}	mg ai plot^{-1} (BV;QT)	g ai ha^{-1} (BV;QT)
2015 FS Soybean	Thiamethoxam + Fungicide	Cruiser 5FS	Thiamethoxam	0.0756	400.52; 387.60	28.96; 28.02
		Maxim 4FS	Fludioxonil	0.0038	20.13; 19.48	1.46; 1.41
		Apron XL	Mefenoxam	0.0113	59.87; 57.93	4.33; 4.19
		Vibrance	Sedaxane	0.0038	20.13; 19.48	1.46; 1.41
	Imidacloprid + Fungicide	Gaucho 600	Imidacloprid	0.1000	529.78; 512.69	38.30; 37.07
		Allegiance FL	Metalaxyl	0.0244	129.27; 125.10	9.35; 9.04
		Evergol Energy	Prothioconazole	0.0081	42.91; 41.53	3.10; 3.00
			Penflufen	0.0045	23.84; 23.07	1.72; 1.67
			Metalaxyl	0.0064	33.91; 32.81	2.45; 2.37
	Fungicide Only	Maxim 4FS	Fludioxonil	0.0038	20.13; 19.48	1.46; 1.41
		Apron XL	Mefenoxam	0.0113	59.87; 57.93	4.33; 4.19
		Vibrance	Sedaxane	0.0038	20.13; 19.48	1.46; 1.41
2015-2016 Winter Wheat	Thiamethoxam + Fungicide	Cruiser 5FS	Thiamethoxam	0.0143	854.56	61.78
		Vibrance Extreme	Sedaxane	0.0013	78.62	5.68
			Difenoconazole	0.0063	377.35	27.28
			Mefenoxam	0.0016	94.37	6.82
	Imidacloprid + Fungicide	Gaucho 600	Imidacloprid	0.0217	1303.98	93.63
		Allegiance FL	Metataxyl	0.0017	102.93	7.39
		Evergol Energy	Prothioconazole	0.0018	106.69	7.66
			Penflufen	0.0009	53.35	3.83
			Metataxyl	0.0014	85.30	6.12
	Fungicide Only	Vibrance Extreme	Sedaxane	0.0013	78.62	5.68
			Difenoconazole	0.0063	377.35	27.28
			Mefenoxam	0.0016	94.37	6.82

Table A2. Seed treatment (Trt) active ingredients (ai) used in 2016 double-cropped (DC) soybean and 2017 corn. Soybean was treated at a low rate and corn was treated at a medium rate. Soybean

variety P39T67R (Pioneer) was treated at a rate of 494,210 seeds per hectare at Beltsville (BV) and 303,939 seeds per hectare at Queenstown (QT). Corn variety TA506-22SPRIb (T.A. Seeds) was treated at 74,132 seeds per hectare at Beltsville and 81,545 seeds per hectare at Queenstown.

Crop	Trt	Product	Active ingredient (ai)	mg ai seed^{-1}	mg ai plot^{-1} (BV;QT)	g ai ha^{-1} (BV;QT)
2016 DC Soybean	Thiamethoxam + Fungicide	Cruiser 5FS	Thiamethoxam	0.0756	516.80; 317.83	37.36; 22.98
		Maxim 4FS	Fludioxonil	0.0038	25.98; 15.98	1.88; 1.15
		Apron XL	Mefenoxam	0.0113	77.25; 47.51	5.58; 3.43
		Vibrance	Sedaxane	0.0038	25.98; 15.98	1.88; 1.15
	Imidacloprid + Fungicide	Gaucho 600	Imidacloprid	0.1000	683.59; 420.41	49.42; 30.39
		Allegiance FL	Metalaxyl	0.0244	166.80; 102.58	12.06; 7.42
		Evergol Energy	Prothioconazole	0.0081	55.37; 34.05	4.00; 2.46
			Penflufen	0.0045	30.76; 18.92	2.22; 1.37
			Metalaxyl	0.0064	43.75; 26.91	3.16; 1.95
	Fungicide Only	Maxim 4FS	Fludioxonil	0.0038	25.98; 15.98	1.88; 1.15
		Apron XL	Mefenoxam	0.0113	77.25; 47.51	5.58; 3.43
		Vibrance	Sedaxane	0.0038	25.98; 15.98	1.88; 1.15
2017 Corn	Thiamethoxam + Fungicide	Cruiser 5FS	Thiamethoxam	0.5000	512.69; 563.96	37.07; 40.77
		Vibrance	Sedaxane	0.0125	12.82; 14.10	0.93; 1.02
		Maxim Quattro	Fludioxonil	0.0063	6.46; 7.11	0.47; 0.51
			Mefenoxam	0.0050	5.13; 5.64	0.37; 0.41
			Azoxystrobin	0.0025	2.56; 2.82	0.19; 0.20
			Thiabendazole	0.0501	51.37; 56.51	3.71; 4.09
	Imidacloprid + Fungicide	Gaucho 600	Imidacloprid	0.5000	512.69; 563.96	37.07; 40.77
		Vortex FL	Ipconazole	0.0063	6.46; 7.10	0.47; 0.51
		Allegiance FL	Metalaxyl	0.0050	5.17; 5.68	0.37; 0.41
		Trilex Flowable	Trifloxystrobin	0.0126	12.91; 14.21	0.93; 1.03
	Fungicide Only	Vibrance	Sedaxane	0.0125	12.82; 14.10	0.93; 1.02
		Maxim Quattro	Fludioxonil	0.0063	6.46; 7.11	0.47; 0.51
			Mefenoxam	0.0050	5.13; 5.64	0.37; 0.41
			Azoxystrobin	0.0025	2.56; 2.82	0.19; 0.20
			Thiabendazole	0.0501	51.37; 56.51	3.71; 4.09

Table A3. Sampling timeline for the Solvita field test at Beltsville (BT) and Queenstown (QT) in full-season (FS) soybean, winter wheat, double-cropped (DC) soybean and corn.

Crop	Growth Stage/Date	Planting		Sample 1		Sample 2		Sample 3	
		BV	QT	BV	QT	BV	QT	BV	QT
2015 FS Soybean	Stage	-		VC-V2		V5		R3	
	Date	5/14	5/26	6/3	6/12	6/23	7/1	8/3	8/14
2015-2016 Winter Wheat	Stage (Feekes)	-		5-6		9-10		11	
	Date	10/26	10/27	3/2	3/7	4/13	4/14	5/26	5/25
2016 DC Soybean	Stage	-		V1-V3		R1		R3	
	Date	7/8	7/8	7/28	7/26	8/18	8/17	9/6	9/8
2017 Corn	Stage	-		V3-V4		V10-V12		R3	
	Date	5/4	5/8	5/30	5/31	6/23	6/21	7/24	7/25

Table A4. Timeline for crop and arthropod sampling in 2015 full-season soybean. The two dates represent the sampling date at Beltsville (BV) and Queenstown (QT), respectively. Soybean was planted on 5/14 at Beltsville and 5/26 at Queenstown and harvested on 10/22 at both sites.

Sample Type	Growth Stage (BV, QT)		
	VC-V2	V5	R3
Stand Count	5/28, 6/5		
Height		6/23, 7/1	
Visual Count		6/23, 7/1	
Sticky Card	6/2, 6/12	6/24, 7/1	8/3, 8/14
Pitfall Trap	6/2, 6/12	6/24, 7/1	8/3, 8/14
Litter Extraction	6/2, 6/12	6/26, 7/1	8/3, 8/14
Sweep Net			8/7, 8/14

Table A5. Timeline for crop and arthropod sampling in 2015–2016 winter wheat. October to December dates are from 2015 while March to June dates are from 2016. The two dates represent the sampling date at Beltsville (BV) and Queenstown (QT), respectively. Growth stages were measured using the Feekes scale. Wheat was planted on 10/26 at Beltsville and 10/27 at Queenstown and harvested on 6/30 at Beltsville and 6/29 at Queenstown. Two sets of dates in a single cell indicate that sampling occurred twice during that growth stage.

Sample Type	Growth Stage (BV, QT)					
	Stage 1	Stage 2	Stage 4	Stage 5-6	Stage 9-10	Stage 11
Stand Count	11/11, 11/11					
Height		12/4, 12/4				
NDVI	11/11, 11,11	12/4, 12/4 12/16, 12/16	3/2, 3/7			
Tiller Count		12/16, 12/16		4/15, 4/14		
Visual Count		12/4, 12/4 12/16, 12/16		4/15, 4/14	5/19, 5/16	6/10, 6/7
Sticky Card				4/28, 4/25	5/26, 5/25	6/17, 6/14
Pitfall Trap				4/28, 4/25	5/26, 5/25	6/17, 6/14
Litter Extraction				4/28, 4/25	5/26, 5/25	6/17, 6/14

Table A6. Timeline for crop and arthropod sampling in 2016 double-cropped soybean. The two dates represent the sampling date at Beltsville (BV) and Queenstown (QT), respectively. Soybean was planted on 7/8 and harvested on 11/2 at both sites.

Sample Type	Growth Stage (BV, QT)			
	VE-VC	V1-V3	R1	R3
Stand Count	7/21, 7/19			
Height			8/25, 8/23	
Visual Count		7/28, 7/26	8/25, 8/23	
Sticky Card		7/28, 7/26	8/25, 8/23	9/12, 9/13
Pitfall Trap		7/28, 7/26	8/25, 8/23	9/12, 9/13
Litter Extraction		7/28, 7/26	8/25, 8/23	9/12, 9/13
Sweep Net			8/25, 8/23	

Table A7. Timeline for crop and arthropod sampling in 2017 corn. The two dates represent the sampling date at Beltsville (BV) and Queenstown (QT), respectively. Corn was planted on 5/4 at Beltsville and 5/8 at Queenstown and harvested on 10/5 at Beltsville and 9/27 at Queenstown.

Sample Type	Growth Stage (BV, QT)				
	V3-V4	V7	V10-V12	R1	R3
Stand Count	5/22, 5/24				
Height	5/22, 5/24				
Visual Count		6/6, 6/7		7/10, 7/11	8/4, 8/9
Sticky Card	5/29, 5/31		6/30, 6/28		8/1, 8/3
Pitfall Trap	5/29, 5/31		6/30, 6/28		8/1, 8/3
Litter Extraction	5/29, 5/31		6/30, 6/28		8/1, 8/3

Appendix B: Supplementary Results for Chapters 1

Table B1. Mean relative abundance of the 20 most abundant classes within the prokaryotic community in full-season soybean, combined across Beltsville and Queenstown sites. CON = Control; FUN = Fungicide; IMI = Imidacloprid; THI = Thiamethoxam.

Phylum	Class	Relative Abundance (%) ± Standard Error			
		CON	FUN	IMI	THI
Acidobacteria	Acidobacteriia	5.55 ± 1.87	3.76 ± 0.87	4.77 ± 1.52	4.50 ± 1.56
	Blastocatellia (Sbgrp_4)	4.48 ± 0.95	5.22 ± 0.79	5.10 ± 0.92	4.68 ± 0.69
	Subgroup_6	7.28 ± 1.21	8.42 ± 0.77	8.44 ± 1.33	8.63 ± 1.29
Actinobacteria	Actinobacteria	4.54 ± 0.30	4.80 ± 0.34	4.35 ± 0.69	4.65 ± 0.31
	Thermoleophilia	3.50 ± 0.35	3.42 ± 0.23	3.78 ± 0.29	3.13 ± 0.38
Bacteroidetes	Bacteroidia	2.16 ± 0.28	2.32 ± 0.21	2.13 ± 0.26	2.19 ± 0.37
Chloroflexi	AD3	1.55 ± 0.41	1.71 ± 0.46	1.73 ± 0.43	1.60 ± 0.41
	Chloroflexia	1.74 ± 0.27	1.93 ± 0.31	1.67 ± 0.36	1.64 ± 0.27
	KD4-96	3.34 ± 0.76	3.44 ± 0.39	3.53 ± 0.51	3.35 ± 0.51
	Ktedonobacteria	5.47 ± 2.17	3.70 ± 1.48	3.92 ± 1.65	5.92 ± 2.46
Cyanobacteria	Oxyphotobacteria	2.39 ± 0.66	2.70 ± 0.46	1.34 ± 0.34	2.74 ± 0.64
Firmicutes	Bacilli	3.31 ± 0.39	3.15 ± 0.26	3.48 ± 0.39	2.74 ± 0.31
Gemmatimonadetes	Gemmatimonadetes	1.78 ± 0.09	1.96 ± 0.15	2.25 ± 0.13	1.75 ± 0.16
Planctomycetes	Phycisphaerae	2.65 ± 0.19	2.65 ± 0.13	3.02 ± 0.27	2.85 ± 0.21
	Planctomycetacia	6.26 ± 0.31	6.70 ± 0.40	5.87 ± 0.47	6.25 ± 0.3
Proteobacteria	Alphaproteobacteria	7.99 ± 0.39	8.28 ± 0.31	8.34 ± 0.42	7.93 ± 0.67
	Deltaproteobacteria	2.95 ± 0.18	2.71 ± 0.08	2.91 ± 0.20	2.46 ± 0.31
	Gammaproteobacteria	6.18 ± 0.25	6.00 ± 0.30	6.23 ± 0.23	5.68 ± 0.41
Thaumarchaeota	Nitrososphaeria	7.96 ± 0.47	7.06 ± 0.52	7.33 ± 0.54	7.95 ± 0.88
Verrucomicrobia	Verrucomicrobiae	6.58 ± 0.40	7.03 ± 0.62	6.58 ± 0.49	6.69 ± 0.55

Table B2. Mean relative abundance of the 20 most abundant classes within the prokaryotic community in winter wheat, combined across Beltsville and Queenstown sites. CON = Control; FUN = Fungicide; IMI = Imidacloprid; THI = Thiamethoxam.

Phylum	Class	Relative Abundance (%) ± Standard Error			
		CON	FUN	IMI	THI
Acidobacteria	Acidobacteriia	6.14 ± 1.97	4.97 ± 1.41	5.36 ± 1.73	6.08 ± 2.35
	Blastocatellia (Sbgrp_4)	9.19 ± 1.41	8.42 ± 1.48	8.03 ± 0.95	6.30 ± 1.00
	Holophagae	1.58 ± 0.31	1.41 ± 0.12	1.33 ± 0.08	1.21 ± 0.15
	Subgroup_6	7.77 ± 1.25	7.94 ± 0.86	8.28 ± 1.21	7.65 ± 1.20
Actinobacteria	Actinobacteria	3.10 ± 0.26	3.39 ± 0.29	3.29 ± 0.29	3.25 ± 0.49
	Thermoleophilia	2.63 ± 0.19	2.79 ± 0.27	2.72 ± 0.20	2.74 ± 0.26
Bacteroidetes	Bacteroidia	3.39 ± 0.26	3.00 ± 0.25	3.92 ± 0.42	3.13 ± 0.68
Chloroflexi	KD4-96	2.14 ± 0.27	2.46 ± 0.26	2.52 ± 0.41	2.29 ± 0.40
Firmicutes	Ktedonobacteria	3.51 ± 1.59	2.94 ± 1.11	2.72 ± 1.17	3.30 ± 1.50
	Bacilli	1.42 ± 0.17	1.89 ± 0.27	1.50 ± 0.21	1.93 ± 0.45
Gemmatimonadetes	Gemmatimonadetes	1.69 ± 0.26	1.81 ± 0.11	1.99 ± 0.13	1.67 ± 0.23
Patescibacteria	Saccharimonadia	1.34 ± 0.24	1.39 ± 0.26	1.34 ± 0.23	1.29 ± 0.20
Planctomycetes	Phycisphaerae	3.03 ± 0.34	2.86 ± 0.22	2.88 ± 0.19	2.63 ± 0.34
	Planctomycetacia	7.37 ± 0.48	7.55 ± 0.27	7.35 ± 0.69	6.26 ± 0.62
Proteobacteria	Alphaproteobacteria	7.62 ± 0.32	7.75 ± 0.37	7.91 ± 0.55	12.33 ± 3.69
	Deltaproteobacteria	2.21 ± 0.15	2.47 ± 0.13	2.51 ± 0.15	2.63 ± 0.23
	Gammaproteobacteria	6.72 ± 0.44	6.73 ± 0.31	6.64 ± 0.46	8.05 ± 1.26
Rokubacteria	NC10	1.59 ± 0.31	1.74 ± 0.27	1.61 ± 0.28	1.73 ± 0.38
Thaumarchaeota	Nitrososphaeria	7.12 ± 0.86	6.92 ± 0.94	6.61 ± 0.88	5.95 ± 0.64
Verrucomicrobia	Verrucomicrobiae	8.47 ± 0.62	8.44 ± 0.30	9.03 ± 0.63	7.56 ± 0.98

Table B3. Mean relative abundance of the 20 most abundant classes within the prokaryotic community in corn, combined across Beltsville and Queenstown sites. CON = Control; FUN = Fungicide; IMI = Imidacloprid; THI = Thiamethoxam.

Phylum	Class	Relative Abundance (%) ± Standard Error			
		CON	FUN	IMI	THI
Acidobacteria	Acidobacteriia	5.51 ± 1.66	4.92 ± 1.71	6.08 ± 1.62	7.27 ± 2.27
	Blastocatellia (Sbgrp_4)	10.57 ± 1.58	10.27 ± 2.47	7.74 ± 1.37	7.88 ± 1.40
	Subgroup_6	7.26 ± 1.02	6.77 ± 1.03	7.42 ± 1.17	6.43 ± 1.00
Actinobacteria	Actinobacteria	3.24 ± 0.22	3.65 ± 0.24	2.92 ± 0.23	3.05 ± 0.20
	Thermoleophilia	2.56 ± 0.13	2.97 ± 0.26	2.87 ± 0.15	2.50 ± 0.18
Bacteroidetes	Bacteroidia	2.94 ± 0.24	3.16 ± 0.41	2.94 ± 0.48	2.90 ± 0.42
Chloroflexi	AD3	1.16 ± 0.40	1.38 ± 0.49	1.94 ± 0.50	1.24 ± 0.40
	Chloroflexia	1.22 ± 0.16	1.08 ± 0.20	1.00 ± 0.19	1.22 ± 0.17
	KD4-96	2.28 ± 0.40	2.26 ± 0.38	2.44 ± 0.30	1.90 ± 0.34
	Ktedonobacteria	4.16 ± 1.69	3.64 ± 1.77	3.63 ± 1.37	4.85 ± 1.82
Firmicutes	Bacilli	1.76 ± 0.16	2.30 ± 0.38	1.60 ± 0.25	1.61 ± 0.18
Gemmatimonadetes	Gemmatimonadetes	1.66 ± 0.20	1.48 ± 0.26	1.79 ± 0.20	1.61 ± 0.15
Planctomycetes	Phycisphaerae	2.86 ± 0.16	2.52 ± 0.10	2.83 ± 0.27	3.09 ± 0.19
	Planctomycetacia	6.90 ± 0.35	6.44 ± 0.42	6.33 ± 0.48	6.82 ± 0.40
Proteobacteria	Alphaproteobacteria	6.84 ± 0.35	7.61 ± 0.56	7.94 ± 0.59	7.44 ± 0.30
	Deltaproteobacteria	2.50 ± 0.09	2.53 ± 0.19	2.69 ± 0.32	2.62 ± 0.13
	Gammaproteobacteria	5.94 ± 0.19	6.45 ± 0.52	7.02 ± 0.44	6.43 ± 0.35
Rokubacteria	NC10	1.48 ± 0.31	1.55 ± 0.27	1.55 ± 0.26	1.47 ± 0.31
Thaumarchaeota	Nitrososphaeria	9.15 ± 0.52	8.05 ± 1.02	7.28 ± 1.32	9.96 ± 0.93
Verrucomicrobia	Verrucomicrobiae	6.82 ± 0.48	8.04 ± 0.41	9.16 ± 0.73	7.03 ± 0.57

Appendix C: Supplementary methods for Chapter 2

For all arthropod and crop sampling, the outer 1 m of the plot was excluded on all sides to avoid edge effects.

Residue analysis

Winter annual flower collection

In 2016, flower buds were collected at both sites on March 15 (common henbit *Lamium amplexicaule* L. at Beltsville and common chickweed *Stellaria media* L. Vill. at Queenstown). To collect sufficient material for analysis, samples from two replicates of the same treatment (Column 1+ Column 2; Column 3+ Column 4) (Fig. A1) were combined at both sites. In 2017, common henbit was collected at Beltsville on March 13 and common chickweed at Queenstown on April 12, with samples combined as described previously. At Beltsville in 2017, we also collected common chickweed on April 10, and were able to collect enough material for analysis from each individual plot. Flower buds (3g) were stored at -80°C in falcon tubes until they were sent for neonicotinoid residue analysis.

Soil collection

On each sampling date (see Table 2.1), we took 30 random soil cores (1.9 cm diameter and 12 cm depth) from within and between rows in each plot and mixed them into a single homogenized sample. Due to space restrictions, only a small subsample of each homogenized sample was collected and stored at -80ºC

for subsequent analysis. Because these samples were also used for another experiment, insufficient soil was available to analyze residues within each plot individually. In corn and soybean, soil from two replicates of the same treatment (Col 1+ Col 2; Col 3+ Col 4) (Fig. A1) were combined, and in wheat, soil from all four replicates was combined.

Arthropod sampling

Pitfall traps

Pitfall traps consisted of two stacked 360ml plastic cups buried so that the opening was level with the soil surface. The inner cup contained approximately 60ml of ethylene glycol and was sheltered from weather and wildlife interference with a 30cm square black plastic cover supported by three carriage bolts held approximately 5cm above the soil surface. In 2015 and 2016, we used 50% ethylene glycol, but switched to 100% in 2017 because water from rainfall further diluted the ethylene glycol allowing samples to degrade. Three pitfall traps were set up between the rows within each plot in an evenly spaced diagonal line. On each sampling date, pitfall traps were left in place for one week. After collection, samples were vacuum filtered and transferred into alcohol that was dyed with food coloring to increase visibility of soft bodied arthropods. Data from the three pitfall subsamples per plot were averaged before analysis.

Litter extraction

Litter samples were collected by using masonry trowels to gather all the litter and the soil rhizosphere to a depth of about 1cm from a circular (full-season

soybean and corn) or rectangular (double-cropped soybean and wheat) 0.09m^2 area. Four sets of litter were collected per plot, and the litter was combined into two subsamples for extraction with Berlese funnels. Berlese funnels were constructed using a 19 L painter's bucket with the bottom replaced by a layer of wide mesh, placed over a metal funnel with a cup of 70% ethanol placed underneath. The alcohol was dyed with food coloring to aid in visualization of soft bodied arthropods. 30W incandescent bulbs were used as the heat source during extraction, and samples were placed in the funnel for up to 48 hours, until the soil was completely dry. Data from the two subsamples were averaged before analysis.

Sticky cards

One sided yellow sticky cards (7.62 cm x 12.7 cm) were placed horizontally at a height of 8cm. Three sticky cards were placed in an evenly spaced diagonal line between rows in each plot. On each sampling date, sticky cards were deployed for one week. Data from the three sticky card subsamples was averaged before analysis.

Visual inspection

In soybean, leaf trifoliate inspections were conducted on the newest trifoliate from 10 randomly selected plants (V5 stage in 2015, V2 and R1 in 2016). For soybean analyses, data from the 10 plants was summed because of low arthropod numbers. In wheat, we scouted for pests twice in the winter and three times in the spring and summer. Two subsamples of two linear yards (1.8 m) were

scouted per plot by throwing out a yardstick and counting pests on the plants on either side. Data from the wheat subsamples was averaged for analysis. Earlier in the season, the whole plant was visually examined, but in the last set of samples only the flag leaf and head were included. In corn, foliar arthropods were recorded through visual counts at three time points. At V7, five adjacent plants were scouted from four subsamples taken at randomly selected points, and the whole plant was examined. Data from the subsamples was averaged for analysis. At R1, 10 plants were randomly selected from the middle six rows of each plot and were destructively sampled, with all the leaves stripped off the plant and examined. Insects that may have been present within the stem were not included. At R3-R4, plants were selected the same way as R1, but only the ears were sampled. For corn analyses, the sum of data from all plants was used, due to low arthropod abundance.

Crop sampling

Stand density

In all crops, stand density was measured by throwing out a meter stick at randomly selected points and counting the number of plants along it, and data from subsamples was averaged for analysis. In soybean, stand density was measured at emergence in 2015 and 2016. The meter stick was thrown four times and the number of plants on either side was counted. In wheat, stand density was measured one-week post emergence. The meter stick was thrown four times and the number of seedlings on one side of the stick was counted. In corn, stand

density was measured shortly after emergence with four throws where the number of plants on both sides of the stick was counted.

Plant height

In soybean, plant height was measured concurrently with trifoliate sampling, using the same ten plants per plot, extended to their full height. Data from the 10 plants was averaged for analysis. In wheat, plant height was measured six weeks post planting by randomly throwing out meter sticks at four points and measuring the heights of five randomly selected plants along the meter stick. The heights of the five plants in each subsample were averaged, and then the data from the four subsamples was averaged again. In corn, height was measured along with stand density by measuring the first five plants on one side of the meter stick while fully extended. The height from the five plants was averaged, and then the mean heights from the four subsamples were averaged again.

Tiller counts

In wheat, three one foot (30.4 cm) sections of plants were selected randomly and dug up at Feekes stages 2 and 6 in each plot. The plants from the three sections were combined and brought back to the lab to count the number of tillers per three row feet.

Normalized difference vegetation index (NDVI)

NDVI is a measure of photosynthetic activity that is calculated using the variation in reflectance of light by different surfaces. These measurements were taken three times in the winter and once in the spring using a Crop Circle optical sensor (Holland Scientific, Lincoln, Nebraska, USA). Measurements were taken

by walking the length of each plot between the two center rows, while holding the sensor out at shoulder height.

Yield

Yield was measured directly by either a five- or ten-foot combine harvester in all cases except wheat and double-cropped soybean at Beltsville, where the harvested grain was transferred to a weigh wagon. In full-season soybean, the whole plot was harvested at both sites through three passes of a 10-foot combine harvester. In wheat, half of each plot was harvested through three passes of a 5-foot combine harvester through the center of the plot. The wheat yield data from Beltsville was more variable due to a horseweed *Erigeron canadensis* outbreak throughout the field. In double-cropped soybean, whole plots were harvested at Beltsville (three 10-foot passes) and half of each plot was harvested at Queenstown (three 5-foot passes in the center of the plot). In corn, half of each plot was harvested at both sites (three 5-foot passes in the center of the plot). The data was corrected to the appropriate moisture content for each crop (13% for soybean, 14% for wheat, and 15.5% for corn) and converted to kilograms per hectare for analysis. To calculate corrected moisture content, sample moisture content was measured separately within a day of harvest using a GAC 2100 grain tester (Dickey-john Corporation, Auburn, IL, USA), in all cases except for full season soybean at Queenstown, where it was measured directly by the combine harvester.

Appendix D: Supplementary results for Chapter 2

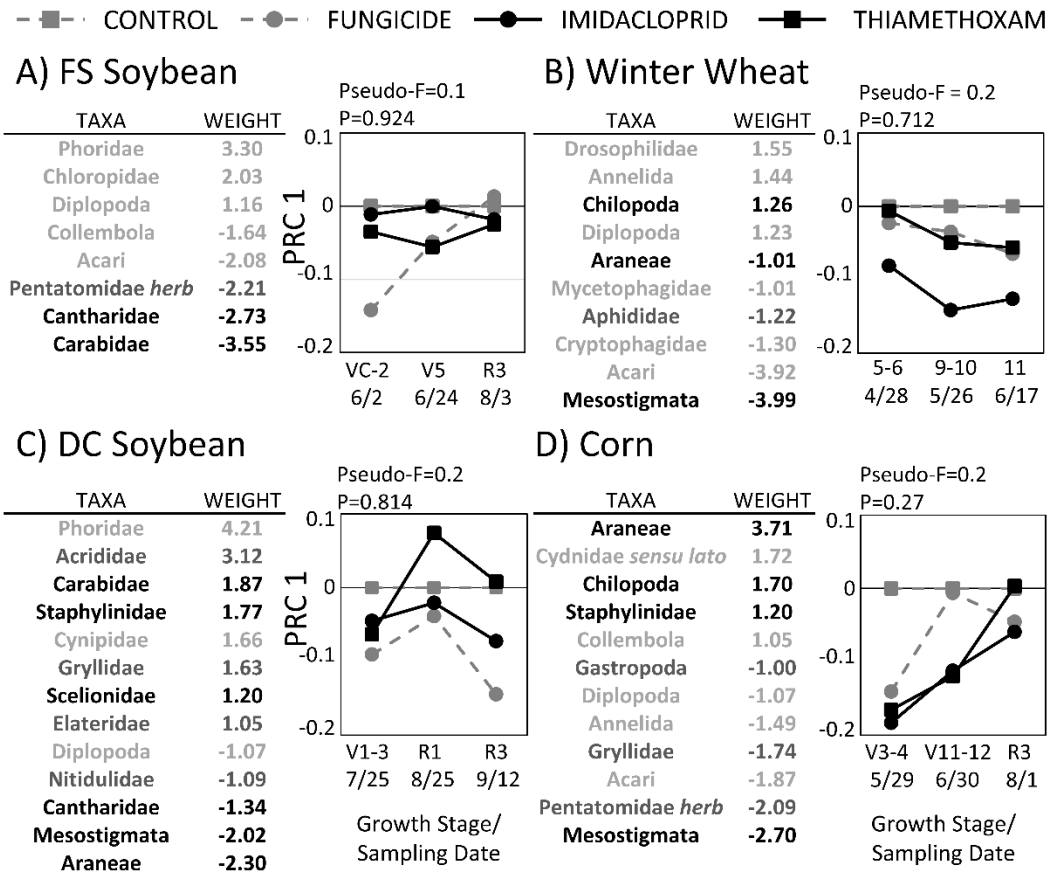

Fig. D1. Principal Response Curve analysis of pitfall trap data for all crops. For each crop, date*treatment served as the explanatory variable, with date and site*column used as covariates. Subsamples were averaged for each replicate, and only taxa with overall means greater than one were included. A Monte-Carlo permutation procedure with N=499 was used to calculate the Pseudo-F statistic. Taxon weights indicate which groups most contributed to the observed community response. Higher positive weights indicate that taxon abundances in the treated plots followed the trend depicted by the response curve, whereas higher negative values indicate the opposite. Taxon weights between -1 and 1 were excluded due to weak response or lack of correlation with the trends shown. Beneficial groups are shown in black, herbivore pests in dark grey, and other groups in light grey. Acari refers specifically to the mite order Oribatida and the family Tarsonemidae. FS = full-season, DC = double-cropped.

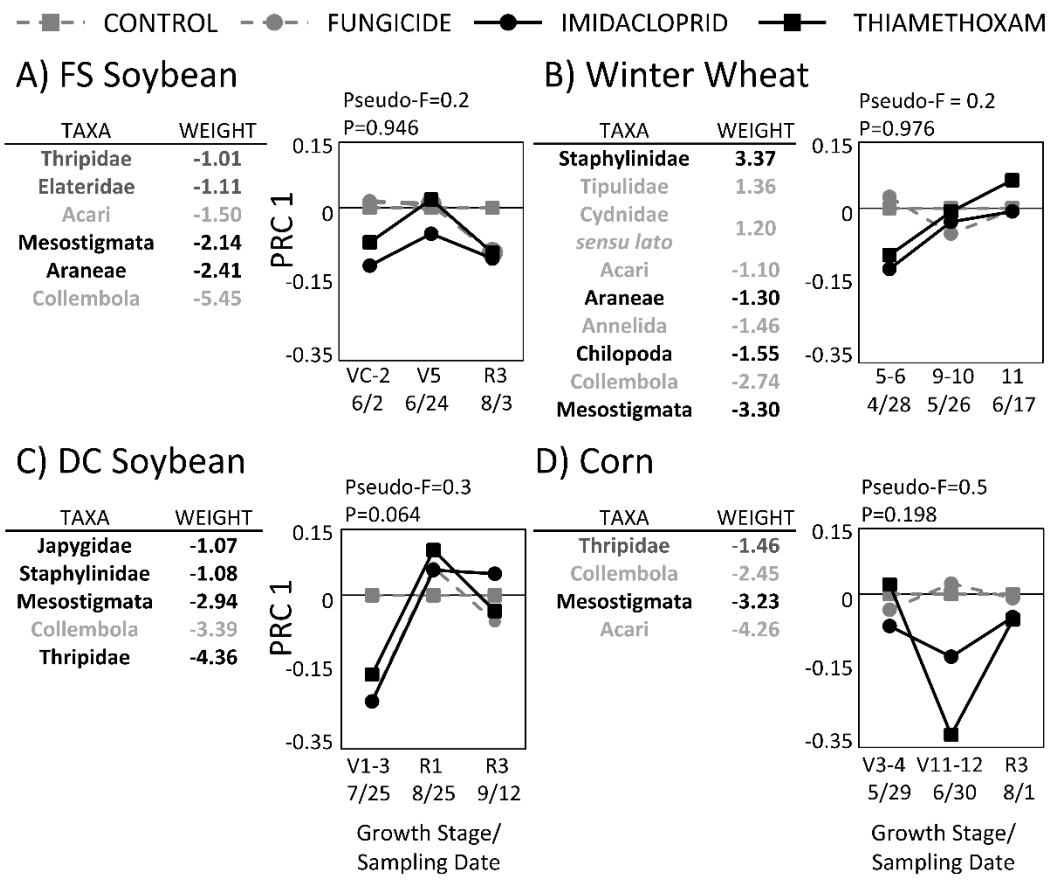

Fig. D2. Principal Response Curve analysis of litter extraction data for all crops. For each crop, date*treatment served as the explanatory variable, with date and site*column used as covariates. Subsamples were averaged for each replicate, and only taxa with overall means greater than one were included. A Monte-Carlo permutation procedure with N=499 was used to calculate the Pseudo-F statistic. Taxon weights indicate which groups most contributed to the observed community response. Higher positive weights indicate that taxon abundances in the treated plots followed the trend depicted by the response curve, whereas higher negative values indicate the opposite. Taxon weights between -1 and 1 were excluded due to weak response or lack of correlation with the trends shown. Beneficial groups are shown in black, herbivore pests in dark grey, and other groups in light grey. Acari refers specifically to the mite order Oribatida and the family Tarsonemidae. FS = full-season, DC = double-cropped.

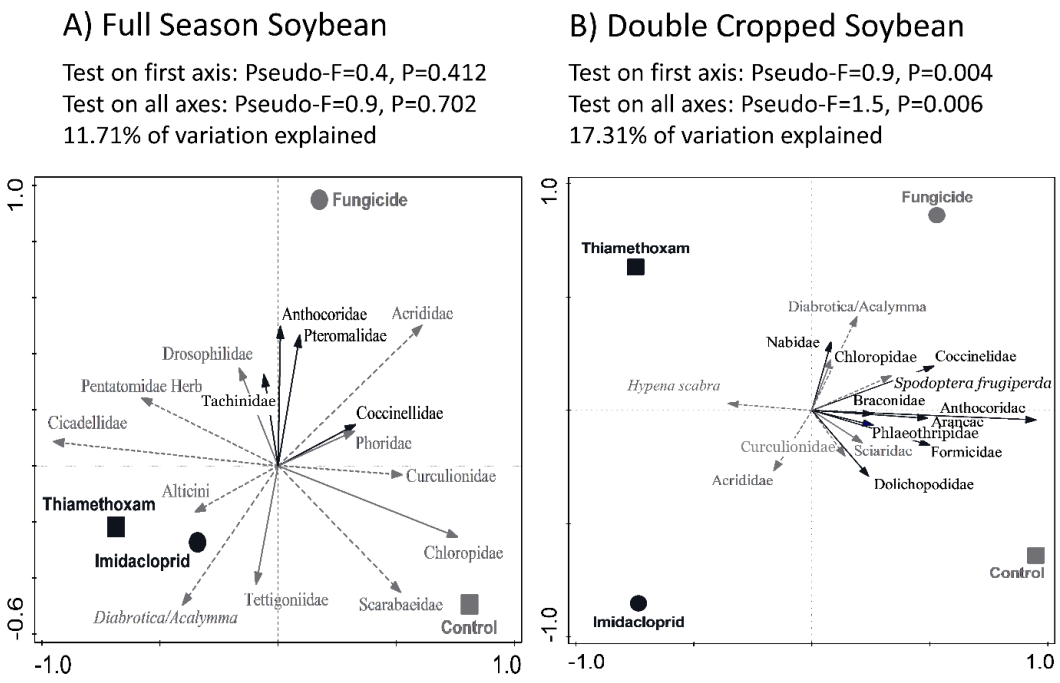

Fig. D3. Redundancy analysis of sweep net data from A) 2015 full-season soybean and B) 2016 double-cropped soybean. Treatment served as the explanatory variable and the site*column interaction was used as a covariate. The horizontal axis is the first axis. Only the 15 taxa that most contributed are shown. A Monte-Carlo permutation procedure with N=499 was used to calculate a Pseudo-F statistic. Beneficial groups are shown in black, economic pests in grey with dotted lines, and other groups in grey with solid lines.

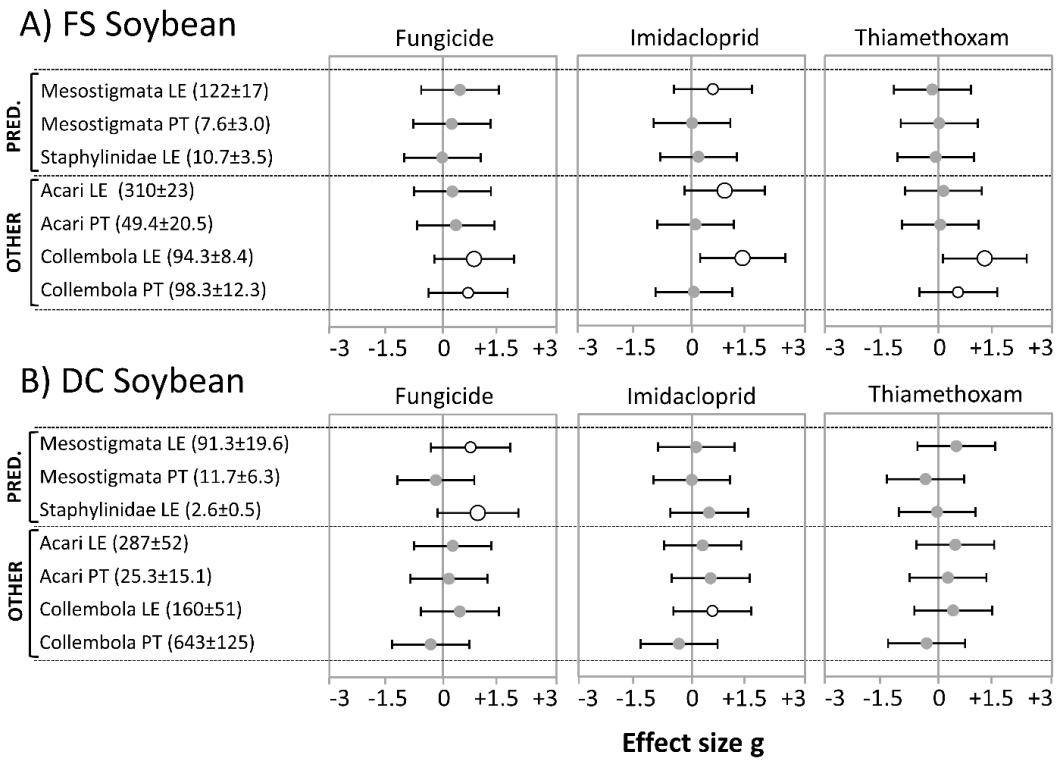

Fig. D4. Comparisons of arthropod abundances in the fungicide, imidacloprid and thiamethoxam treatments to the control through Analysis of Variance followed by Hedge's g effect test (±95% confidence intervals) for litter (LE) and pitfall trap (PT) taxa in full-season (FS) and double-cropped (DC) soybean. The values in parentheses indicate mean taxon abundance ± standard error for the control. The ANOVA treatment effect was P>0.05 for all soil taxa. Small grey circles represent a negligible or small effect size (between -0.5 and 0.5), small and large black circles represent medium (between -0.5 and -0.8) and large (less than -0.8) negative effect sizes, respectively, while small and large white circles represent medium (between 0.5 and 0.8) and large (greater than 0.8) positive effect sizes, respectively. Acari refers specifically to the mite order Oribatida and the family Tarsonemidae.

Table D1. Taxa collected through pitfall traps that comprised at least 1% of total abundance in one or more crops Data from subsamples was averaged and data was totaled across locations and sampling dates. # Organisms Total includes ants (Formicidae) and insects from the orders Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera that could not be identified beyond order, which were excluded from analysis. FS = full-season, DC = double-cropped.

Guild	Taxa		% Total			
			2015 FS Soybean	2016 DC Soybean	2015-2016 Wheat	2017 Corn
Predator	Chilopoda		0.8	0.4	1.0	0.6
	Araneae		9.4	2.7	7.9	3.9
	Mesostigmata		2.5	1.2	5.7	1.2
	Coleoptera	Staphylinidae	4.3	3.9	3.2	2.2
		Carabidae	1.6	0.6	1.3	1.5
		Cantharidae	5.2	0.1	<0.1	<0.1
Parasitoid	Hymenoptera	Scelionidae	0.8	1.0	0.5	0.8
Pest	Gastropoda		0.9	<0.1	0.8	3.7
	Hemiptera	Pentatomidae *herbivorous*	2.1	0.1	0.1	2.2
	Odonata	Gryllidae	2.5	4.5	0.9	5.7
Other	Annelida		0.3	<0.1	0.7	1.1
	Diplopoda		0.9	0.1	1.2	0.5
	Acari	Tarsonemidae & Oribatida	16.9	5.1	4.4	11.1
	Collembola		32.1	68.4	53.8	48.9
	Coleoptera	Mycetophagidae	0.3	0.3	1.9	0.2
		Cryptophagidae	<0.1	0.1	2.1	0.2
	Diptera	Chloropidae	3.8	0.1	0.1	0.4
		Sciaridae	0.6	0.5	2.0	0.7
		Phoridae	0.7	1.1	1.5	0.2
	Hymenoptera	Formicidae	10.2	5.7	3.9	11.7
# Organisms Analyzed			**9750**	**24760**	**9438**	**9448**
# Organisms Total			**11051**	**26552**	**10030**	**10772**

Table D2. Taxa collected through litter extraction that comprised at least 1% of total abundance in one or more crops. Data from subsamples was averaged and data was totaled across locations and sampling dates. # Organisms Total includes ants (Formicidae) and insects from the orders Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera that could not be identified beyond order, which were excluded from analysis. FS = full-season, DC = double-cropped.

Guild	Taxa		% Total			
			2015 FS Soybean	2016 DC Soybean	2015-2016 Wheat	2017 Corn
Predator	Araneae		1.2	1.6	1.5	2.1
	Mesostigmata		19.6	15.0	18.8	8.1
	Coleoptera	Staphylinidae	1.5	0.5	0.9	1.6
Pest	Thysanoptera	Thripidae	0.7	2.5	2.4	0.8
Other	Annelida		0.7	0.4	0.5	2.3
	Diplopoda		0.5	0.1	0.7	1.5
	Acari	Tarsonemidae & Oribatida	47.5	43.8	46.4	48.9
	Collembola		20.5	29.2	20.6	18.2
	Hymenoptera	Formicidae	1.9	1.4	0.8	5.7
# Organisms Analyzed			**22112**	**23135**	**18529**	**5536**
# Organisms Total			**23305**	**24250**	**19497**	**6113**

Table D3. Taxa collected through sticky cards that comprised at least 1% of total abundance in one or more crops. Data from subsamples was averaged and data was totaled across locations and sampling dates. # Organisms Total includes ants (Formicidae) and insects from the orders Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera that could not be identified beyond order, which were excluded from analysis. Sticky cards from the first double-cropped soybean sampling date at Queenstown were misplaced, and so only Beltsville data from the first date is included. FS = full-season, DC = double-cropped.

Guild	Taxon		% Total			
			2015 FS Soybean	2016 DC Soybean	2015-2016 Wheat	2017 Corn
Predator	Coleoptera	Coccinellidae	0.1	0.3	0.5	1.3
	Hemiptera	Anthocoridae	1.2	0.9	<0.1	4.3
Parasitoid	Hymenoptera	Scelionidae	1.7	3.0	4.2	2.6
		Ceraphronidae	3.8	2.4	8.5	5.0
		Aphelinidae	3.4	1.6	7.6	1.5
		Mymaridae	1.4	3.5	2.2	4.4
		Eulophidae	0.1	0.2	3.2	0.6
		Trichogrammatidae	0.4	1.5	0.6	1.1
		Braconidae	0.3	1.0	3.5	0.6
	Diptera	Tachinidae	0.7	1.1	0.6	0.9
Pest	Coleoptera	Chrysomelidae - Alticini	0.4	0.8	0.5	2.4
	Hemiptera	Cicadellidae	7.5	16.5	8.3	15.9
		Aphididae	1.8	1.8	1.8	4.3
		Aleyrodidae	<0.1	2.0	0.4	0.7
	Thysanoptera	Thripidae	32.7	10.2	31.8	12.5
Other	Coleoptera	Phalacridae	0.1	0.1	0.9	2.7
	Diptera	Chloropidae	32.4	38.3	10.3	20.2
		Sciaridae	2.0	2.5	3.1	4.5
		Phoridae	1.0	0.9	0.5	0.1
		Cecidomyiidae	0.8	2.8	3.5	6.9
	Hymenoptera	Cynipidae	0.3	2.4	1.4	1.5
# Organisms Analyzed			**13979**	**9790**	**5273**	**5238**
# Organisms Total			**14008**	**9916**	**5313**	**5269**

Table D4. Taxa collected through sweep net sampling that comprised at least 1% of total abundance in full-season (FS) and double-cropped (DC) soybean. Data was totaled across locations. The percent total is included for each group, as well as the overall abundance for that crop. # Organisms Total includes insects from the orders Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera that could not be identified beyond order, which were excluded from analysis.

Guild	**Taxa**		**% Total**	
			2015 FS Soybean	**2016 DC Soybean**
Predator	Araneae		8.6	7.0
	Coleoptera	Coccinellidae	0.6	1.6
	Hemiptera	Anthocoridae	11.7	5.1
		Geocoridae	0.3	1.3
Parasitoid	Hymenoptera	Braconidae	0.6	2.4
		Scelionidae	1.3	0.8
Pest	Coleoptera	Chrysomelidae	1.0	0.0
		Chryosmelidae - Alticini	0.2	1.1
		Curculionidae	3.7	2.9
		Diabrotica/Acalymma spp.	1.7	1.8
		Epilachna varivestis	1.3	0.0
		Scarabaeidae	1.7	0.0
	Hemiptera	Aphididae	2.9	0.5
		Cicadellidae	3.6	5.8
		Pentatomidae *herbivorous*	2.5	0.3
	Lepidoptera	*Hypena scabra*	3.1	12.3
		Spodoptera frugiperda	0.0	4.6
	Orthoptera	Acrididae	0.0	2.4
Other	Diptera	Chloropidae	46.6	30.9
		Phoridae	0.4	1.0
		Sciaridae	0.0	1.6
	Hymenoptera	Formicidae	0.2	1.3
# Organisms Analyzed			**2320**	**1549**
# Organisms Total			**2358**	**1591**

Appendix E: Evaluating host-mediated impacts of neonicotinoid seed treatments on an aphid parasitoid *Aphidius colemani* in winter wheat

This appendix is a brief overview of an experiment that was designed to be part of Chapter 3 but could not be completed due to COVID-19 related campus closure. It includes background information, methods, and a summary of the partial data collected.

Background

In winter wheat (*Triticum aestivum*), neonicotinoid seed treatments (NSTs) greatly reduced the activity density of Aphelinid wasps in the spring (Dubey et al., 2020). Aphelinidae includes many aphid parasitoids, which play an important role in controlling cereal aphids in wheat (Pike et al., 1997; Schmidt et al., 2003). The impacts of neonicotinoids on parasitoids are less well-understood than their impact on predators (Pisa et al., 2015), and little is known with regard to the effects of neonicotinoids on parasitoids of cereal aphids.

Parasitoids may be exposed to neonicotinoids in different ways; adults can be exposed by feeding on contaminated nectar or through direct contact with treated foliage, while developing parasitoid larvae may be exposed to neonicotinoids through both ingestion and direct contact if females lay their eggs in or on a host that fed on a plant which received foliar or systemic neonicotinoid application (EASAC, 2015). Several studies have found that neonicotinoids may be acutely toxic to adult parasitoids at high doses and can have sublethal

behavioral and physiological impacts at lower doses (Frewin et al., 2014; Krischik et al., 2007; Paine et al., 2011; Prabhaker et al., 2011; Preetha et al., 2010; Stara et al., 2011; Tappert et al., 2017; Whitehorn et al., 2015). However, fewer studies have evaluated host-mediated tritrophic impacts on developing parasitoids. Two parasitoids of the tobacco budworm, *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae), varied in their host mediated responses to imidacloprid, with one parasitoid species exhibiting significantly lower parasitism rates and adult longevity, while the other was not impacted (Taylor et al. 2015). Even within a single system, impacts of neonicotinoids on parasitoids can be variable and unpredictable.

To better understand potential host-mediated impacts of NSTs on parasitoids of cereal aphids, I designed a laboratory study evaluating sub-lethal effects of imidacloprid and thiamethoxam seed treatments on *Aphidius colemani* Viereck, (Hymenoptera: Braconidae), a solitary parasitoid of aphid nymphs. *A. colemani* has been introduced in many parts of the world as a biocontrol agent, including North and South America, and attacks several economically important species of aphids including *Rhopalosiphum padi* (Elliott et al., 1994; Takada, 1998). Because it is commonly used as a biological control agent, it is commercially available and its physiology is well understood (van Steenis, 1993; Zamani et al., 2007). Imidacloprid was found to be highly toxic to *A. colemani* on direct exposure (Stara et al., 2011), so it may be susceptible to lower doses of neonicotinoids present in hosts reared on treated plants.

Materials & Methods

Insects & wheat: The methods for rearing *R. padi* and growing wheat were the same as those described in Chapter 3. A colony of *A. colemani* wasps (Hymenoptera: Braconidae) was established using wasps purchased from Evergreen Growers Supply (Clackamas, OR, USA). Species identity was confirmed by Dr. Greg Evans from the USDA National Identification Service. Wasps were reared using *R. padi* as the host on untreated wheat plants at 22°C with a 16:8 light: dark photoperiod.

Timeline: The study was originally designed to include three time points in the fall (3, 6- and 9-weeks post planting), and three in the spring/summer (24-, 27- and 30-weeks post planting). The first time point was started but was dropped partway due to contamination of experimental cages by wasps. The second and third time point were completed successfully, but the experiment had to be terminated before the spring/summer time points due to COVID-19.

Experimental Design: The experiment included six wheat treatments: control or untreated; fungicide only; fungicide + imidacloprid; fungicide + thiamethoxam; fungicide + imidacloprid positive control; fungicide + thiamethoxam positive control. Positive control treatments were included to provide a point of comparison for the insecticide treatments during later time points, when insecticides may no longer be active in the plant tissue. Five replicate plants were used for each treatment at every time point. Products and treatment rates for the fungicide only, fungicide + imidacloprid, and fungicide + thiamethoxam treatments were the same as those used in Chapter 3. For the positive control

treatments, plants of each insecticide treatment received an additional dose of active ingredient equivalent to the dose applied per seed. Active ingredients were delivered through dilutions of the soil drench products Admire Pro (imidacloprid, Bayer Crop Science) and Platinum 75 SG (thiamethoxam, Syngenta AG). The soil drench products were added to plants ~48 hours prior to the aphids for each time point.

Experimental Procedure: During each experimental time point, plants and insects were maintained at 22°C with a 16:8 light: dark photoperiod. At each time point, 75 aphid nymphs were placed on each plant (day 1) (5 plants per treatment, 30 plants total) by placing the nymphs in the soil near the base of the plant. After adding aphids, plants were caged using a mesh sleeve supported by wooden dowels. The following day (day 2), wasp mating pairs were created by pairing unmated male and female wasps that had emerged within the previous 4 days. To generate wasps for mating pairs, 200-300 aphid mummies from the wasp colony were individually placed in 1 oz plastic cups over the course of the previous week. When wasps emerged, they were sexed and given honey water (a cotton wick soaked in a 2:1 solution of honey and water by volume was placed in the cup). Twenty four hours after creating wasp mating pairs (day 3), wasps were added to the caged plants, by placing the cup containing the mating pair inside the cage and then removing the lid through the mesh, to prevent wasps from escaping. After 48 hours (day 5), wasps were removed from cages. If the wasps could not be found after three search attempts, they were presumed dead.

Five days after wasps were removed (day 10), we began checking the cages for aphid mummies. Cages were searched carefully, and mummies were removed and placed in individual 1 oz cups. At this stage, nearly all the aphids in the thiamethoxam positive control cages had died, and so no further measurements were recorded for that treatment. Plants were checked daily until no more mummies or parasitized aphids could be found. If no parasitized aphids or mummies were found on a plant by day 15, it was marked as infertile and excluded from analyses.

Five days after we began checking cages for mummies (day 15), we began checking individual mummies for wasp emergence. When wasps emerged, they were sexed and given honey water within 24 hours and honey water was replenished as needed. Within 72 hours of emergence, wasp body length was measured using a dissecting microscope equipped with a reticule (KR-207, Klarmann Rulings, Litchfield, NH, USA). During measurement, wasps were anesthetized with carbon dioxide and their bodies were straightened out for consistent measurement. After measurement, wasps were returned to individual cups and checked daily until they died. Wasps that had not emerged within 2 weeks of the mummy being found were marked as unemerged.

The following wasp response parameters were measured:

Percent parasitism: Number of mummies found in each cage as a percentage of the 75 aphids originally added to the cage. This metric captures variation in the survival of aphids from different treatments in addition to wasp fecundity. The

thiamethoxam treatment had a lower percent parasitism than the others because fewer aphids survived relative to the other treatments.

Percent emergence: Number of wasps produced by each cage as a percentage of the total number of mummies found in that cage.

Percent female wasps: Number of female wasps from each cage as a percentage of the total number of emerged wasps from that cage.

Development time: Number of days from when parent wasps were added to the cages (parent wasps were added on day 3 and removed on day 5; for this measurement, we started counting from day 4) to when offspring wasps emerged from a mummy.

Adult lifespan: Number of days from when an adult wasp emerged from a mummy to when it died.

Body length: the length of the adult wasp, from the top of the head, to the tip of the abdomen.

Results

Because the experiment was terminated partway and could not be replicated, data was not analyzed. Instead, I present figures summarizing preliminary results from the 6- and 9-weeks post planting time points. Fig. E1 depicts percent parasitism, percent emergence and percent female wasps for each time point. Fig. E2 depicts development time, Fig. E3 adult lifespan, and Fig. E4 body length for male and female wasps for each time point. Due to variation in

parasitism, emergence, sex ratio and lifespan, as well as some individuals being lost or killed at different stages, the number of individuals measured for development time, adult lifespan and body length varied considerably between treatments. Therefore, the total number of individuals measured across all replicates for each treatment are included in Fig. E2-E4.

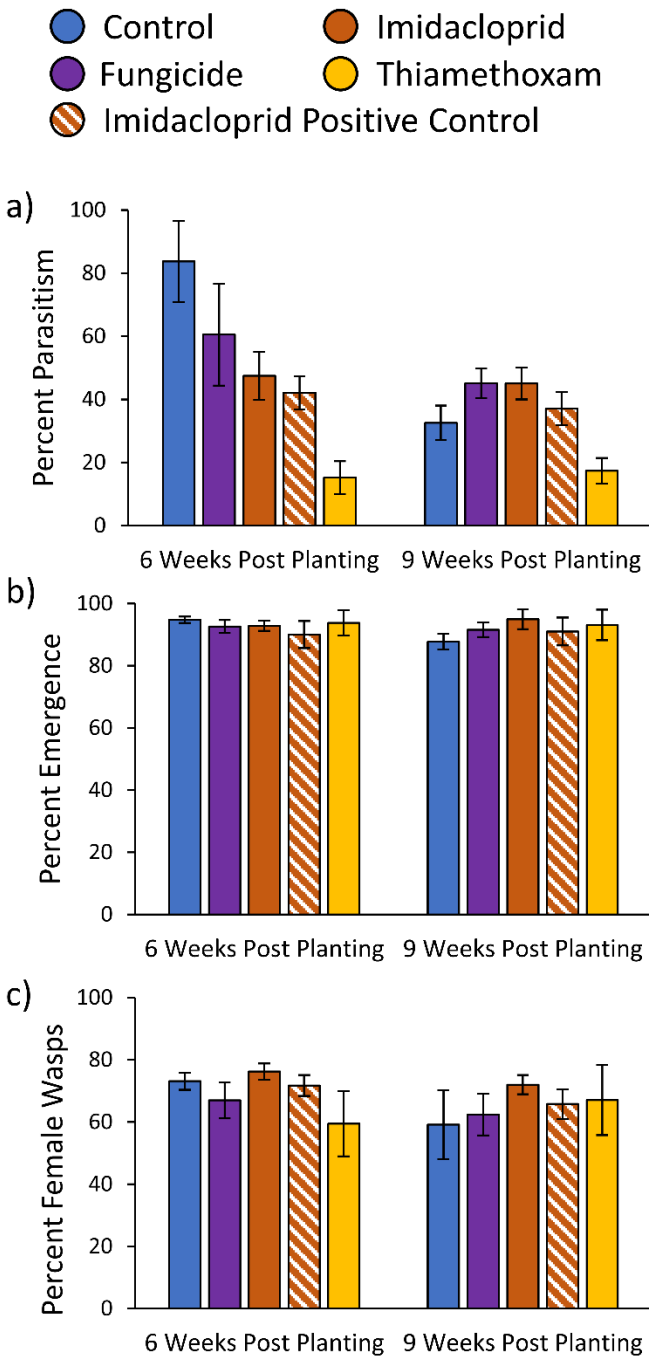

Fig. E1. Mean a) percent parasitism b) percent emergence and c) percent of female wasps measured at 6- and 9-weeks post planting. Errors bars depict standard error.

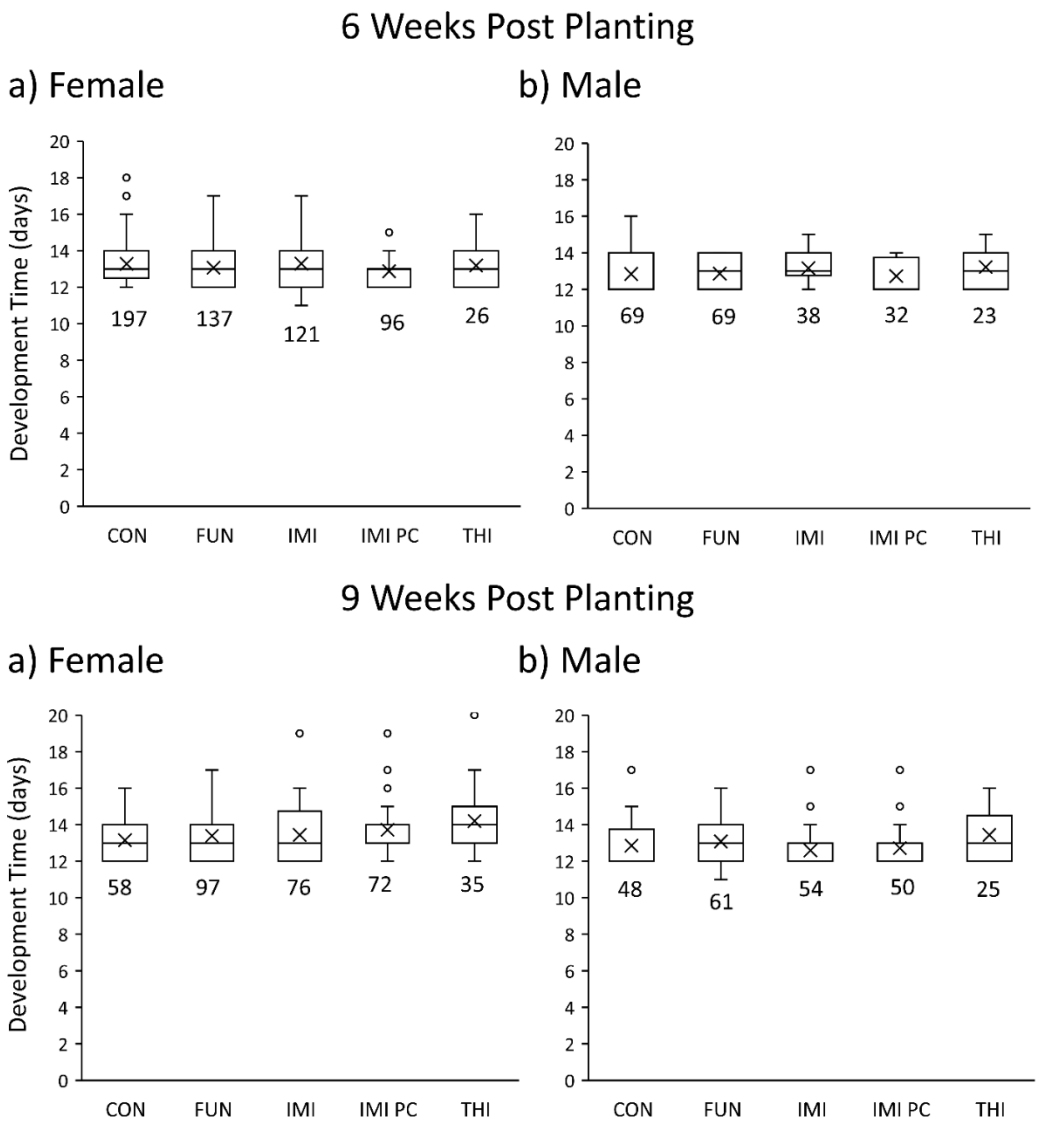

Fig. E2. Development time of a) female and b) male wasps measured at 6- and 9-weeks post planting in control (CON), fungicide (FUN), imidacloprid (IMI), imidacloprid positive control (IMI PC) and thiamethoxam (THI) treatments. Lower and upper box boundaries represent 25th and 75th percentiles, respectively, the line and cross inside the box represent the median and mean respectively, lower and upper error lines represent 10th and 90th percentiles, respectively, and circles represent data falling outside 10th and 90th percentiles. The number below each box represents the total number of individuals included in the measurement across replicates.

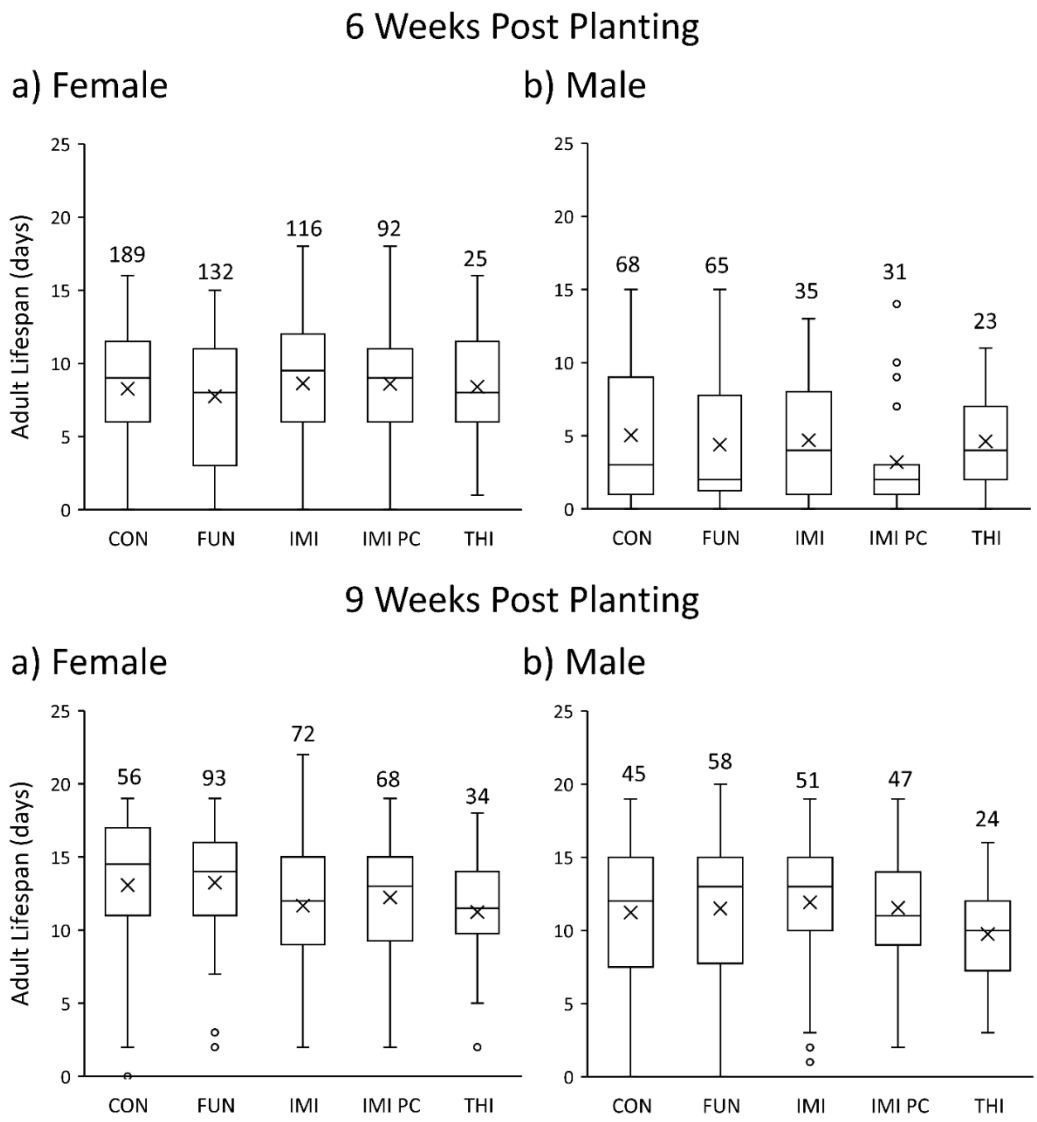

Fig. E3. Adult lifespan of a) female and b) male wasps measured at 6- and 9-weeks post planting in control (CON), fungicide (FUN), imidacloprid (IMI), imidacloprid positive control (IMI PC) and thiamethoxam (THI) treatments. Lower and upper box boundaries represent 25th and 75th percentiles, respectively, the line and cross inside the box represent the median and mean respectively, lower and upper error lines represent 10th and 90th percentiles, respectively, and circles represent data falling outside 10th and 90th percentiles. The number above each box represents the total number of individuals included in the measurement across replicates.

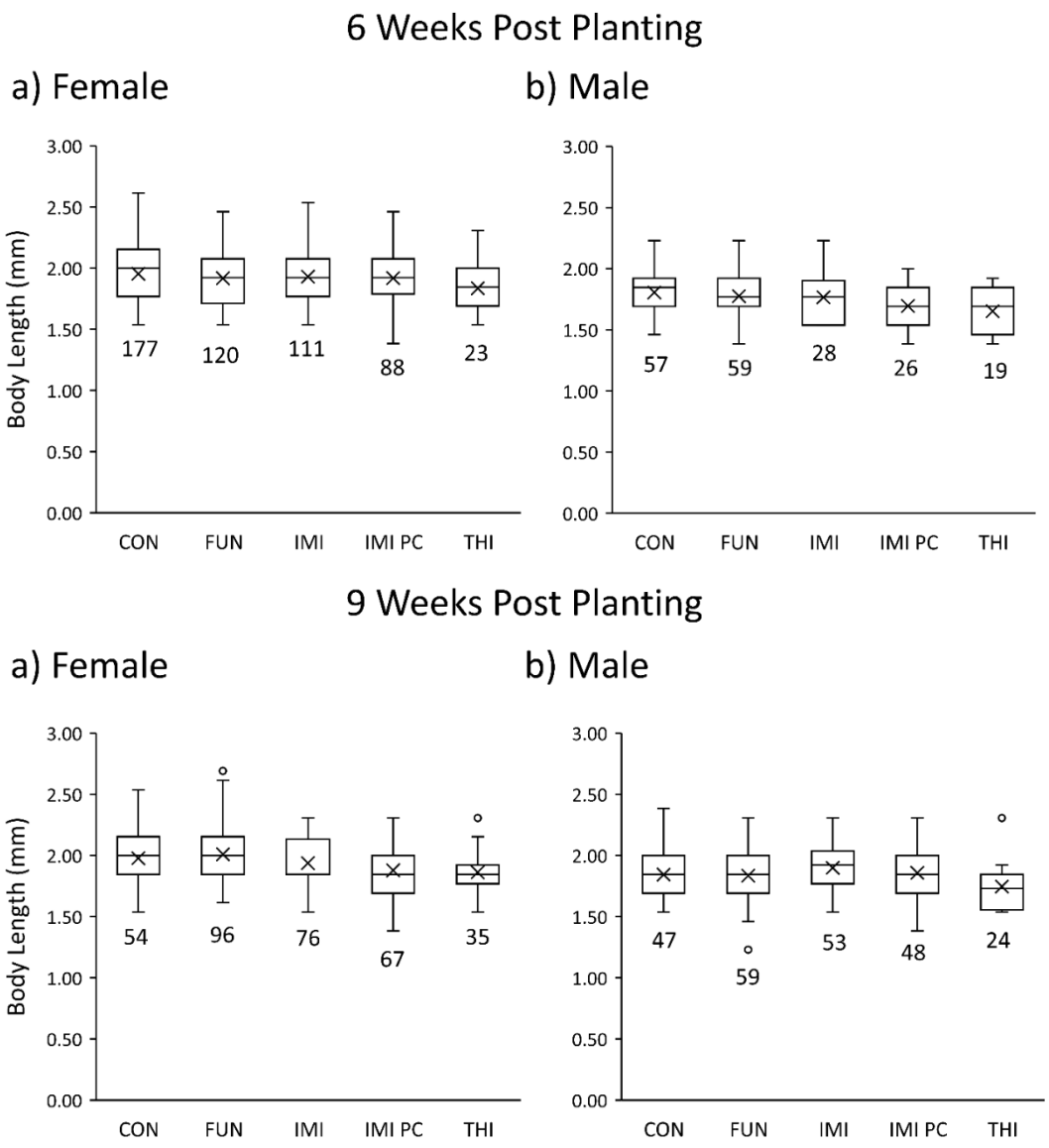

Fig. E4. Body length of a) female and b) male wasps measured at 6- and 9-weeks post planting in control (CON), fungicide (FUN), imidacloprid (IMI), imidacloprid positive control (IMI PC) and thiamethoxam (THI) treatments. Lower and upper box boundaries represent 25th and 75th percentiles, respectively, the line and cross inside the box represent the median and mean respectively, lower and upper error lines represent 10th and 90th percentiles, respectively, and circles represent data falling outside 10th and 90th percentiles. The number below each box represents the total number of individuals included in the measurement across replicates.

Appendix F: Temperature records for wheat plants used in Chapter 3

Tables F1-F4 show the temperature values recorded by data loggers placed within the two chambers in which wheat plants were grown during the pre- and post- vernalization periods. Because the loggers collected data every 30 minutes, two values per hour were averaged to obtain a single hourly temperature value. Then, hourly data were averaged across the two-week period for each temperature cycle to obtain a single set of hourly temperatures per two-week period. Data from the days on which the program was reset were excluded as I do not know the exact time at which the greenhouse staff changed the settings. The number of days over which temperature was averaged are as follows: Late October 14/10 (different for the first and second chambers); Early November 2; Late November 6; Early December 11; Late March; 13; Early April 13; Late April; 13; Early May: 13. Plants were kept at the Late October, Early November and Late November temperatures for the entire two-week period, but temperature data could not be collected over the entire period. Plants were only kept at the Early December temperature for 12 days as they had to be moved to the cold chamber slightly early for logistical reasons.

Table F1. Mean recorded temperature values for the first of the two growth chambers in which wheat plants were grown during the pre-vernalization period.

Hour (24:00)	Temperature (°C) (Mean ± Standard Error)			
	Late October	Early November	Late November	Early December
0:00	11.87 ± 0.07	9.01 ± 0.06	6.39 ± 0.01	6.39 ± 0.02
1:00	11.65 ± 0.08	9.02 ± 0.04	6.38 ± 0.01	6.42 ± 0.03
2:00	10.86 ± 0.07	8.93 ± 0.06	5.87 ± 0.01	6.37 ± 0.01
3:00	10.85 ± 0.06	8.98 ± 0.04	5.38 ± 0.01	6.38 ± 0.02
4:00	10.83 ± 0.06	8.23 ± 0.02	5.38 ± 0.01	5.89 ± 0.02
5:00	10.84 ± 0.05	7.95 ± 0.09	6.35 ± 0.02	5.38 ± 0.01
6:00	10.85 ± 0.05	7.97 ± 0.07	8.45 ± 0.02	6.46 ± 0.02
7:00	12.22 ± 0.20	7.95 ± 0.05	10.03 ± 0.03	8.70 ± 0.02
8:00	14.59 ± 0.19	11.71 ± 0.09	10.56 ± 0.03	10.54 ± 0.06
9:00	16.31 ± 0.19	12.38 ± 0.41	10.51 ± 0.02	10.58 ± 0.03
10:00	17.39 ± 0.18	13.04 ± 0.00	10.54 ± 0.03	10.57 ± 0.02
11:00	18.45 ± 0.16	14.51 ± 0.01	10.99 ± 0.02	10.56 ± 0.02
12:00	19.48 ± 0.18	15.70 ± 0.01	11.52 ± 0.03	10.56 ± 0.02
13:00	20.18 ± 0.23	16.87 ± 0.09	11.52 ± 0.02	10.57 ± 0.03
14:00	20.1 ± 0.26	17.02 ± 0.02	11.53 ± 0.02	10.57 ± 0.03
15:00	19.29 ± 0.26	17.02 ± 0.05	11.04 ± 0.05	10.07 ± 0.03
16:00	19.09 ± 0.26	16.42 ± 0.08	9.35 ± 0.02	8.35 ± 0.04
17:00	18.14 ± 0.29	15.35 ± 0.04	8.42 ± 0.04	6.9 ± 0.03
18:00	16.04 ± 0.24	12.22 ± 0.04	7.92 ± 0.05	6.38 ± 0.02
19:00	14.73 ± 0.10	10.35 ± 0.03	7.4 ± 0.02	6.44 ± 0.04
20:00	13.88 ± 0.08	10.00 ± 0.00	7.34 ± 0.02	6.38 ± 0.01
21:00	13.84 ± 0.05	9.26 ± 0.01	7.38 ± 0.03	6.38 ± 0.02
22:00	13.71 ± 0.06	9.03 ± 0.04	6.87 ± 0.02	6.41 ± 0.03
23:00	12.7 ± 0.11	9.01 ± 0.01	6.41 ± 0.01	6.38 ± 0.01

Table F2. Mean recorded temperature values for the first of the two growth chambers in which wheat plants were grown during the post-vernalization period.

Hour (24:00)	Temperature (°C) (Mean ± Standard Error)			
	Late March	Early April	Late April	Early May
0:00	5.82 ± 0.04	12.22 ± 0.03	13.32 ± 0.03	16.22 ± 0.09
1:00	5.3 ± 0.04	11.72 ± 0.05	12.79 ± 0.02	15.04 ± 0.10
2:00	4.78 ± 0.04	11.27 ± 0.03	12.42 ± 0.03	14.71 ± 0.09
3:00	4.79 ± 0.04	10.77 ± 0.03	11.82 ± 0.02	14.38 ± 0.02
4:00	4.8 ± 0.04	10.77 ± 0.04	11.35 ± 0.02	13.99 ± 0.04
5:00	4.84 ± 0.04	10.28 ± 0.02	10.88 ± 0.01	13.96 ± 0.12
6:00	6.14 ± 0.05	10.75 ± 0.04	12.3 ± 0.02	14.65 ± 0.24
7:00	8.24 ± 0.03	12.69 ± 0.03	14.82 ± 0.03	15.84 ± 0.35
8:00	10.3 ± 0.03	15.27 ± 0.03	16.42 ± 0.04	17.08 ± 0.25
9:00	11.3 ± 0.03	16.29 ± 0.03	16.85 ± 0.03	18.38 ± 0.32
10:00	11.27 ± 0.02	17.24 ± 0.02	17.4 ± 0.03	19.50 ± 0.24
11:00	11.27 ± 0.02	18.25 ± 0.02	18.33 ± 0.03	20.52 ± 0.25
12:00	11.28 ± 0.03	18.76 ± 0.02	19.83 ± 0.03	21.54 ± 0.23
13:00	11.26 ± 0.03	19.24 ± 0.02	20.32 ± 0.03	22.20 ± 0.19
14:00	11.78 ± 0.02	19.23 ± 0.03	20.3 ± 0.03	22.63 ± 0.17
15:00	12.28 ± 0.02	19.26 ± 0.02	20.34 ± 0.02	22.87 ± 0.10
16:00	11.71 ± 0.04	19.28 ± 0.02	19.81 ± 0.04	22.56 ± 0.03
17:00	11.21 ± 0.03	18.75 ± 0.02	19.33 ± 0.03	22.10 ± 0.04
18:00	11.06 ± 0.03	17.22 ± 0.03	18.37 ± 0.02	21.25 ± 0.06
19:00	9.86 ± 0.04	15.21 ± 0.03	16.41 ± 0.03	19.81 ± 0.18
20:00	8.38 ± 0.04	14.7 ± 0.02	15.8 ± 0.03	18.18 ± 0.13
21:00	7.34 ± 0.04	13.76 ± 0.03	14.83 ± 0.01	17.18 ± 0.02
22:00	6.84 ± 0.05	12.8 ± 0.04	13.79 ± 0.03	16.89 ± 0.12
23:00	6.33 ± 0.04	12.76 ± 0.03	13.79 ± 0.04	16.91 ± 0.12

Table F3. Mean recorded temperature values for the second of the two growth chambers in which wheat plants were grown during the pre-vernalization period.

Hour (24:00)	Temperature (°C) (Mean ± Standard Error)			
	Late October	Early November	Late November	Early December
0:00	12.75 ± 0.06	9.63 ± 0.07	5.45 ± 0.03	5.69 ± 0.02
1:00	12.24 ± 0.08	9.71 ± 0.05	5.48 ± 0.04	5.7 ± 0.02
2:00	11.67 ± 0.07	9.74 ± 0.10	4.87 ± 0.04	5.69 ± 0.02
3:00	11.69 ± 0.06	9.75 ± 0.20	4.45 ± 0.02	5.68 ± 0.02
4:00	11.59 ± 0.04	8.67 ± 0.03	4.52 ± 0.02	5.14 ± 0.02
5:00	11.63 ± 0.04	8.61 ± 0.08	5.58 ± 0.04	4.66 ± 0.02
6:00	11.67 ± 0.05	8.83 ± 0.24	7.82 ± 0.05	6.09 ± 0.04
7:00	12.96 ± 0.28	8.52 ± 0.05	9.32 ± 0.05	8.19 ± 0.05
8:00	14.48 ± 0.14	11.07 ± 0.19	9.8 ± 0.06	9.63 ± 0.04
9:00	16.13 ± 0.14	11.72 ± 0.09	9.81 ± 0.05	9.96 ± 0.16
10:00	17.17 ± 0.07	11.86 ± 0.03	9.83 ± 0.03	10.07 ± 0.05
11:00	18.11 ± 0.07	13.79 ± 0.06	10.47 ± 0.07	10.08 ± 0.06
12:00	19.11 ± 0.10	14.69 ± 0.06	10.86 ± 0.04	10.11 ± 0.05
13:00	19.77 ± 0.03	15.81 ± 0.12	10.8 ± 0.06	10.11 ± 0.05
14:00	19.5 ± 0.11	16.27 ± 0.04	10.83 ± 0.04	10.11 ± 0.05
15:00	18.95 ± 0.07	15.94 ± 0.10	10.21 ± 0.06	9.49 ± 0.09
16:00	18.48 ± 0.13	15.34 ± 0.20	8.26 ± 0.07	7.43 ± 0.03
17:00	17.63 ± 0.18	13.88 ± 0.10	7.49 ± 0.04	6.1 ± 0.03
18:00	16.46 ± 0.17	12.11 ± 0.06	7.01 ± 0.04	5.66 ± 0.02
19:00	15.38 ± 0.14	11.05 ± 0.06	6.49 ± 0.04	5.65 ± 0.02
20:00	14.71 ± 0.06	10.65 ± 0.06	6.5 ± 0.05	5.66 ± 0.03
21:00	14.59 ± 0.03	9.84 ± 0.20	6.51 ± 0.05	5.69 ± 0.03
22:00	14.33 ± 0.12	9.75 ± 0.05	5.96 ± 0.05	5.7 ± 0.02
23:00	13.46 ± 0.17	9.64 ± 0.15	5.48 ± 0.04	5.68 ± 0.02

Table F4. Mean recorded temperature values for the second of the two growth chambers in which wheat plants were grown during the post-vernalization period.

Hour (24:00)	Temperature (°C) (Mean ± Standard Error)			
	Late March	Early April	Late April	Early May
0:00	4.42 ± 0.06	10.83 ± 0.06	11.90 ± 0.04	14.33 ± 0.25
1:00	3.70 ± 0.05	10.56 ± 0.04	11.63 ± 0.03	13.55 ± 0.12
2:00	3.39 ± 0.05	9.81 ± 0.05	10.85 ± 0.04	13.26 ± 0.04
3:00	3.44 ± 0.04	9.52 ± 0.04	10.61 ± 0.03	12.72 ± 0.12
4:00	3.47 ± 0.04	9.52 ± 0.03	9.84 ± 0.04	12.31 ± 0.07
5:00	3.50 ± 0.04	8.79 ± 0.05	10.39 ± 0.09	12.48 ± 0.07
6:00	5.50 ± 0.04	9.31 ± 0.02	13.00 ± 0.12	13.97 ± 0.37
7:00	7.89 ± 0.08	13.77 ± 0.13	16.02 ± 0.08	15.20 ± 0.37
8:00	10.02 ± 0.05	16.06 ± 0.15	16.70 ± 0.13	16.39 ± 0.30
9:00	10.30 ± 0.06	16.56 ± 0.19	17.48 ± 0.13	18.10 ± 0.42
10:00	10.25 ± 0.06	18.13 ± 0.17	17.75 ± 0.14	18.92 ± 0.24
11:00	10.17 ± 0.06	18.62 ± 0.19	19.36 ± 0.11	20.35 ± 0.38
12:00	10.15 ± 0.05	19.34 ± 0.19	20.58 ± 0.12	21.10 ± 0.26
13:00	10.21 ± 0.07	19.63 ± 0.20	20.82 ± 0.12	21.73 ± 0.29
14:00	10.82 ± 0.08	19.64 ± 0.18	20.75 ± 0.12	22.10 ± 0.19
15:00	11.17 ± 0.05	19.63 ± 0.17	20.75 ± 0.13	22.08 ± 0.14
16:00	10.33 ± 0.07	19.59 ± 0.18	19.88 ± 0.17	21.64 ± 0.05
17:00	10.03 ± 0.07	18.92 ± 0.18	19.64 ± 0.14	21.24 ± 0.10
18:00	9.70 ± 0.06	16.81 ± 0.19	18.12 ± 0.18	20.09 ± 0.12
19:00	7.91 ± 0.05	14.00 ± 0.06	15.12 ± 0.07	17.92 ± 0.30
20:00	6.64 ± 0.05	13.72 ± 0.04	14.82 ± 0.03	16.22 ± 0.23
21:00	5.61 ± 0.06	12.14 ± 0.06	13.18 ± 0.06	15.40 ± 0.05
22:00	5.40 ± 0.05	11.58 ± 0.04	12.66 ± 0.03	15.31 ± 0.03
23:00	4.65 ± 0.05	11.59 ± 0.03	12.64 ± 0.03	15.29 ± 0.02

Appendix G: Approval of previously published work

UNIVERSITY OF MARYLAND
COLLEGE OF COMPUTER, MATHEMATICAL AND NATURAL SCIENCES
DEPARTMENT OF ENTOMOLOGY

4112 Plant Sciences Building
College Park, Maryland 20742-4454
301.405.3911 TEL 301.314.9290 FAX
www.entomology.umd.edu

November 3, 2020

Dr. Steve Fetter
Associate Provost and Dean, The Graduate School
2123 Lee Building
College Park, MD 20742

Dear Dr. Fetter,

Aditi Dubey, UID 114307002, has the approval of her examining committee to include her previously published work in her dissertation. This work, Dubey, A., Lewis, M.T., Dively, G.P., Hamby, K.A., 2020. Ecological impacts of pesticide seed treatments on arthropod communities in a grain crop rotation. J. Appl. Ecol. 57, 936–951. https://doi.org/10.1111/1365-2664.13595, has been cited appropriately throughout the dissertation and a preface discussing her contributions has been included and approved by the committee. Aditi conceived the manuscript, conducted the work, and prepared the manuscript; her committee has determined that she made a substantial contribution to the work.

Please do not hesitate to contact me if you have further questions.

Sincerely,

Kelly A. Hamby
Dissertation Director
Associate Professor/Extension Specialist
Department of Entomology

Jeffrey W. Shultz
Director of Graduate Studies
Associate Professor
Department of Entomology

Bibliography

2019 Agricultural Statistics Annual [WWW Document], 2019. URL https://www.nass.usda.gov/Publications/Ag_Statistics/2019/index.php (accessed 7.10.20).

2019 State Agriculture Overview Maryland [WWW Document], n.d. . USDA NASS. URL https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=MARYLAND (accessed 10.9.20).

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18, 265–267.

Alford, A., Krupke, C.H., 2017. Translocation of the neonicotinoid seed treatment clothianidin in maize. PLoS One 12, 1–19. https://doi.org/10.1371/journal.pone.0173836

Amjad, A., Azam, I., Sarwar, M.K., Malik, M.F., Sattar, A., 2018. A review of imidacloprid toxicity in coccinellids. Arthropods 7, 1–10.

Anderson, N.L., Harmon-Threatt, A.N., 2019. Chronic contact with realistic soil concentrations of imidacloprid affects the mass, immature development speed, and adult longevity of solitary bees. Sci. Rep. 9, 1–9. https://doi.org/10.1038/s41598-019-40031-9

Atwood, L.W., Mortensen, D.A., Koide, R.T., Smith, R.G., 2018. Evidence for multi-trophic effects of pesticide seed treatments on non-targeted soil fauna.

Soil Biol. Biochem. 125, 144–155. https://doi.org/10.1016/j.soilbio.2018.07.007

Baskaran, S., Kookana, R.S., Naidu, R., 1999. Degradation of bifenthrin, chlorpyrifos and imidacloprid in soil and bedding materials at termiticidal application rates. Pestic. Sci. 55, 1222–1228. https://doi.org/10.1002/(SICI)1096-9063(199912)55:12<1222::AID-PS83>3.0.CO;2-7

Basley, K., Goulson, D., 2018. Effects of field-relevant concentrations of clothianidin on larval development of the butterfly *Polyommatus icarus* (Lepidoptera, Lycaenidae). Environ. Sci. Technol. 52, 3990–3996. https://doi.org/10.1021/acs.est.8b00609

Bass, C., Denholm, I., Williamson, M.S., Nauen, R., 2015. The global status of insect resistance to neonicotinoid insecticides. Pestic. Biochem. Physiol. 121, 78–87. https://doi.org/10.1016/j.pestbp.2015.04.004

Beare, M., Coleman, D., Hendrix, P.F., Odum, E.P., 1995. A hierarchical approach to evaluating the significance of soil biodiversity to biogeochemical cycling. Plant Soil 170, 5–22. https://doi.org/10.1007/bf02183051

Bonmatin, J.M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E.A., Noome, D.A., Simon-Delso, N., Tapparo, A., 2015. Environmental fate and exposure; neonicotinoids and fipronil. Environ. Sci. Pollut. Res. 22, 35–67.

https://doi.org/10.1007/s11356-014-3332-7

Bonmatin, J.M., Moineau, I., Charvet, R., Colin, M.E., Fleche, C., Bengsch, E.R., 2005. Behaviour of imidacloprid in fields. Toxicity for honey bees. Environ. Chem. - Green Chem. Pollut. Ecosyst. 483–94. https://doi.org/10.1007/3-540-26531-7_44

Botias, C., David, A., Hill, E., Goulson, D., 2016. Contamination of wild plants near neonicotinoid seed-treated crops, and implications for non-target invertebrates. Sci. Total Environ. 566–567, 269–278.

Botías, C., David, A., Horwood, J., Abdul-Sada, A., Nicholls, E., Hill, E., Goulson, D., 2015. Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. Environ. Sci. Technol. 49, 12731–12740. https://doi.org/10.1021/acs.est.5b03459

Bredeson, M.M., Lundgren, J.G., 2019. Neonicotinoid insecticidal seed-treatment on corn contaminates interseeded cover crops intended as habitat for beneficial insects. Ecotoxicology 28, 222–228. https://doi.org/10.1007/s10646-018-02015-9

Bredeson, M.M., Lundgren, J.G., 2015. Thiamethoxam seed treatments have no impact on pest numbers or yield in cultivated sunflowers. J. Econ. Entomol. 108, 2665–2671. https://doi.org/10.1093/jee/tov249

Bredeson, M.M., Reese, R.N., Lundgren, J.G., 2015. The effects of insecticide dose and herbivore density on tri-trophic effects of thiamethoxam in a system involving wheat, aphids, and ladybeetles. Crop Prot. 69, 70–76.

https://doi.org/10.1016/j.cropro.2014.12.010

Bretagnolle, V., Gaba, S., 2015. Weeds for bees? A review. Agron. Sustain. Dev. 35, 891–909. https://doi.org/10.1007/s13593-015-0302-5

Callahan, B.J., n.d. DADA2 Pipeline Tutorial (1.16) [WWW Document]. URL https://benjjneb.github.io/dada2/tutorial.html (accessed 8.30.20).

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869

Calvo-agudo, M., González-cabrera, J., Picó, Y., Calatayud-vernich, P., Urbaneja, A., 2019. Neonicotinoids in excretion product of phloem-feeding insects kill beneficial insects. PNAS 116, 16817–16822. https://doi.org/10.1073/pnas.1904298116

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624. https://doi.org/10.1038/ismej.2012.8

Capowiez, Y., Bérard, A., 2006. Assessment of the effects of imidacloprid on the behavior of two earthworm species (*Aporrectodea nocturna* and *Allolobophora icterica*) using 2D terraria. Ecotoxicol. Environ. Saf. 64, 198–206. https://doi.org/10.1016/j.ecoenv.2005.02.013

Capowiez, Y., Dittbrenner, N., Rault, M., Triebskorn, R., Hedde, M., Mazzia, C., 2010. Earthworm cast production as a new behavioural biomarker for toxicity testing. Environ. Pollut. 158, 388–393. https://doi.org/10.1016/j.envpol.2009.09.003

Castle, S., Naranjo, S.E., 2009. Sampling plans, selective insecticides and sustainability: the case for IPM as ‘ informed pest management .’ Pest Manag. Sci. 65, 1321–1328. https://doi.org/10.1002/ps.1857

Chagnon, M., Kreutzweiser, D., Mitchell, E.A.D., Morrissey, C.A., Noome, D.A., Van der Sluijs, J.P., 2015. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. Environ. Sci. Pollut. Res. Int. 22, 119–134. https://doi.org/10.1007/s11356-014-3277-x

Chapin, J.W., Thomas, J.S., Gray, S.M., Smith, D.M., Halbert, S.E., Chapin, J.A.Y.W., Thomas, J.S., Gray, S.M., Smith, D.M., Halbert, S.E., 2001. Seasonal abundance of aphids (Homoptera: Aphididae) in wheat and their role as barley yellow dwarf virus vectors in the South Carolina coastal plain. J. Econ. Entomol. 94, 410–421.

Chevillot, F., Convert, Y., Desrosiers, M., Cadoret, N., Veilleux, É., Cabana, H., Bellenger, J.P., 2017. Selective bioaccumulation of neonicotinoids and sub-lethal effects in the earthworm *Eisenia andrei* exposed to environmental concentrations in an artificial soil. Chemosphere 186, 839–847. https://doi.org/10.1016/j.chemosphere.2017.08.046

Cloyd, R.A., Bethke, J.A., 2011. Impact of neonicotinoid insecticides on natural

enemies in greenhouse and interiorscape environments. Pest Manag. Sci. 67, 3–9. https://doi.org/10.1002/ps.2015

Cox, W.J., Cherney, J.H., Shields, E., 2007. Clothianidin seed treatments inconsistently affect corn forage yield when following soybean. Agron. J. 99, 543–548. https://doi.org/10.2134/agronj2006.0170

Cycoń, M., Markowicz, A., Borymski, S., Wójcik, M., Piotrowska-Seget, Z., 2013. Imidacloprid induces changes in the structure, genetic diversity and catabolic activity of soil microbial communities. J. Environ. Manage. 131, 55–65. https://doi.org/10.1016/j.jenvman.2013.09.041

Cycoń, M., Piotrowska-Seget, Z., 2015. Biochemical and microbial soil functioning after application of the insecticide imidacloprid. J. Environ. Sci. 27, 147–158. https://doi.org/10.1016/j.jes.2014.05.034

de Lima e Silva, C., Brennan, N., Brouwer, J.M., Commandeur, D., Verweij, R.A., van Gestel, C.A.M., 2017. Comparative toxicity of imidacloprid and thiacloprid to different species of soil invertebrates. Ecotoxicology 26, 555–564. https://doi.org/10.1007/s10646-017-1790-7

de Lima e Silva, C., Mariette, J., Verweij, R.A., van Gestel, C.A.M., 2018. Assessing the toxicity of thiamethoxam, in natural LUFA 2.2 soil, through three generations of *Folsomia candida*. Ecotoxicology In Press. https://doi.org/10.1007/s10646-018-1922-8

DeCant, J., Barrett, M., 2010. Clothianidin registration of Prosper T400 seed treatment on mustard seed (oilseed and condiment) and Poncho/Votivo seed

treatment on cotton, United States Environmental Protection Agency. https://doi.org/EPA 832-F-99-062

Decourtye, A., Armengaud, C., Renou, M., Devillers, J., Cluzeau, S., Gauthier, M., Pham-Delègue, M.H., 2004. Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). Pestic. Biochem. Physiol. 78, 83–92. https://doi.org/10.1016/j.pestbp.2003.10.001

Deng, D., Duan, W., Wang, H., Zhang, K., Guo, J., Yuan, L., Wang, L., Wu, S., 2019. Assessment of the effects of lethal and sublethal exposure to dinotefuran on the wheat aphid *Rhopalosiphum padi* (Linnaeus). Ecotoxicology 28, 825–833. https://doi.org/10.1007/s10646-019-02080-8

DiBartolomeis, M., Kegley, S., Mineau, P., Radford, R., Klein, K., 2019. An assessment of acute insecticide toxicity loading (AITL) of chemical pesticides used on agricultural land in the United States. PLoS One 14, e0220029. https://doi.org/10.1371/journal.pone.0220029

Disque, H.H., Hamby, K.A., Dubey, A., Taylor, C., Dively, G.P., 2018. Effects of clothianidin-treated seed on the arthropod community in a mid-Atlantic no-till corn agroecosystem. Pest Manag. Sci. 75, 969–978. https://doi.org/10.1002/ps.5201

Dittbrenner, N., Triebskorn, R., Moser, I., Capowiez, Y., 2010. Physiological and behavioural effects of imidacloprid on two ecologically relevant earthworm species (*Lumbricus terrestris* and *Aporrectodea caliginosa*). Ecotoxicology 19, 1567–1573. https://doi.org/10.1007/s10646-010-0542-8

Dively, G.P., Kamel, A., 2012. Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. J. Agric. Food Chem. 60, 4449–4456. https://doi.org/10.1021/jf205393x

Donnarumma, L., Pulcini, P., Pochi, D., Rosati, S., Lusco, L., Conte, E., 2011. Preliminary study on persistence in soil and residues in maize of imidacloprid. J. Environ. Sci. Heal. Part. B 46, 469–72. https://doi.org/10.1080/03601234.2011.583848

Doran, J., Kettler, T., Tsivou, M., 1997. Field and laboratory Solvita Soil Test evaluation, University of Nebraska USDA-ARS.

Douglas, M.R., Rohr, J.R., Tooker, J.F., 2015. Neonicotinoid insecticide travels through a soil food chain, disrupting biological control of non-target pests and decreasing soya bean yield. J. Appl. Ecol. 52, 250–260. https://doi.org/10.1111/1365-2664.12372

Douglas, M.R., Tooker, J.F., 2016. Meta-analysis reveals that seed-applied neonicotinoids and pyrethroids have similar negative effects on abundance of arthropod natural enemies. PeerJ 4, e2776. https://doi.org/10.7717/peerj.2776

Douglas, M.R., Tooker, J.F., 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in U.S. Field crops. Environ. Sci. Technol. 49, 5088–5097. https://doi.org/10.1021/es506141g

Dubey, A., Lewis, M.T., Dively, G.P., Hamby, K.A., 2020. Ecological impacts of pesticide seed treatments on arthropod communities in a grain crop rotation.

J. Appl. Ecol. 57, 936–951. https://doi.org/10.1111/1365-2664.13595

EASAC, 2015. Ecosystem services, agriculture, and neonicotinoids.

Easton, A.H., Goulson, D., 2013. The Neonicotinoid insecticide imidacloprid repels pollinating flies and beetles at field-realistic concentrations. PLoS One 8, 8–11. https://doi.org/10.1371/journal.pone.0054819

Edwards, C.A., 2002. Assessing the effects of environmental pollutants on soil organisms, communities, processes and ecosystems. Eur. J. Soil Biol. 38, 225–231. https://doi.org/10.1016/S1164-5563(02)01150-0

Edwards, C.A., Bohlen, P.J., 1996. Biology and Ecology of Earthworms, 3rd ed. Chapman & Hall, London.

Elliott, N.C., French, B.W., Burd, J.D., Kindler, S.D., Reed, D.K., 1994. Parasitism, adult emergence, sex ratio, and size of *Aphidius colemani* (Hymenoptera: Aphidiidae) on several aphid species. Gt. Lakes Entomol. 27, 137–142.

Erdle, K., Mistele, B., Schmidhalter, U., 2011. Comparison of active and passive spectral sensors in discriminating biomass parameters and nitrogen status in wheat cultivars. F. Crop. Res. 124, 74–84. https://doi.org/10.1016/j.fcr.2011.06.007

Fievet, V., Dedryver, C., Plantegenest, M., Simon, J., 2007. Aphid colony turn-over influences the spatial distribution of the grain aphid *Sitobion avenae* over the wheat growing season 125–134.

Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N., Snyder, P.K., 2005. Global consequences of land use. Science (80-.). 309, 570–574. https://doi.org/10.1126/science.1111772

Frank, S.D., Tooker, J.F., 2020. Opinion: Neonicotinoids pose undocumented threats to food webs. Proc. Natl. Acad. Sci. 117, 202017221. https://doi.org/10.1073/pnas.2017221117

Franklin, R.B., Mills, A.L., 2003. Multi-scale variation in spatial heterogeneity for microbial community structure in an eastern Virginia agricultural field. FEMS Microbiol. Ecol. 44, 335–346. https://doi.org/10.1016/S0168-6496(03)00074-6

Frewin, A.J., Schaafsma, A.W., Hallett, R.H., 2014. Susceptibility of *Aphelinus certus* (Hymenoptera: Aphelinidae) to neonicotinoid seed treatments used for soybean pest management. J. Econ. Entomol. 107, 1450–1457. https://doi.org/10.1603/EC13523

Godfray, H.C.J., Blacquière, T., Field, L.M., Hails, R.S., Petrokofsky, G., Potts, S.G., Raine, N.E., Vanbergen, a. J., McLean, a. R., 2014. A restatement of the natural science evidence base concerning neonicotinoid insecticides and insect pollinators. Proc. R. Soc. B Biol. Sci. 281, 20140558. https://doi.org/10.1098/rspb.2014.0558

Gontijo, P.C., Moscardini, V.F., Michaud, J., Carvalho, G.A., 2015. Non-target effects of two sunflower seed treatments on *Orius insidiosus* (Hemiptera: Anthocoridae). Pest Manag. Sci. 71, 515–522. https://doi.org/10.1002/ps.3798

Goulson, D., 2013. An overview of the environmental risks posed by neonicotinoid insecticides. J. Appl. Ecol. 50, 977–987. https://doi.org/10.1111/1365-2664.12111

Gourmet, C., Kolb, F.L., Smyth, C.A., Pedersen, W.L., 1996. Use of imidacloprid as a seed-treatment insecticide to control Barley Yellow Dwarf Virus (BYDV) in oat and wheat. Plant Dis. https://doi.org/10.1094/PD-80-0136

Gupta, S., Gajbhiye, V.T., Gupta, R.K., 2008a. Effect of light on the degradation of two neonicotinoids viz acetamiprid and thiacloprid in soil. Bull. Environ. Contam. Toxicol. 81, 185–189. https://doi.org/10.1007/s00128-008-9405-x

Gupta, S., Gajbhiye, V.T., Gupta, R.K., 2008b. Soil dissipation and leaching behavior of a neonicotinoid insecticide thiamethoxam. Bull. Environ. Contam. Toxicol. 80, 431–437. https://doi.org/10.1007/s00128-008-9420-y

Hallmann, C. a., Foppen, R.P.B., Turnhout, C. a M. Van, Kroon, H. De, Jongejans, E., van Turnhout, C. a. M., de Kroon, H., Jongejans, E., 2014. Declines in insectivorous birds are associated with high neonicotinoid concentrations. Nature 511, 341–343. https://doi.org/10.1038/nature13531

Haney, R.L., Brinton, W.F., Evans, E., 2008. Soil CO2 respiration: Comparison of chemical titration, CO2 IRGA analysis and the Solvita gel system. Renew.

Agric. Food Syst. 23, 171–176. https://doi.org/10.1017/S174217050800224X

Henry, M., Béguin, M., Requier, F., Rollin, O., Odoux, J., Aupinel, P., Aptel, J., Tchamitchian, S., Decourtye, A., 2012. A common pesticide decreases foraging success and survival in honey bees. Science 336, 3–5.

Hesler, L.S., Sappington, T.W., Luttrell, R.G., Allen, K.C., Papiernik, S.K., 2018. Selected early-season insect pests of wheat in the United States and factors affecting their risks of infestation. J. Integr. Pest Manag. 9, 1–8. https://doi.org/10.1093/jipm/pmx023

Hladik, M.L., Corsi, S.R., Kolpin, D.W., Baldwin, A.K., Blackwell, B.R., Cavallin, J.E., 2018. Year-round presence of neonicotinoid insecticides in tributaries to the Great Lakes, USA. Environ. Pollut. 235, 1022–1029. https://doi.org/10.1016/j.envpol.2018.01.013

Hladik, M.L., Kolpin, D.W., 2016. First national-scale reconnaissance of neonicotinoid insecticides in streams across the USA. Environ. Chem. 13, 12–20. https://doi.org/10.1071/EN15061

Hladik, M.L., Kolpin, D.W., Kuivila, K.M., 2014. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region , USA. Environ. Pollut. 193, 189–196. https://doi.org/10.1016/j.envpol.2014.06.033

Hunger, R.M., Olson, B., Siegerist, W.C., 2000. Evaluation of Gaucho 480F seed treatment to control aphids and barley yellow dwarf (BYD) in hard red

winter wheat (HRWW). F&N Tests 566, ST37.

Hussain, S., Hartley, C.J., Shettigar, M., Pandey, G., 2016. Bacterial biodegradation of neonicotinoid pesticides in soil and water systems. FEMS Microbiol. Lett. 363, 1–13. https://doi.org/10.1093/femsle/fnw252

Irwin, M.E., Thresh, J.M., 1990. Epidemiology of barley yellow dwarf: a study in ecological complexity. Annu. Rev. Phytopathol. 28, 393–424. https://doi.org/10.1146/annurev.py.28.090190.002141

Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2011. Overview of the status and global strategy for neonicotinoids. J. Agric. Food Chem. 1–7. https://doi.org/10.1021/jf101303g

Johnson, M.W., Tabashnik, B.E., 1999. Enhanced biological control through pesticide selectivity, in: Bellows, T., Fisher, T. (Eds.), Handbook of Biological Control. San Diego, pp. 297–317.

Kathage, J., Castañera, P., Alonso-Prados, J.L., Gómez-Barbero, M., Rodríguez-Cerezo, E., 2018. The impact of restrictions on neonicotinoid and fipronil insecticides on pest management in maize, oilseed rape and sunflower in eight European Union regions. Pest Manag. Sci. 74, 88–99. https://doi.org/10.1002/ps.4715

Kennedy, T.F., Connery, J., 2012. Control of barley yellow dwarf virus in minimum-till and conventional-till autumn-sown cereals by insecticide seed and foliar spray treatments. J. Agric. Sci. 150, 249–262. https://doi.org/10.1017/S0021859611000505

Khani, A., Ahmadi, F., Ghadamyari, M., 2012. Side effects of imidcloprid and abamectin on the mealybug destroyer *Cryptolaemus montrouzieri*. Trakia J. Sci. 10, 30–35.

Kibblewhite, M.G., Ritz, K., Swift, M.J., 2008. Soil health in agricultural systems. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 363, 685–701. https://doi.org/10.1098/rstb.2007.2178

Kieckhefer, R.W., Gellner, J.L., 1992. Yield losses in winter wheat caused by low-density cereal aphid populations. Agron. J. 84, 180. https://doi.org/10.2134/agronj1992.00021962008400020011x

Kirkland, L.S., Pirtle, E.I., Umina, P.A., 2018. Responses of the Russian wheat aphid (*Diuraphis noxia*) and bird cherry oat aphid (*Rhopalosiphum padi*) to insecticide seed treatments in wheat. Crop Pasture Sci. 69, 966–973.

Kreutzweiser, D.P. et al. et al., 2008. Effects on litter-dwelling earthworms and microbial decomposition of soil-applied imidacloprid for control of wood-boring insects. Pest Manag. Sci. 64, 112–118. https://doi.org/10.1002/ps

Krischik, V.A., Landmark, A.L., Heimpel, G.E., 2007. Soil-applied imidacloprid is translocated to nectar and kills nectar-feeding *Anagyrus pseudococci* (Girault) (Hymenoptera: Encyrtidae). Environ. Entomol. 36, 1238–45. https://doi.org/10.1603/0046-225X(2007)36

Krupke, C.., Holland, J.D., Long, E.Y., Eitzer, B.D., 2017. Planting of neonicotinoid-treated maize poses risks for honey bees and other non-target organisms over a wide area without consistent crop yield benefit. J. Appl.

Ecol. 54, 1449–1458.

Krupke, C.H., Hunt, G.J., Eitzer, B.D., Andino, G., Given, K., 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. PLoS One 7. https://doi.org/10.1371/journal.pone.0029268

Kunkel, B.A., Held, D.W., Potter, D.A., Kunkel, B.A., Held, D.W., 2001. Lethal and sublethal effects of bendiocarb, halofenozide, and imidacloprid on *Harpalus pennsylvanicus* (Coleoptera: Carabidae) following different modes of exposure in turfgrass. J. Econ. Entomol. 94, 60–67.

Labrie, G., Gagnon, A.È., Vanasse, A., Latraverse, A., Tremblay, G., 2020. Impacts of neonicotinoid seed treatments on soil-dwelling pest populations and agronomic parameters in corn and soybean in Quebec (Canada). PLoS One 15, 1–20. https://doi.org/10.1371/journal.pone.0229136

Lagnaoui, A., Radcliffe, E.B., 2009. Potato fungicides interfere with entomopathogenic fungi impacting population dynamics of green peach aphid. Am. J. Potato Res. 75, 19–25. https://doi.org/10.1007/bf02883513

Lamichhane, J.R., You, M.P., Laudinot, V., Barbetti, M.J., Aubertot, J.N., 2020. Revisiting sustainability of fungiside seed treatments for field crops. Plant Dis. 104, 610–623. https://doi.org/10.1094/PDIS-06-19-1157-FE

Leiva, J.A., Nkedi-Kizza, P., Morgan, K.T., Qureshi, J.A., 2015. Imidacloprid sorption kinetics, equilibria, and degradation in sandy soils of Florida. J. Agric. Food Chem. 63, 4915–4921. https://doi.org/10.1021/acs.jafc.5b00532

Li, Yaofa, An, J., Dang, Z., Lv, H., Pan, W., 2018. Treating wheat seeds with neonicotinoid insecticides does not harm the rhizosphere microbial community. PLoS One 13, e0205200.

Li, Y., An, J., Dang, Z., Pan, W., Gao, Z., 2018. Systemic control efficacy of neonicotinoids seeds dressing on English grain aphid (Hemiptera: Aphididae). J. Asia. Pac. Entomol. 21, 430–435. https://doi.org/10.1016/j.aspen.2018.01.003

Li, Yang, Su, P., Li, Yadong, Wen, K., Bi, G., Cox, M., 2018. Chemosphere Adsorption-desorption and degradation of insecticides clothianidin and thiamethoxam in agricultural soils. Chemosphere 207, 708–714. https://doi.org/10.1016/j.chemosphere.2018.05.139

Lister, B.C., Garcia, A., 2018. Climate-driven declines in arthropod abundance restructure a rainforest food web. Proc. Natl. Acad. Sci. 115, E10397–E10406. https://doi.org/10.1073/pnas.1722477115

Liu, Z., Dai, Y., Huang, G., Gu, Y., Ni, J., Wei, H., Yuan, S., 2011. Soil microbial degradation of neonicotinoid insecticides imidacloprid, acetamiprid, thiacloprid and imidaclothiz and its effect on the persistence of bioefficacy against horsebean aphid *Aphis craccivora* Koch after soil application. Pest Manag. Sci. 67, 1245–1252. https://doi.org/10.1002/ps.2174

Lundgren, J.G., Fausti, S.W., 2015. Trading biodiversity for pest problems. Sci. Adv. 1, e1500558.

Magalhaes, L.C., Hunt, T.E., Siegfried, B.D., 2009. Efficacy of neonicotinoid

seed treatments to reduce soybean aphid populations under field and controlled conditions in Nebraska. J. Econ. Entomol. 102, 187–195. https://doi.org/10.1603/029.102.0127

Main, A.R., Webb, E.B., Goyne, K.W., Mengel, D., 2018. Neonicotinoid insecticides negatively affect performance measures of non-target terrestrial arthropods: a meta-analysis. Ecol. Appl. 28, 1232–1244. https://doi.org/10.1002/eap.1723

Mandelik, Y., Winfree, R., Neeson, T., Kremen, C., 2016. Complementary habitat use by wild bees in agro-natural landscapes 22, 1535–1546. https://doi.org/10.1890/11-1299.1

Mccornack, B.P., Ragsdale, D.W., 2006. Efficacy of thiamethoxam to suppress soybean aphid populations in Minnesota soybean crop management. Crop Manag. 5, doi:10.1094/CM-2006-0915-01-RS. https://doi.org/10.1094/CM-2006-0915-01-RS.Abstract

Mckirdy, S.J., Jones, R.A.C., 1996. Use of imidacloprid and newer generation synthetic pyrethroids to control the spread of Barley yellow dwarf luteovirus in cereals . Plant Dis. Plant Dis. 80, 895–901. https://doi.org/10.1094/PD-80-0895

McMurdie, P.J., Holmes, S., 2013. Phyloseq: An R Package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8. https://doi.org/10.1371/journal.pone.0061217

Miles, J.C., Hua, J., Sepulveda, M.S., Krupke, C.H., Hoverman, J.T., 2017.

Effects of clothianidin on aquatic communities: Evaluating the impacts of lethal and sublethal exposure to neonicotinoids. PLoS One 12, 1–24. https://doi.org/10.1371/journal.pone.0174171

Morrissey, C.A., Mineau, P., Devries, J.H., Sanchez-Bayo, F., Liess, M., Cavallaro, M.C., Liber, K., 2015. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. Environ. Int. 74, 291–303. https://doi.org/10.1016/j.envint.2014.10.024

Moscardini, V.F., Gontijo, P.C., Michaud, J.P., Carvalho, G.A., 2014. Sublethal effects of chlorantraniliprole and thiamethoxam seed treatments when *Lysiphlebus testaceipes* feed on sunflower extrafloral nectar. BioControl 59, 503–511. https://doi.org/10.1007/s10526-014-9588-5

Moser, S.E., Obrycki, J.J., 2009. Non-target effects of neonicotinoid seed treatments; mortality of coccinellid larvae related to zoophytophagy. Biol. Control 51, 487–492. https://doi.org/10.1016/j.biocontrol.2009.09.001

Mourtzinis, S., Krupke, C.H., Esker, P.D., Varenhorst, A., Arneson, N.J., Bradley, C.A., Byrne, A.M., Chilvers, M.I., Giesler, L.J., Herbert, A., Kandel, Y.R., Kazula, M.J., Hunt, C., Lindsey, L.E., Malone, S., Mueller, D.S., Naeve, S., Nafziger, E., Reisig, D.D., Ross, W.J., Rossman, D.R., Taylor, S., Conley, S.P., 2019. Neonicotinoid seed treatments of soybean provide negligible benefits to US farmers. Sci. Rep. 9, 1–7. https://doi.org/10.1038/s41598-019-47442-8

Mullin, C.A., Saunders, M.C., Leslie, T.W., Biddinger, D.J., Fleischer, S.J., 2005.

Toxic and behavioral effects to Carabidae of seed treatments used on Cry3Bbl- and CrylAb/c-protected corn. Environ. Entomol. 34, 1626–1636. https://doi.org/10.1603/0046-225X-34.6.1626

Myers, C., Hill, E., 2014. Benefits of neonicotinoid seed treatments to soybean production, Environmental Protection Agency.

Nauen, R., Jeschke, P., Copping, L., 2008. In Focus: Neonicotinoid insecticides. Pest Manag. Sci. 64, 1081. https://doi.org/10.1002/ps.1659

Naveed, M., Salam, A., Saleem, M.A., Rafiq, M., Hamza, A., 2010. Toxicity of thiamethoxam and imidacloprid as seed treatments to parasitoids associated to control *Bemisia tabaci*. Pak. J. Zool. 42, 559–565.

Nettles, R., Watkins, J., Ricks, K., Boyer, M., Licht, M., Atwood, L.W., Peoples, M., Smith, R.G., Mortensen, D.A., Koide, R.T., 2016. Influence of pesticide seed treatments on rhizosphere fungal and bacterial communities and leaf fungal endophyte communities in maize and soybean. Appl. Soil Ecol. 102, 61–69. https://doi.org/10.1016/j.apsoil.2016.02.008

New Active Ingredient Review: Sedaxane [WWW Document], 2012. . Minnesota Dep. Agric. URL https://www.mda.state.mn.us/sites/default/files/inline-files/nair-sedaxane.pdf (accessed 7.10.20).

North, J.H., Gore, J., Catchot, A.L., Stewart, S.D., Lorenz, G.M., Musser, F.R., Cook, D.R., Kerns, D.L., Dodds, D.M., 2016. Value of neonicotinoid insecticide seed treatments in mid-South soybean (*Glycine max*) production systems. J. Econ. Entomol. 109, tow035. https://doi.org/10.1093/jee/tow035

Nuyttens, D., Devarrewaere, W., Verboven, P., Foqué, D., 2013. Pesticide-laden dust emission and drift from treated seeds during seed drilling: A review. Pest Manag. Sci. 69, 564–575. https://doi.org/10.1002/ps.3485

Oksanen, J., Guillame Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2019. vegan: Community Ecology Package.

Paine, T.D., Hanlon, C.C., Byrne, F.J., 2011. Potential risks of systemic imidacloprid to parasitoid natural enemies of a cerambycid attacking Eucalyptus. Biol. Control 56, 175–178. https://doi.org/10.1016/j.biocontrol.2010.08.007

Papachristos, D.P., Milonas, P.G., 2008. Adverse effects of soil applied insecticides on the predatory coccinellid *Hippodamia undecimnotata* (Coleoptera: Coccinellidae). Biol. Control 47, 77–81. https://doi.org/10.1016/j.biocontrol.2008.06.009

Papiernik, S.K., Sappington, T.W., Luttrell, R.G., Hesler, L.S., Allen, K.C., 2018. Overview: Risk factors and historic levels of pressure from insect pests of seedling corn, cotton, soybean, and wheat in the United States. J. Integr. Pest Manag. 9. https://doi.org/10.1093/jipm/pmx026

Pecenka, J.R., Lundgren, J.G., 2015. Non-target effects of clothianidin on monarch butterflies. Sci. Nat. 102. https://doi.org/10.1007/s00114-015-1270-y

Peck, D.C., 2009a. Comparative impacts of white grub (Coleoptera: Scarabaeidae) control products on the abundance of non-target soil-active arthropods in turfgrass. Pedobiologia (Jena). 52, 287–299. https://doi.org/10.1016/j.pedobi.2008.10.003

Peck, D.C., 2009b. Long-term effects of imidacloprid on the abundance of surface- and soil-active nontarget fauna in turf. Agric. For. Entomol. 11, 405–419. https://doi.org/10.1111/j.1461-9563.2009.00454.x

Pelosi, C., Barot, S., Capowiez, Y., Hedde, M., Vandenbulcke, F., 2014. Pesticides and earthworms. A review. Agron. Sustain. Dev. 34, 199–228. https://doi.org/10.1007/s13593-013-0151-z

Pettis, J.S., Vanengelsdorp, D., Johnson, J., Dively, G., 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen Nosema. Naturwissenschaften 99, 153–158. https://doi.org/10.1007/s00114-011-0881-1

Philippot, L., Spor, A., Hénault, C., Bru, D., Bizouard, F., Jones, C.M., Sarr, A., Maron, P.-A., 2013. Loss in microbial diversity affects nitrogen cycling in soil. ISME J. 7, 1609–1619. https://doi.org/10.1038/ismej.2013.34

Pike, K.S., Starý, P., Miller, T., Allison, D., Boydston, L., Graf, G., Gillespie, R., 1997. Small-grain aphid parasitoids (Hymenoptera: Aphelinidae and Aphidiidae) of Washington: distribution, relative abundance, seasonal occurrence, and key to known North American species. Environ. Entomol. 26, 1299–1311. https://doi.org/10.1093/ee/26.6.1299

Pisa, L.W., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Downs, C.A., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., McField, M., Morrissey, C.A., Noome, D.A., Settele, J., Simon-Delso, N., Stark, J.D., Van der Sluijs, J.P., Van Dyck, H., Wiemers, M., 2015. Effects of neonicotinoids and fipronil on non-target invertebrates. Environ. Sci. Pollut. Res. Int. 22, 68–102. https://doi.org/10.1007/s11356-014-3471-x

Porter, B.Y.J.R., Kirby, E.J.M., Day, W., Adam, J.S., Appleyard, M., Ayling, S., Baker, C.K., Beale, P., Belford, R.K., Bscoe, P.V., Chapman, A., Fuller, M.P., Hampson, J., Hay, R.K.M., Hough, M.N., Matthews, S., Thompson, W.J., Weir, A.H., Willington, V.B.A., Wood, D.W., 1987. An analysis of morphological development stages in Avalon winter wheat crops with different sowing dates and at ten sites in England and Scotland. J. Agric. Sci. 109, 107–121.

Prabhaker, N., Castle, S.J., Naranjo, S.E., Toscano, N.C., Morse, J.G., 2011. Compatibility of two systemic neonicotinoids, imidacloprid and thiamethoxam, with various natural enemies of agricultural pests. J. Econ. Entomol. 104, 773–781. https://doi.org/10.1603/ec10362

Preetha, G., Manoharan, T., Stanley, J., Kuttalam, S., 2010. Impact of chloronicotinyl insecticide, imidacloprid on egg, egg-larval and larval parasitoids under laboratory conditions. J. Plant Prot. Res. 50, 535–540. https://doi.org/10.2478/v10045-010-0088-z

Puglisi, E., 2012. Response of microbial organisms (aquatic and terrestrial) to

pesticides.

Qu, Y., Xiao, D., Li, J., Chen, Z., Biondi, A., Desneux, N., Gao, X., Song, D., 2015. Sublethal and hormesis effects of imidacloprid on the soybean aphid *Aphis glycines*. Ecotoxicology 24, 479–487. https://doi.org/10.1007/s10646-014-1396-2

Reisig, D.D., Herbert, D.A., Malone, S., 2012. Impact of neonicotinoid seed treatments on thrips (Thysanoptera: Thripidae) and soybean yield in Virginia and North Carolina. J. Econ. Entomol. 105, 884–889. https://doi.org/10.1603/EC11429

Royer, T.A., Giles, K.L., Nyamanzi, T., Hunger, R.M., Krenzer, E.G., Elliot, N.C., Kindler, S.D., Payton, M., 2005. Economic evaluation of the effects of planting date and application rate of imidacloprid for management of cereal aphids and barley yellow dwarf in winter wheat. J. Econ. Entomol. 98, 95–102. https://doi.org/10.1603/0022-0493-98.1.95

Rundlöf, M., Andersson, G.K.S., Bommarco, R., Fries, I., Hederström, V., Herbertsson, L., Jonsson, O., Klatt, B.K., Pedersen, T.R., Yourstone, J., Smith, H.G., 2015. Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature 521, 77–80. https://doi.org/10.1038/nature14420

Santos, J.B., Jakelaitis, A., Silva, A.A., Costa, M.D., Manabe, A., 2006. Action of two herbicides on the microbial activity of soil cultivated with common bean (*Phaseolus vulgaris*) in conventional-till and no-till systems. Eur. Weed Res. Soc. 46, 284–289.

Sappington, T.W., Hesler, L.S., Clint Allen, K., Luttrell, R.G., Papiernik, S.K., 2018. Prevalence of sporadic insect pests of seedling corn and factors affecting risk of infestation. J. Integr. Pest Manag. 9. https://doi.org/10.1093/jipm/pmx020

Schloter, M., Dilly, O., Munch, J.C., 2003. Indicators for evaluating soil quality. Agric. Ecosyst. Environ. 98, 255–262. https://doi.org/10.1016/S0167-8809(03)00085-9

Schmidt, M.H., Lauer, A., Purtauf, T., Thies, C., Schaefer, M., Tscharntke, T., 2003. Relative importance of predators and parasitoids for cereal aphid control. Proc. R. Soc. B Biol. Sci. 270, 1905–1909. https://doi.org/10.1098/rspb.2003.2469

Schmidt, R., Gravuer, K., Bossange, A. V, Mitchell, J., Scow, K., 2018. Long-term use of cover crops and no-till shift soil microbial community life strategies in agricultural soil. PLoS One 13, e0192953.

Schroeder, P.J., Jenkins, D.G., 2018. How robust are popular beta diversity indices to sampling error. Ecosphere 9, e02100. https://doi.org/10.1002/ecs2.2100

Seagraves, M.P., Lundgren, J.G., 2012. Effects of neonicotinoid seed treatments on soybean aphid and its natural enemies. J. Pest Sci. (2004). 85, 125–132. https://doi.org/10.1007/s10340-011-0374-1

Sgolastra, F., Medrzycki, P., Bortolotti, L., Renzi, M.T., Tosi, S., Bogo, G., Teper, D., Porrini, C., Molowny-Horas, R., Bosch, J., 2017. Synergistic

mortality between a neonicotinoid insecticide and an ergosterol-biosynthesis-inhibiting fungicide in three bee species. Pest Manag. Sci. 73, 1236–1243. https://doi.org/10.1002/ps.4449

Sial, M.U., Zhao, Z., Zhang, L., Zhang, Y., Mao, L., Jiang, H., 2018. Evaluation of Insecticides induced hormesis on the demographic parameters of Myzus persicae and expression changes of metabolic resistance detoxification genes. Sci. Rep. 8, 4–11. https://doi.org/10.1038/s41598-018-35076-1

Simon-Delso, N., Amaral-Rogers, V., Belzunces, L.P., Bonmatin, J.M., Chagnon, M., Downs, C., Furlan, L., Gibbons, D.W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C.H., Liess, M., Long, E., Mcfield, M., Mineau, P., Mitchell, E.A., Morrissey, C.A., Noome, D.A., Pisa, L., Settele, J., Stark, J.D., Tapparo, A., Van Dyck, H., Van Praagh, J., Van Der Sluijs, J.P., Whitehorn, P.R., Wiemers, M., 2015. Systemic insecticides (Neonicotinoids and fipronil): Trends, uses, mode of action and metabolites. Environ. Sci. Pollut. Res. 22, 5–34. https://doi.org/10.1007/s11356-014-3470-y

Smalling, K.L., Hladik, M.L., Sanders, C.J., Kuivila, K.M., 2018. Leaching and sorption of neonicotinoid insecticides and fungicides from seed coatings. J. Environ. Sci. Heal. - Part B Pestic. Food Contam. Agric. Wastes 53, 176–183. https://doi.org/10.1080/03601234.2017.1405619

Stapel, J.O., Cortesero, A.M., Lewis, W.J., 2000. Disruptive sublethal effects of insecticides on biological control: Altered foraging ability and life span of a

parasitoid after feeding on extrafloral nectar of cotton treated with systemic insecticides. Biol. Control 17, 243–249. https://doi.org/10.1006/bcon.1999.0795

Stara, J., Ourednickova, J., Kocourek, F., 2011. Laboratory evaluation of the side effects of insecticides on *Aphidius colemani* (Hymenoptera: Aphidiidae), *Aphidoletes aphidimyza* (Diptera: Cecidomyiidae), and *Neoseiulus cucumeris* (Acari: Phytoseidae). J. Pest Sci. (2004). 84, 25–31. https://doi.org/10.1007/s10340-010-0322-5

Starner, K., Goh, K.S., 2012. Detections of the neonicotinoid insecticide imidacloprid in surface waters of three agricultural regions of California, USA, 2010-2011. Bull. Environ. Contam. Toxicol. 88, 316–321. https://doi.org/10.1007/s00128-011-0515-5

Stewart, S.D., Lorenz, G.M., Catchot, A.L., Gore, J., Cook, D., Skinner, J., Mueller, T.C., Johnson, D.R., Zawislak, J., Barber, J., 2014. Potential exposure of pollinators to neonicotinoid insecticides from the use of insecticide seed treatments in the mid-southern United States. Environ. Sci. Technol. 48, 9762–9769. https://doi.org/10.1021/es501657w

Sur, R., Stork, A., 2003. Uptake, translocation and metabolism of imidacloprid in plants. Bull. Insectology 56, 35–40.

Takada, H., 1998. A review of *Aphidius colemani* (Hymenoptera: Braconidae; Aphidiinae) and closely related species indigenous to Japan. Appl. Entomol. Zool. 33, 59–66.

Tapparo, A., Giorio, C., Marzaro, M., Marton, D., Soldà, L., Girolami, V., 2011. Rapid analysis of neonicotinoid insecticides in guttation drops of corn seedlings obtained from coated seeds. J. Environ. Monit. 13, 1564–8. https://doi.org/10.1039/c1em10085h

Tappert, L., Pokorny, T., Hofferberth, J., Ruther, J., 2017. Sublethal doses of imidacloprid disrupt sexual communication and host finding in a parasitoid wasp. Sci. Rep. 7, 42756. https://doi.org/10.1038/srep42756

Tatsumi, E., Takada, H., 2006. Overwintering of the aphid parasitoids *Aphelinus asychis* and *A. albipodus* (Hymenoptera: Aphelinidae) under natural conditions in Kyoto, Japan. Appl. Entomol. Zool. 41, 139–144. https://doi.org/10.1303/aez.2006.139

Taylor, S., Laub, C., 2020. Insect control in field crops [WWW Document]. Virginia Coop. Ext. URL https://www.pubs.ext.vt.edu/content/dam/pubs_ext_vt_edu/456/456-016/ENTO-335C.pdf (accessed 1.10.20).

Taylor, S. V., Burrack, H.J., Michael Roe, R., Bacheler, J.S., Sorenson, C.E., 2015. Systemic imidacloprid affects intraguild parasitoids differently. PLoS One 10, 1–13. https://doi.org/10.1371/journal.pone.0144598

Team, R.C., 2018. R: A language and environment for statistical computing. Vienna, Austria.

Teder, T., Knapp, M., 2019. Sublethal effects enhance detrimental impact of insecticides on non-target organisms: A quantitative synthesis in parasitoids.

Chemosphere 214, 371–378. https://doi.org/10.1016/j.chemosphere.2018.09.132

Tooker, J.F., Douglas, M.R., Krupke, C.H., 2017. Neonicotinoid seed treatments: Limitations and compatibility with Integrated Pest Management. Agric. Environ. Lett. 2, 170026. https://doi.org/10.2134/ael2017.08.0026

Torchiano, M., 2019. effsize: Efficient effect size computation [WWW Document]. https://doi.org/10.5281/zenodo.1480624

Ullah, F., Gul, H., Desneux, N., Gao, X., Song, D., 2019. Imidacloprid-induced hormesis effects on demographic traits of the melon aphid, *Aphis gossypii*. Entomol. Gen. 39, 325–337. https://doi.org/10.1127/entomologia/2019/0892

Van den Brink, P.J., Ter Braak J. F., C., 1999. Principal Response Curves: Analysis of time-dependent multivariate responses of biological community to stress. Environ. Toxicol. Chem. 18, 138–148.

van der Sluijs, J.P., Amaral-Rogers, V., Belzunces, L.P., Bijleveld van Lexmond, M.F.I.J., Bonmatin, J.M., Chagnon, M., Downs, C.A., Furlan, L., Gibbons, D.W., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C., Liess, M., Long, E., McField, M., Mineau, P., Mitchell, E.A.D., Morrissey, C.A., Noome, D.A., Pisa, L., Settele, J., Simon-Delso, N., Stark, J.D., Tapparo, A., Van Dyck, H., van Praagh, J., Whitehorn, P.R., Wiemers, M., 2015. Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. Environ. Sci. Pollut. Res. Int. 22, 148–154. https://doi.org/10.1007/s11356-

014-3229-5

Van der Sluijs, J.P., Simon-Delso, N., Goulson, D., Maxim, L., Bonmatin, J.M., Belzunces, L.P., 2013. Neonicotinoids, bee disorders and the sustainability of pollinator services. Curr. Opin. Environ. Sustain. 5, 293–305. https://doi.org/10.1016/j.cosust.2013.05.007

van Gestel, C.A.M., de Lima e Silva, C., Lam, T., Koekkoek, J.C., Lamoree, M.H., Verweij, R.A., 2017. Multigeneration toxicity of imidacloprid and thiacloprid to *Folsomia candida*. Ecotoxicology 1–9. https://doi.org/10.1007/s10646-017-1765-8

Van Hoesel, W., Tiefenbacher, A., König, N., Dorn, V.M., Hagenguth, J.F., Prah, U., Widhalm, T., Wiklicky, V., Koller, R., Bonkowski, M., Lagerlöf, J., Ratzenböck, A., Zaller, J.G., 2017. Single and combined effects of pesticide seed dressings and herbicides on earthworms, soil microorganisms, and litter decomposition. Front. Plant Sci. 8. https://doi.org/10.3389/fpls.2017.00215

van Steenis, M.J., 1993. Intrinsic rate of increase of *Aphidius colemani* Vier. (Hym., Braconidae), a parasitoid of *Aphis gossypii* Glov. (Hom., Aphididae), at different temperatures. J. Appl. Entomol. 116, 192–198.

van Toor, R.F., Butler, R.C., Stufkens, M.A.W., Teulon, D.A.J., 2016. Lower incidence of yellow dwarf disease in autumn-sown wheat crops in New Zealand is linked with sowing dates , insecticide regimes and aerial aphid numbers. Australas. Plant Pathol. 45, 609–619. https://doi.org/10.1007/s13313-016-0450-3

Vidau, C., Diogon, M., Aufauvre, J., Fontbonne, R., Viguès, B., Brunet, J.L., Texier, C., Biron, D.G., Blot, N., Alaoui, H., Belzunces, L.P., Delbac, F., 2011. Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. PLoS One 6. https://doi.org/10.1371/journal.pone.0021550

Walls, J., Rajotte, E., Rosa, C., 2019. The past, present, and future of barley yellow dwarf management. Agriculture 9, 23. https://doi.org/10.3390/agriculture9010023

Wang, F., Yao, J., Chen, H., Yi, Z., Choi, M.M.F., 2014. Influence of short-time imidacloprid and acetamiprid application on soil microbial metabolic activity and enzymatic activity. Environ. Sci. Pollut. Res. 21, 10129–10138. https://doi.org/10.1007/s11356-014-2991-8

Wang, S.Y., Qi, Y.F., Desneux, N., Shi, X.Y., Biondi, A., Gao, X.W., 2017. Sublethal and transgenerational effects of short-term and chronic exposures to the neonicotinoid nitenpyram on the cotton aphid *Aphis gossypii*. J. Pest Sci. (2004). 90, 389–396. https://doi.org/10.1007/s10340-016-0770-7

Wang, Y., Wu, S., Chen, L., Wu, C., Yu, R., Wang, Q., Zhao, X., 2012. Toxicity assessment of 45 pesticides to the epigeic earthworm *Eisenia fetida*. Chemosphere 88, 484–491. https://doi.org/10.1016/j.chemosphere.2012.02.086

Whalen, J.M., Spellman, M.P., Kline, W.L., Kline, S.T., n.d. Winter wheat IPM field guide for Delaware and Maryland [WWW Document]. Univ. Delaware

Coop. Ext. URL https://nj-vegetable-crops-online-resources.rutgers.edu/wp-content/uploads/2015/06/Wheat-IPM-Insect-Disease-Field-Guide-for-DE.pdf (accessed 1.10.20).

Whitehorn, P.R., Cook, N., Blackburn, C. V, Gill, S.M., Green, J., Shuker, D.M., Whitehorn, P.R., 2015. Sex allocation theory reveals a hidden cost of neonicotinoid exposure in a parasitoid wasp. Proc. R. Soc. B 282, 20150389. https://doi.org/http://dx.doi.org/10.1098/rspb.2015.0389

Whitehorn, P.R., O'Connor, S., Wackers, F.L., Goulson, D., 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science (80-.). 336, 351–352. https://doi.org/10.1126/science.1215025

Wilde, G., Roozeboom, K., Ahmad, A., Claassen, M., Gordon, B., Heer, W., Maddux, L., Martin, V., Evans, P., Kofoid, K., Long, J., Schlegel, A., Witt, M., 2007. Seed treatment effects on early-season pests of corn and on corn growth and yield in the absence of insect pests. J. Agric. Urban Entomol. 24, 177–193. https://doi.org/10.3954/1523-5475-24.4.177

Winder, L., Colin, J., Holland, J.M., Woolley, C., Perry, J.N., 2001. Modelling the dynamic spatio-temporal response of predators to transient prey patches in the field. Ecol. Lett. 4, 568–576.

Winder, L., Perry, J.N., Holland, J.M., 1999. The spatial and temporal distribution of the grain aphid Sitobion avenae in winter wheat. Entomol. Exp. Appl. 93, 277–290.

Yamamuro, M., Komuro, T., Kamiya, H., Kato, T., Hasegawa, H., Kameda, Y.,

2019. Neonicotinoids disrupt aquatic food webs and decrease fishery yields. Science 366, 620–623. https://doi.org/10.1126/science.aax3442

Yu, B., Chen, Z., Lu, X., Huang, Y., Zhou, Y., Zhang, Q., Wang, D., Li, J., 2020. Effects on soil microbial community after exposure to neonicotinoid insecticides thiamethoxam and dinotefuran. Sci. Total Environ. 725. https://doi.org/10.1016/j.scitotenv.2020.138328

Yu, Y., Shen, G., Zhu, H., Lu, Y., 2010. Imidacloprid-induced hormesis on the fecundity and juvenile hormone levels of the green peach aphid Myzus persicae (Sulzer). Pestic. Biochem. Physiol. 98, 238–242. https://doi.org/10.1016/j.pestbp.2010.06.013

Zaller, J.G., König, N., Tiefenbacher, A., Muraoka, Y., Querner, P., Ratzenböck, A., Bonkowski, M., Koller, R., 2016. Pesticide seed dressings can affect the activity of various soil organisms and reduce decomposition of plant material. BMC Ecol. 16, 37. https://doi.org/10.1186/s12898-016-0092-x

Zamani, A.A., Talebi, A., Fathipour, Y., Baniameri, V., 2007. Effect of temperature on life history of *Aphidius colemani* and *Aphidius matricariae* (Hymenoptera: Braconidae), two parasitoids of *Aphis gossypii* and *Myzus persicae* (Homoptera: Aphididae). Environ. Entomol. 36, 263–271.

Zhang, P., Ren, C., Sun, H., Min, L., 2018. Sorption, desorption and degradation of neonicotinoids in four agricultural soils and their effects on soil microorganisms. Sci. Total Environ. 615, 59–69. https://doi.org/10.1016/j.scitotenv.2017.09.097

Zhang, P., Zhang, X., Zhao, Y., Wei, Y., Mu, W., Liu, F., 2016. Effects of imidacloprid and clothianidin seed treatments on wheat aphids and their natural enemies on winter wheat. Pest Manag. Sci. 72, 1141–1149. https://doi.org/10.1002/ps.4090

Zhang, Q., Xue, C., Wang, C., 2015. Effects of imidacloprid on soil microbial communities in different saline soils. Environ. Sci. Pollut. Res. 22, 19667–19675. https://doi.org/10.1007/s11356-015-5154-7

Zhou, G.C., Wang, Y., Zhai, S., Ge, F., Liu, Z.H., Dai, Y.J., Yuan, S., Hou, J.Y., 2013. Biodegradation of the neonicotinoid insecticide thiamethoxam by the nitrogen-fixing and plant-growth-promoting rhizobacterium *Ensifer adhaerens* strain TMX-23. Appl. Microbiol. Biotechnol. 97, 4065–4074. https://doi.org/10.1007/s00253-012-4638-3

Zwiener, C.M., Conley, S.P., Bailey, W.C., Sweets, L.E., 2005. Influence of aphid species and barley yellow dwarf virus on soft red winter wheat yield. J. Econ. Entomol. 98, 2013–2019. https://doi.org/10.1603/0022-0493-98.6.2013