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Diversifying Cornfields by Interseeding Cover Crops: Practical Implications and the Response of Invertebrate Communities

Michael Bredeson

South Dakota State University

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DIVERSIFYING CORNFIELDS BY INTERSEEDING COVER CROPS: PRACTICAL

IMPLICATIONS AND THE RESPONSE OF INVERTEBRATE COMMUNITIES

BY

MICHAEL BREDESON

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MICHAEL BREDESON

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Jonathan Lundgren, Ph.D.
Thesis Advisor   Date

Alexander Smart, Ph.D.
Thesis Advisor   Date

Michele Dudash, Ph.D.
Head, Department of Communications   Date

Dean, Graduate School   Date

"One of the penalties of an ecological education is that one lives alone in a world of wounds. Much of the damage inflicted on land is quite invisible to laymen. An ecologist must either harden his shell and make believe that the consequences of science are none of his business, or he must be the doctor who sees the marks of death in a community that believes itself well and does not want to be told otherwise."
-Aldo Leopold

This dissertation is dedicated to those agricultural producers who live by the words of history's greatest conservationists and, with a sense of urgency, work each day to better conserve natural resources on the land they steward.

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My wife, Tera, will receive the final acknowledgement because it is she who has been the most important person in my life throughout graduate school. Tera has been a remarkably supportive and positive partner, ever-understanding when my research and schoolwork pulled me away from home. Without her this process would have been a much grander challenge, and I cannot thank her enough for her boundless confidence in me.

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ABSTRACT

DIVERSIFYING CORNFIELDS BY INTERSEEDING COVER CROPS: PRACTICAL

IMPLICATIONS AND THE RESPONSE OF INVERTEBRATE COMMUNITIES

MICHAEL BREDESON

2019

The lack of suitable habitat conditions for beneficial organisms in simplified

agroecosystems leads to unstable invertebrate communities and overreliance on chemical

control of herbivores. It is possible to manage pest populations without agrichemicals by

manipulating farmland so that herbivores are impaired by plant-driven bottom-up and

enemy-driven top-down antagonisms. Interseeding cover crops between established crop

rows is a method used by farmers to improve habitat suitability for natural enemies and

hinder host-finding, feeding and movement by herbivores. Here I address three important

research gaps related to interseeding cover crops. A calcium carbonate seed coating, used

to improve seed-broadcasting efficiency, was tested to determine if arthropod granivores

are deterred from consuming cover crop seeds. Invertebrate communities were compared

between monoculture cornfields and cornfields possessing a mixture of cover crop

species. Finally, neonicotinoid seed treatment, thiamethoxam, and metabolite,

clothianidin, were quantified within vegetative tissue of cover crops growing between

seed-treated corn to examine a potential route of exposure by non-target organisms. The

addition of a calcium carbonate seed coating reduced seed granivory by invertebrates.

Hairy vetch (*Vicia villosa*) and sorghum × sudan (*Sorghum × drummondii*) were

especially protected. Gryllidae, Carabidae and Staphylinidae were the most abundantly-

collected granivores. Corn interseeded with cover crops possessed a larger abundance of surface-dwelling predators, herbivores, numerous individual taxa and total invertebrates than monocultures. Greater epigeic species richness was also recorded in cover-cropped fields. With the exception of four individual taxa, subterranean invertebrate abundances were unchanged between interseeded and monoculture corn, however, cover crops did increase species diversity below the soil surface. Interseeding did not affect species richness, diversity or arthropod abundance on corn foliage. Thiamethoxam and toxic metabolite, clothianidin, were detected in interseeded hairy vetch and cereal rye (*Secale cereale*) on all but one sampling dates during the corn growing season with highest concentrations in earlier samplings. On each collection date clothianidin was found at a higher level than thiamethoxam for both species. As management techniques improve interseeding cover crops has potential to become an increasingly important tool for restoring agroecosystem functions if the incompatibility of added plant diversity and existing pesticide strategies is addressed.

INTRODUCTORY STATEMENT FROM THE AUTHOR

Industrialized agriculture in developed nations has led to a simplification of the agrolandscape, a phenomenon impossible to ignore when traveling through United States farm country. Substitution of native vegetation with a small number of crop species grown in monoculture has resulted in substantial degradation of ecosystem services and functions once fully supported by native plant communities. Production of food, fuel and fiber to sustain a burgeoning human population has come at the cost of biodiversity, climate and landscape change, and environmental intoxication, to name a few. The question becoming ever-more present in the minds of agroecologists is, how can we design food production systems which drastically reduce the currently-observed negative environmental footprint, and furthermore, how do we restore the damage done?

Within the area of food production and land management there are a great number of things which can be done to improve the functionality of land crops are grown on. Disturbing earth less-frequently by eliminating mechanical soil turbation and drastically curtailing pesticide use are both ways we can soften our negative production impacts. In concert with limiting disturbances, the addition of plant diversity on field and landscape scales shows promise for restoring important ecosystem services.

Transitioning from crop monocultures to mixed-cropping systems is a feasible method for bolstering cropland biodiversity and may be applicable on an extensive scale. Whether it be multiple harvestable crops grown simultaneously, or one crop in companionship with non-crop supporting plant species a growing number of farmers are diversifying cropland through these techniques despite a lack of research in this area to reinforce decision making.

Invertebrate communities, especially those species beneficial to agricultural producers stand to gain suitable habitat conditions from the plant-derived resources provided under a mixed-cropping regimen. Complex plant communities provide nutritive and abiotic elements necessary for sustained inhabitance by these organisms. Persistent beneficial arthropod populations in farmland perform a plethora of services such as nutrient cycling, soil aeration, weed seed granivory, pollination, and predation of crop herbivores. It is the biological control of pests which drives many producers to adopt techniques for plant diversification, as chemical control has both high economic and environmental costs.

There is an urgent need for research observations and on-farm trial-and-error to elucidate farming techniques which go beyond ameliorating the negative effects of production agriculture. Restoration of functionality and health to working lands is also in order. Interseeding plant diversity into crops which are usually grown as monocultures might be one of these techniques.

This dissertation addresses numerous factors related to the response of cornfield (*Zea mays*) invertebrate communities to the addition of plant diversity via interseeding cover crops. It is the goal of the author to provide transferable information on how top-down and bottom-up forces in a diversified habitat serve to prevent herbivore outbreaks. In the following chapters readers will be informed of the previous works and developed theories explaining the mechanistic response of arthropod communities to cropland plant diversification. Primary research results will also be revealed addressing several previously unanswered questions regarding interseeding cover crops into an extensively-grown plant species, corn. First, the effect of coating cover crop seeds in calcium

carbonate to prevent arthropod granivory of surface-broadcasted seeds is documented. Second, invertebrate community responses and generalist predator activity are compared in corn monocultures and corn possessing interseeded cover crops. Finally, the possible intoxication of cover crops by neonicotinoid seed-treated corn is explored by quantifying pesticide levels in plants meant to provide resources to beneficial invertebrates.

CHAPTER ONE: TOP-DOWN AND BOTTOM-UP MECHANISMS FOR

INVERTEBRATE PEST MANAGEMENT IN MIXED-CROPPING SYSTEMS

Abstract

Conventional agricultural land is often managed as large-scale monocultures experiencing frequent disturbances in the form of tillage, pesticides, and fallow periods. Cropland managed in this fashion is often subject to damage via herbivore populations which function relatively unchallenged in an environment where natural enemy habitat requirements are not met. As a result, both prophylactic and reactive insecticides are utilized to prevent such outbreaks, not without some negative non-targeted effects. To remediate this phenomenon, agricultural producers have begun to integrate management practices which mimic the diversity and stability of natural ecological systems where pest outbreaks are rare due to a combination of predator-driven top-down, and plant-mediated bottom-up antagonisms. Many methods exist for the diversification of cropland plant communities, but there still often remains a substantial period during the growing season where plants are gown in monoculture. One method gaining attention is the use of interseeding technology to plant cover crops between rows of established focal crops. The added plant diversity of interseeded cover crops can change agroecosystem conditions to become more favorable for maintaining natural enemy communities (top-down) and provide a less hospitable environment for herbivorous arthropods (bottom-up). Predatory and parasitic natural enemies can persist in croplands depauperate of specialist crop herbivores if non-prey foods items such as nectar, pollen, and alternative prey are provided as a nutritional substitute from non-crop plants. In addition to meeting the

nutritional needs of top-down antagonists, physical conditions of the farmscape must also be conducive for survival of these species. Alternative plant species can ameliorate extreme abiotic conditions, and provide structures for shelter, prey capture, and oviposition. Impedance of an herbivore recognizing the visual and olfactory ques necessary in host plant finding, and physical impairment of pest movement are important effects of diversifying the cropland plant communities. Here we discuss the underlying mechanistic effects of diversifying annual cropland plant communities through interseeding on the management of herbivores and conservation of beneficial arthropods.

Keywords: Conservation biological control, cover crops, integrated pest management (IPM), intercropping, interseeding, natural enemies

1.0 Introduction

Though drastically altered and simplified in comparison to its pre-agricultural

state, farmland is nonetheless an ecosystem governed by biological processes, and to the

dismay of many land managers, is not a simple medium by which crops grow

unchallenged by antagonists. Instead, farmland abides by the same rules as natural

ecosystems. When populations of a single plant species becomes abundant and

monoculture-like in natural habitats, a diverse suite of herbivores, diseases, and plant

competitors act to correct the imbalance (Hunter and Price, 1992). Within simplified

cropping systems these biological actors enter as "weeds", providing ecosystem services

such as erosion prevention, accumulating excess nutrients and offering resources to

pollinators and predators, to name a few (Blaix et al., 2018). Cropland monocultures are

attractive to herbivores and pathogens. These crop antagonists are undesirable for

agricultural producers because they are often expensive to suppress and can result in

reduced crop productivity. As annual cropland is repeatedly brought back to a state of

early secondary succession each growing season, the natural progression of plant

community diversification and habitat stabilization is quashed by inputs of tillage,

pesticides, and fertilizers to support the favored growing conditions for a single plant

species (Altieri, 1999; Odum, 1966). Such conditions are ripe for supporting populations

of damage-inflicting invertebrates whose host plant is abundantly available, and poor for

supporting beneficial invertebrates that require the resources offered in plant-diverse

habitats (Altieri and Nicholls, 2003).

Suppression of ecological succession is not a trivial task, and maintenance of

herbivore-free monocultures through regular implementation of anthropogenic inputs can

be economically (Johnson et al., 2009) and energetically (Deike et al., 2008) costly for farmers as well as environmentally degrading (Goulson, 2013). Insect pest management on conventionally managed cropland uses a combination of prophylactic and reactive pesticide applications (Douglas and Tooker, 2015; Johnson et al., 2009), and genetically engineered (GE) crops that confer resistance to herbivores (Fausti et al., 2011). However, these management techniques are frequently cited as having detrimental effects on beneficial and non-targeted organisms either through direct toxicity (Moser et al., 2008; Pisa et al., 2015) or indirectly by simplifying landscapes and limiting nutritional resources (Fausti, 2015; Meehan et al., 2011).

Pressure from consumer groups desiring pesticide-free food and fiber (Magnusson and Cranfield, 2005), and political entities banning insecticides and GE crops is encouraging farmers to adapt their management practices, but existing barriers are slowing progress. The current agricultural paradigm in developed countries overwhelmingly relies on large-scale monocultures for production. As a result, government regulation, agricultural equipment, as well as on- and off-farm infrastructure has been developed to support this paradigm (Fausti, 2015). Additionally, advanced management strategies for achieving maximal crop yield and suppressing plant competition has resulted in a farmer ethic which socially rewards producers whom are achieving high yields (via regional yield contests) in insect- and weed-free monocultures. Clearly, social, regulatory, and infrastructural changes must be addressed to support producers and speed the transition toward natural resource conservation in a diversified agricultural ecosystem.

Pre-industrialization of agriculture in developed nations [arguably with the advent of synthetic fertilizers in the early 1900s (Erisman et al., 2008)], farmers managed cropland hosting much greater diversity than is seen in today's farming landscapes. This is partly because access to synthetic fertilizers and pesticides was limited, but also because individual family-owned and operated farms were supported through multiple sources of revenue (i.e. livestock, dairy, vegetables, forage, bedding, etc.) (Berglund et al., 2014). To maintain these different farm assets producers had to incorporate different types of plants and plant mixtures in a more complex rotation, including periods of perenniality. In concert with rotational complexity and perennial crops, plant diversity was undoubtedly bolstered through the presence of weeds (Timmons, 2017). In terms of integrated pest management (IPM), increased diversity across the agricultural landscape through this "older" way of farming would have provided a hospitable environment for beneficial arthropods, while making it difficult for specialist herbivore populations to increase to outbreak levels (Barzman et al., 2015). With recent advances in seeding technology, and a clearer understanding of the beneficial associations between plant diversity and pest management the ecological benefits of a more diverse "older" style of farming can once again be realized in the modern production regimen without sacrificing productivity (Martens et al., 2015).

Agricultural producers pioneering conservation in agriculture recognize the futility of resisting ecological succession and have begun to explore alternative cropping system designs which are less reliant on inputs for the control of crop pests (LaCanne and Lundgren, 2018). Instead, farmers are beginning to implement production regimens which mimic ecosystems that naturally resist pest outbreaks. A defining characteristic of

most natural habitats is the presence of multi-species, genetically diverse plant communities (Schmid, 2014). Plant diversification is a prerequisite for sustained, simultaneous top-down and bottom-up control of herbivores. Commenting on the importance of complexity and diversity within mature ecological habitats for the prevention of pest outbreaks Odum (1966) states, "Such mechanisms enable the biological community to maintain the large and complex organic structure that mitigates perturbations of the physical environment. Severe stress or rapid changes (i.e. tillage, pesticides and fertilizer) brought about by outside forces can of course, rob the system of these protective mechanisms and allow irruptive, cancerous growths of certain species (i.e. herbivores, weeds and pathogens) to occur, as man too often finds to his sorrow."

Conservation of natural enemies is an important top-down method to prevent the "cancerous growth" of pest populations, and is a component of ecologically-based pest management that land managers can directly affect through the manipulation of cropland plant assemblages (Landis et al., 2000). Habitat suitability for biological control agents largely depends on the organism's access to vital nutritional resources and refugia. Establishing suitable habitat conditions through plant diversification is necessary for maintaining persistent natural enemy populations and supporting their ecosystem services (Holland et al., 2016; Landis et al., 2005; Landis et al., 2000).

Diverse plant assemblages also hinder herbivore population growth and feeding efficiency through plant-mediated, bottom-up forces (Moreira et al., 2016). Though there is much to be discovered regarding the effects of chemical ecology and multi-trophic interactions on herbivory in diversified agricultural habitats a few mechanisms have been identified. For example, interference of a pest's host-finding capabilities by impairing

clear recognition of visual (Degen and Städler, 1996) and olfactory cues (Togni et al., 2010) and subsequent limited locomotion due to the presence of non-host plants (Mazzi and Dorn, 2012) can reduce herbivore success in mixed cropping systems.

Augmenting plant and genetic diversity in cropland can be accomplished through numerous methods (Altieri and Letourneau, 1982; Landis et al., 2005; Landis et al., 2000). Current strategies are aimed at diversification of cropland temporally [crop rotation (Barzman et al., 2015)], during periods when cropland would otherwise be fallow [fallow cover cropping (Lundgren and Fergen, 2010)], or in non-cropped habitats (Tschumi et al., 2015). Though these are important strategies inclusive in a holistic pest management framework, and shouldn't be ignored, a significant portion of the growing season remains un-diverse as focal crop species grow in monoculture. Interseeding additional plant species between crop rows has become a viable management tool used by farmers to diversify would-be monocultures and mimic the conditions found in late-succession, self-regulating natural habitats (Belfry and Van Eerd, 2016; Noland et al., 2018).

Interseeded cover crops (CCs) are used in farmland habitats to meet several agronomic goals. As a management tool interseeding can positively affect weed (Uchino et al., 2015) and disease suppression (Uzokwe et al., 2016), soil microbial communities (Lange et al., 2015), erosion prevention (Sij et al., 2016), water infiltration and water use efficiency (Gulick et al., 1994; Hu et al., 2016), nutrient fixation and carbon sequestration (Lange et al., 2015), and pollinator and other wildlife populations (Pereira et al., 2015). In addition to these benefits, interseeding CCs shows great promise for maintaining

herbivorous pest populations to below economically-threatening levels (Manandhar and Wright, 2016), potentially curtailing or even eliminating the need for chemical control. As we further understand the negative environmental significance of simplified agricultural landscapes and non-targeted effects of chemical pest control the need to identify reliable alternative methods is prodigious. Here we address the implications of current insect pest control in conventional monocultures and review the known mechanisms for herbivore suppression in cropland augmented with plant diversity during the growing season via interseeding additional plant species. It is our aim that this document will make land managers aware of potential hazards associated with insecticide usage and assist farmers in developing plans to diversify cropland in their own unique growing situations by gaining a mechanistic understanding of how plant diversification through interseeding can inhibit arthropod pests.

2.0 Replacement of biological control with human capital, and consequences of doing so

Despite published evidence of higher yields in some mixed versus monocropping systems (Putnam and Allan, 1992; Qiao et al., 2016; Reiss and Drinkwater, 2018; Vandermeer, 1992), modern agricultural practices have trended toward a simplified system of monocultures having negative environmental consequences. Examples of this are especially evident across much of USA's "corn belt", where 37 million ha in 2017 was planted to a single species, corn (Zea mays), (NASS, 2017). Extensification and simplification of farmland has led to a replacement of naturally occurring biological control of pests, pathogens and weeds (natural capital) with anthropogenic substitutes like

tillage, pesticides, etc. (human capital). Prior to commercially available pesticides and GE crops farmers largely relied on ecological principals to avert herbivorous insect outbreaks (Barzman et al. 2015). Insecticides and genetically-modified plants possessing insecticidal proteins frequently replace crop rotation, scouting, and other IPM practices in making farmland unsuitable for herbivore proliferation (Pilcher et al., 2002).

2.1 GM crops

In 1994 potatoes (*Solanum tuberosum*) became the first commercially available crop to possess genes from the bacterium *Bacillus thuringiensis* (*Bt*) for controlling the Colorado potato beetle (*Leptinotarsa decemlineata*) (Perlak et al., 1993). Numerous other extensively grown crops have since been modified to possess *Bt* genes encoding for insecticidal proteins and are now commercially available for on-farm use (Huesing and English, 2004). Corn farmers were first able to purchase seeds genetically engineered to resist the lepidopteran insect pest, *Ostrinia nubilalis* (Hübner) using *Bt* technology in 1996 (Pilcher et al., 2002). Seven years later, corn varieties engineered to possess toxic proteins against western corn rootworm (*Diabrotica virgifera virgifera*) also became available (Gassmann et al., 2011). *Bt* corn effectively controlled pests (Keweshan et al., 2015), which has led to a steady increase in adoption by growers across the Midwest, regardless of evidence for the development of resistance by some targeted species (Wangila et al., 2015). In 2018, *Bt* hybrids constituted 82% of planted corn (USDA-ERS, 2018). This large-scale acceptance of GM crops by producers has not been without some unintended direct and indirect consequences.

There is little evidence for negative effects of GM crops on entire beneficial invertebrate communities (Lozzia, 1999; Wolfenbarger et al., 2008) or in other groups of soil biota (Saxena and Stotzky, 2001). However, there are numerous documented cases where harm to individual species is observed. For instance, Moser et al. (2008) measured prolonged development in fourth-instar zoophytophagous ladybeetles [*Coleomegilla maculata* (DeGeer)] after consuming *Bt* corn seedling tissue compared to individuals feeding on non-*Bt* seedlings. Monarch (*Danaus plexippus*) larvae consuming milkweed (*Asclepias curassavica*) tissue dusted with pollen from *Bt* corn exhibited slower development, reduced feeding, and greater mortality compared to larvae consuming undusted milkweed (Losey et al., 1999). Interestingly, predatory lacewing (*Chrysoperla carnea*) larvae reared on larval cotton leafworms (Noctuidae: *Spodoptera littoralis*) which had been fed an artificial diet containing *Bt* toxins resulted in significantly higher lacewing mortality compared to individuals consuming unintoxicated prey (Hilbeck et al., 1999).

Beyond the direct effects of GM corn on non-targeted invertebrates, widescale adoption of this pest management tool has been followed by an overreliance on *Bt* technology. In fact, fields are often planted to *Bt* corn without being rotated with another species for several years (Gassmann et al., 2012), with some midwestern fields having continuous corn for >10 consecutive seasons (personal communication with Minnesota corn grower, Jacob Bredeson). *Bt* dependence by producers has resulted in the development of resistance by herbivores (Gassmann et al., 2011), poor IPM technique by land managers (Pilcher et al., 2002) and simplified farmscapes (Fausti, 2015; Lundgren and Fausti, 2015).

The effects of transgenic *Bt* plants and pollen on non-targeted invertebrates is hotly debated within the scientific literature (Gatehouse et al., 2002; Scriber, 2001), with studies describing toxicity to beneficial organisms (as described above) as well as studies documenting no ill ecological effects (Gatehouse et al., 2002; Li and Romeis, 2010). Direct toxicity of pesticidal GM crops to pollinators, predators and other beneficial organisms seems to be situational and warrants further discussion and research that especially considers multi-trophic interactions (Hilbeck et al., 1999).

2.2 Insecticides

Synthetic insecticide use persists as a popular tool for conventional farmers despite wide-spread adoption of insect-resistant GM crops. Though GM crops were developed, in part, to reduce synthetic insecticide use (Romeis et al., 2006), the opposite has occurred, and insecticide use has increased in corn-dominated regions. As described in Fausti et al. (2011), the percentage of corn planted in South Dakota possessing insecticidal GM traits grew from 33.0% in 2000, to 59.0% in 2007. Interestingly, the same study concluded that for each 1.0% increase of GM corn acres planted in South Dakota, there was a subsequent increase of 0.24% in the number of corn acres treated with an insecticide (Fausti et al., 2011). Though increased insecticide use in corn identified by Fausti et al. (2011) is concerning their study didn't capture the more recent adoption of insecticidal seed treatments, which has further increased the quantity of insecticides applied in cornfields. In fact, Douglas and Tooker (2015) estimate that in 2010 as much as 594,036 kg neonicotinoid active ingredient was applied to corn as a seed treatment, and by 2011, 87.0% of US corn acres were planted with a neonicotinoid seed

treatment, a remarkable adoption rate considering that these seed treatments only became widely available to farmers in the early 2000's (Douglas and Tooker, 2015).

Replacement of natural capital and ecologically-based cropland management by neonicotinoids and other synthetic insecticides has led to several unintended and negative consequences in both agricultural and non-agricultural habitats. For example, two worm species, one anecic (*Aporrectodea nocturna*) and one endogeic (*Allolobophora icterica*) were observed by Capowiez et al. (2005) to have greater weight loss, higher mortality, and frequent development of abnormal "globular swellings" when subjected to field-collected soils polluted with the neonicotinoid imidacloprid compared to untreated soils. Any detrimental effect of synthetic pesticides to worms or other members of the detritivore community could lead to a disruption of nutrient and organic matter cycling (Altieri, 1999). In thiamethoxam seed-treated sunflower (Helianthus annuus) fields, both pollinator and predator (especially Coleoptera: Coccinellidae) community abundances were significantly reduced when compared to untreated sunflowers (Bredeson and Lundgren, 2018). By hindering beneficial invertebrates, insecticide use can have detrimental effects on the ecosystem services that they provide, including predation (Douglas et al., 2015) and parasitism of herbivores (Moscardini et al., 2014), weed seed granivory (Cutler et al., 2016), nutrient cycling (Capowiez et al., 2005), and pollination of food crops (Stanley et al., 2015). Agritoxins have also been implicated in the global decline of other non-arthropod animals for reasons such as immune suppression and reduced prey abundance (Gibbons et al., 2015; Mason et al., 2013).

Humans are not isolated from exposure to commonly used insecticides. Predictably, farmers and commercial applicators who frequently handle pesticides can

possess measurable levels of agrochemicals within their bodily fluids (Cox, 1994; Kasiotis and Machera, 2015). Exposure to pesticides is difficult to avoid even for those who are not involved in agriculturally-related activities. For example, recent research determined that post-treatment drinking water at the University of Iowa's main campus in Iowa City was contaminated with the neonicotinoids imidacloprid, clothianidin and thiamethoxam (Klarich et al., 2017). Commonly purchased consumer goods can also be contaminated with agricultural pesticides. Chen et al. (2014) quantified neonicotinoid insecticides in fruit and vegetable samples purchased from a local Boston, MA grocery store. Of the 25 produce items examined, 23 possessed measurable amounts of insecticide (tomatoes and nectarines did not), and 45% of vegetables, and 72% of fruits were found to have more than one type of neonicotinoid in their tissues (Chen et al., 2014).

As non-target effects of agricultural toxins become more apparent, producers and researchers are exploring methods and technological advancements to reduce the need for, and negative effects of, pesticides in industrialized farming. Diversifying farmland plant communities and harnessing the naturally-provided services of biological control through 'ecologically-based pest management' is a method gaining traction by farmers for restoration of ecosystem services within managed lands (LaCanne and Lundgren, 2018). The remainder of this manuscript will be a discussion on how the maintenance of invertebrate pests below economic thresholds is aided through diversification of cropland plant communities. Special attention will be given to the effects of diversification via interseeding additional plant species during annual crop growing seasons typically produced as monocultures.

3.0 Non-interseeding methods for row crop agroecosystem diversification

Farmers have access to a wide variety of options for agroecosystem diversification ranging from large-scale landscape-level changes, down to genetic variations between plants of the same species. Augmented plant diversity on all spatial levels results in habitat heterogenization and subsequent development of niches to be occupied by higher organisms, including natural enemies of crop pests (Jimenez-Valverde and Lobo, 2007).

In agriculturally-dominated regions, landscapes are diversified by possessing a wide variety of land use types and different crop species. Regionally, farmscapes may possess little variation, such as those where only one or two crops prevail, whereas in other regions a milieu of natural habitats, pastures, and numerous plants in cultivation can exist simultaneously. As landscapes possessing numerous land-use types also receive varied disturbance regimes over time, there is greater potential for extended resource availability within a locality to support beneficial invertebrates (Tscharntke et al., 2008). Spill-over of invertebrates between natural and agricultural habitats, or between agricultural habitats offering resources at different times allows natural enemies with adequate dispersal capabilities to acquire their basic requirements for survival (Tscharntke et al., 2005). Gardiner et al. (2009) performed a comprehensive study on the effects of landscape diversity on soybean aphid (*Aphis glycines*) biological control in 26 fields across Iowa, Michigan, Minnesota and Wisconsin. As the diversity of crop and non-crop habitat increased in the area surrounding soybean fields biological control of aphids was improved. A possible contributing factor to the observed biological control

was an increase in abundance of an important aphid predator group, Coccinellidae, as the proportion of forests and grasslands increased around study areas.

Within landscapes, small parcels of land set aside for non-crop habitat, such as beetle banks (MacLeod et al., 2004), flowering strips (Tschumi et al., 2015), hedgerows (Morandin et al., 2014) and other natural areas (Holland et al., 2016) function in part as sources for beneficial arthropods which spill over into cropped habitat to provide parasitism and predation of herbivores (Landis et al., 2000). Tschumi et al. (2015) discovered that in Swiss winter wheat fields planted with flowering strip borders, cereal leaf beetle (*Oulema* sp.) larvae and adult populations were reduced by 40% and 53%, respectively, and plant damage was reduced by 61% when compared to unbordered fields. The reduction of cereal leaf beetles was likely a result of increased natural enemy abundance observed within and adjacent to flowering strips (Tschumi et al., 2015). In a similar case, tomato (*Lycopersicon esculentum*) fields planted adjacent to perennial hedgerows had significantly more abundant natural enemy populations than fields without hedgerows. As a result, aphid (primarily *Macrosiphum euphorbiae*) populations on tomato plants were reduced below economically damaging levels (Morandin et al., 2014).

A complex crop rotation can break pest cycles by establishing periodic conditions

> "A diversified crop sequence prevents selection and buildup of the best-adapted pest populations"
> -Barzman et al. (2015)

that are unfavorable for pest establishment and proliferation (Barzman et al., 2015; Lundgren et al., 2017; Reeves, 2017). Indeed, crop rotation has been recommended for managing pests in our most commonly grown crops for more than one hundred years (see

Hill et al,. 1948, for examples with *Diabrotica* spp. in corn). More recently, Kabaluk and

Vernon (2000) found that Canadian potato fields suffered increasing tuber flea beetle

(*Epitrix tuberis*) populations as the number of continuous potato-growing seasons

progressed without rotating to a different plant species. The researchers also found that

potato fields without a history of crop rotation required more insecticide for herbivore

control (Kabaluk and Vernon, 2000). Barzman et al. (2015) highlighted the effectiveness

of crop rotation very clearly, when they stated, "A diversified crop sequence prevents

selection and buildup of the best-adapted pest populations." A possible added benefit to

increasing rotational complexity is the subsequent diversification of land-use types within

an agricultural region, resulting in the benefits described earlier for landscape-level

effects on pest suppression. There is a need for further research aiming to quantify the

potential effects of crop rotation on pest management at the landscape scale. Results of

such work could give direction to land managers for simultaneously establishing multiple

crop types within a geographical area.

      Producers looking to add plant diversity to their farms will frequently adopt the

use of CCs (Reeves, 2017). Typically, CCs grown by farmers in the Midwestern United

States are established in a brief period either before cash crops have been spring planted,

or after crops have been harvested. When livestock is utilized as an economic and

ecological asset CCs can be grown throughout a full growing-season for grazing or

harvestable forage to replace grain production while diversifying farm revenue streams.

Cover crops can also be a valuable tool in terms of IPM. Lundgren and Fergen (2010)

observed greater corn root biomass, fewer third instar corn rootworms (*Diabrotica*

virgifera), and increased epigeic predator abundances in corn fields following a fall-

planted, spring-terminated CC of slender wheatgrass (*Elymus trachycaulus*). In a similar study conducted in Minnesota, USA, aphid (*Aphis glycines*) populations were significantly suppressed on soybeans planted following a winter rye (*Secale cereale*) CC compared to soybeans in non-CC treatments (Koch et al., 2012). Farmers who frequently use CCs view them as an adaptive management tool and CC use is increasing. The 2016-2017 Sustainable Agriculture Research and Education CC survey reported that farmers using CCs increased the area planted to them on their farms by 84.3% between years 2012 and 2016 from an average of 217 acres to nearly 400 acres per farm (CTIC, 2017).

Adding intra-specific diversity into cropland by planting multiple varieties of a single crop species also shows promise in restricting herbivore abundance and increasing crop yield. In a recent two-year study by Grettenburger and Tooker (2016), bird cherry-oat aphid (*Rhopalosiphum padi*) females produced as many as 14.0% (first year) and 10.5% (second year) fewer offspring when isolated on winter wheat (*Triticum aestivum*) planted in close association with genotypically-diverse wheat neighbors compared to a low-diversity stand of wheat. Underlying mechanisms for why aphid populations were reduced in genotypically-diverse wheat stands are not clear and should be addressed in future research. Planting multiple crop varieties simultaneously has benefits beyond herbivore suppression and, for example, has been used to increase crop yield in farmland where plant diseases are problematic (Reiss and Drinkwater, 2018).

Aforementioned techniques successfully increase agroecosystem plant diversity on both local and landscape scales and can contribute to on-farm pest suppression. However, row crops remain a monoculture for most of the growing season. Concurrent cultivation of multiple species shows promise for adding numerous ecosystem services to

agroecosystems lacking the functionality observed in later-successional habitats (Vandermeer, 1992). Interseeding alternative species into an established cash crop is a promising option for diversification of agricultural landscapes during a time of year typically depauperate of plant diversity. Providing resources for natural enemies and creating a less-habitable environment for crop herbivores will likely increase the level of plant protection on farmland (Rusch et al., 2016). Biological control of herbivores and disease vectors can be an effective tool in suppressing pest populations without the use of costly pesticides that can have pernicious effects on non-targeted organisms (Geiger et al., 2010).

4.0 Interseeding to bolster top-down control of herbivores

Natural habitats with diverse plant assemblages support a great variety of invertebrate herbivores and stable predatory and parasitic arthropod communities (Schmid, 2014). Top-down biological control of herbivores in agricultural fields can be accomplished by mimicking the conditions found in highly-functional natural environments (Altieri, 1999). Meeting the habitat and nutritional requirements of biological control agents within cropland allows natural enemies to be present before pests arrive (Gillespie et al., 2016; Landis et al., 2000; Lundgren, 2009).

4.1 Providing non-pest food resources to natural enemies

Very often, predators and parasitoids do not solely attain their nutritional requirements from prey. Nearly all "predatory" arthropods are actually omnivorous (for a thorough review see Lundgren, 2009). Non-prey food sources such as nectar, pollen, or

vegetative tissue can supplement a prey-based diet or can be used in lieu of prey when that resource is limited. In certain cases, a diet of non-prey foods alone can be enough for predatory arthropods to complete development and reproduce (Lundgren and Wiedenmann, 2004). Diversifying plant communities within cropland to have different floral architectures, colors, phenologies, etc. can ensure that floral resources are available to beneficial species throughout the growing season.

Many CC species produce pollen and nectar in excess, and these abundant and nutritional resources are important foods for pollinators and predatory arthropods alike. In a review comparing the nutritional values between various non-prey resources, pollen ranked highest in calories per gram (fresh weight) when compared to seeds, prey, fungus and floral nectar (Lundgren, 2009). Surprisingly, even spiders will consume nectar (Jackson et al., 2001) and pollen (Eggs and Sanders, 2013). In a laboratory setting, Peterson et al. (2016) observed direct consumption of corn pollen by each of three spider species (from families Araneidae, Linyphiidae, Lycosidae), and confirmed, via ELISAs, the presence of corn-derived Cry toxins within cornfield-collected spiders. This result is especially important considering spiders are one of the most abundant predatory groups present in Midwestern agricultural fields (Lundgren and Fausti, 2015). Gut dissections of field-collected generalist predatory ladybeetles [*Coleomegilla maculata* (DeGeer)] from Illinois cornfields during anthesis revealed progressively increasing amounts of pollen in beetle digestive tracts throughout larval development and into adulthood (Lundgren et al., 2005). Cottrell and Yeargan (1998b) observed that the same ladybeetle species mentioned above was more abundant in its egg and larval stages in corn fields where the native weed, *Acalypha ostryaefolia* (Euphorbiaceae), was allowed to persist in comparison to

weed-free fields. Greater abundance of *C. maculata* due to weed-derived floral resources resulted in significantly lower populations of corn earworm larvae (*Helicoverpa zea*, Boddie), an economically important corn pest (Cottrell and Yeargan, 1998b; Olmstead et al., 2016). Similarly, Altieri and Whitcomb (1980) observed the suppression of fall armyworm (*Spodoptera frugiperda*) in diversified fields where weed communities were allowed to persist between alternating corn rows compared to weed-free fields. Although weed-derived resources can bolster insect populations through resource provisioning this is by no means a call for the abandonment of weed management in agriculture as these non-crop plants can prove to be powerful competitors. However, some investigators are of the opinion that the presence of a sub-economically damaging weed population can result in benefits to the ecosystem without jeopardizing farmer profitability (Coble and Mortensen, 1992).

Nectar, though less calorically rich than pollen, is also an important component of predator and parasitoid diets. Inclusion of nectar or other sugary materials from either floral nectar (van Rijn and Wäckers, 2016), extrafloral nectar (Limburg and Rosenheim, 2001), or honeydew (Tena et al., 2018; Wäckers, 2001) can positively affect a species' fitness. For example, Tylianakis et al. (2004) observed that the aphid parasitoid *Aphidius rhopalosiphi* survived significantly longer (3-4 times) and possessed nearly twice as many developing eggs (after 48 days) when provided with a nectar substitute versus pollen or a water control. The same researchers documented a significant, negative correlation between the number of parasitized aphids (*Rhopalosiphum padi*) on wheat plants and increasing distance from nectar-possessing buckwheat (*Fagopyrum*

esculentum) (Tylianakis et al., 2004), a CC species used for interseeding in other vegetable (Gibson et al., 2011) and perennial crops (English-Loeb et al., 2003).

It is important to note that for some species of invertebrates the adult stage is predominantly nectar feeding while larvae are predatory. Such is the case with zoophagous hover flies (Diptera: Syrphidae) and, of course, parasitoid wasps (Hymenoptera). In such scenarios, establishing a population of predatory larvae is highly dependent on providing an appropriate source of nectar to the non-predatory adults (van Rijn and Wäckers, 2016). Those plant species which provide a source of simple carbohydrates unprotected by an inflorescence (via extrafloral nectaries) should especially be considered for use as CCs in mixed cropping circumstances to provide resources for an extended duration (consult Weber et al. 2015 for a list of extrafloral nectary possessing plant species). To highlight the importance of extrafloral nectar for parasitoids and their subsequent control of pests we can consider the work done by Jamont et al. (2013). These researchers observed parasitoid wasps (*Diaeretiella rapae*) surviving for an average of 14 days when given access to extrafloral nectar-possessing faba beans (*Vicia faba*), intercropped between aphid-infested *Brassica oleracea*. Treatments where parasitoids were given infested *B. oleracea* and water, but no nectar-possessing faba beans resulted in a significant reduction in parasitoid survival (longevity: 4 days).

When natural enemies are provided with non-prey food sources, the ecosystem service of pest biological control typically improves. For example, Manandhar and Wright (2016) examined the rate of parasitism of corn earworm (*H. zea*) eggs by native *Tricogramma* spp. wasps, and control of thrips (*Frankliniella spp.*) by minute pirate bugs

(*Orius* spp.) in corn interseeded with various flowering CCs versus corn in monoculture. Corn earworm eggs collected in fields possessing sunnhemp (*Crotolaria juncea* L.) and cowpeas (*Vigna unguiculata* L.) between corn rows were parasitized at significantly higher rates than eggs collected from corn-only fields. Additionally, in one of two study years, thrips populations were reduced in all interseeded treatments compared to corn-only plots (Manandhar and Wright, 2016).

Another mechanism for attracting and retaining predator and parasitoid communities is by supporting an abundant and stable community of alternative prey sources (Gillespie et al., 2016; Landis et al., 2000; Settle et al., 1996). As abundance of herbivores on crop plants can be low during portions of the growing season (LaCanne, 2017) interseeding CC species which attract non-pest herbivores can maintain biological control agents during periods of low prey density. For instance, long-tailed butterfly [*Lampides boeticus* (Lepidoptera: Lycaenidae)] eggs are a viable host for generalist parasitoid Trichogrammatidae wasps and are laid on interseeded cover crops. These alternative hosts allow the parasitoids to maintain their population so that they are able to quickly respond when corn pests arrive (Manandhar and Wright, 2016). These non-crop "trap crops", or "banker-plants" can be susceptible to a number of herbivorous pests and are deliberately established near focal crops to provide alternative prey to biological control agents (Frank, 2010; Huang et al., 2011). Under some pest management regiments, however, trap crops are treated with pesticides once they've been infested with a pest of concern (Vernon, 2005). Such a practice might unintentionally be detrimental to natural enemies utilizing the attracted herbivores as a source of prey.

Many examples of successful trap cropping systems are from greenhouse trials where this pest management tactic is commonly used to support augmentative biological control agents and limit pesticide use under controlled conditions (Payton Miller and Rebek, 2018). Andorno and Lopez (2014) recently studied the effect of adding oat plants (*Avena sativa*) infested with an alternate aphid host (*Rhopalosiphum padi*) on the parasitic biological control agent, *Aphidius colemani*, in greenhouse arugula and sweet pepper production. A significant reduction in pest (*Myzus persicae*) density on arugula occurred in greenhouses supplemented with oats compared to those without (Andorno and López, 2014). There have also been documented successes in implementing trap crops outside of greenhouse conditions. Parker et al. (2016) observed that by establishing a multi-species trap crop near broccoli (*Brassica oleracea* var. italica) crucifer flea beetle (*Phyllotreta cruciferae*) feeding was inhibited, resulting in significantly greater broccoli yield in those fields adjacent to plant-diverse trap plots. Adapting the concept of banker plants and trap crops by interseeding susceptible CCs between row crops is an option which should be further explored to sustain natural enemy communities for long-term pest regulation. Agricultural land managers and the biological control community would benefit from additional field research in this area as there are many questions which need to be resolved. For example, how does the addition of alternative prey resources on non-crop plants affect herbivore control on focal crop species? Which CC species play host to a diversity of non-crop pest herbivores, and are those CC species compatible as companion plants growing adjacent to a primary crop? How much CC diversity leads to positive outcomes in pest management?

Supplementary to the prey and non-prey resources mentioned above, some omnivorous natural enemies of important crop pests gain nutrition through facultative herbivory (Lundgren, 2009; Moser et al., 2008). In a study performed by Lundgren et al. (2011) *C. maculata* larvae, common predatory ladybeetles in corn production systems (Cottrell and Yeargan, 1998a), were observed consuming pinto bean (*Phaseolus vulgaris* L.) leaf tissue regardless of being provided an unrestricted amount of aphids (*Acyrthosiphon pisum*), moth eggs (*Ostrinia nubilalis*) or Lundgren's Super CMAC diet (Lundgren et al., 2011). Increasing plant diversity in polycultures might provide additional resources for beneficial insects to optimize their diets by self-selecting tissues which meet the requirements of an omnivore's complex nutritional profile (Waldbauer and Friedman, 1991).

4.2 Interseeding to support natural enemy structural habitat requirements

In addition to supporting natural enemy communities by providing alternative sources of nutrition, beneficial invertebrates also require a heterogeneous habitat for oviposition, protection from biotic and abiotic factors, and in some cases substrate to assist in acquiring prey. Monocultures often lack the habitat complexity needed to support a suite of arthropod biocontrol agents.

Vegetational complexity offers a variety of microhabitats that predators use in prey capture. Spiders prove to be a good example, as this group of predators has individuals which capture prey through numerous strategies [mimicry and ambush (Théry and Casas, 2002), stalking (Bartos and Szczepko, 2012) and building webs with great morphological diversity (Lubin, 1978)]. In a study of spider habitat suitability, Jimenez-

Valverde and Lobo (2007) found that vegetational complexity was the most important factor in determining spider species richness. Explaining their results, the researchers mention "The availability of structures for attaching a web, and of ambush and refuge sites is probably the most direct effect of vegetation complexity…" (Jimenez-Valverde and Lobo, 2007). Spiders are not the only group of natural enemies which benefit from a heterogeneous habitat. A meta-analysis conducted by Langellotto and Denno (2004) of 62 studies revealed significant increases in overall natural enemy abundances when detritus, vegetation (in some cases through intercropping multiple plant species) or individual plant architecture was made more complex.

Invertebrate biological control agents are not immune to predation themselves, and much like the herbivore pests they control, natural enemies have habitat requirements which allow them to evade antagonists. Vegetational complexity in a diverse environment can provide heterogeneous habitat for refuge from intraguild predators. Wolf spiders (*Pardosa littoralis*) and mirid bugs (*Tytthus vagus*) are important predators of *Prokelisia* spp. planthoppers in Atlantic coast salt marshes, with spiders typically consuming hopper nymphs and adults, and mirids preying upon hopper eggs. In microcosms where spiders, mirids, and planthoppers were present, Finke and Denno (2002) found that the addition of vegetational complexity (thatch) resulted in much greater control (87%) of planthoppers compared to mesocosms completely lacking vegetation. Vegetational complexity allowed predatory mirids to escape intraguild predation by wolf spiders, resulting in greater overall biological control of herbivores. When observed under field conditions, there was a significant positive correlation between higher mirid populations per spider, and vegetational complexity. The authors

explained this phenomenon by stating "… complex vegetation promotes the co-occurrence of these predators in the field by decreasing the intensity of intraguild predation" (Finke and Denno, 2002). Mimicking the natural environment by improving vegetational heterogeneity in agricultural landscapes during crop growth may curb intraguild predation and add efficiency to the biological control-performing community.

Diversification of plant communities within croplands may attract and support gravid natural enemies for oviposition. Invertebrates often prefer or require a specific plant species to deposit their eggs on from which new individuals can disperse in search of resources (Cottrell and Yeargan, 1998b; Lundgren, 2011). The dispersal of beneficial invertebrates from natural and diverse habitats into crops for biological control has been previously documented (Horton et al., 2009; Tscharntke et al., 2005; Tylianakis et al., 2004). Cottrell and Yeargen (1998b) observed ladybeetle (*C. maculata*) preference for ovipositing on the "weed", *Acalypha ostryaefolia*, versus sweetcorn when given the choice. However, when corn earworm (*Helicoverpa zea*) eggs, a prey source for ladybeetles, were presented away from *A. ostryaefolia*, beetle larvae left the plants they had eclosed on in search of nutrition. In field observations, the same researchers also found significantly more ladybeetle larvae on sweetcorn growing in plots possessing *A. ostryaefolia* compared to weed-free corn (Cottrell and Yeargan, 1998b). In 2006 this research was advanced by Seagraves and Yeargen when they replaced *A. ostryaefolia* between sweetcorn rows with a more desirable companion crop, tomatoes. In their two-year field study ladybeetle ovipositional preference on interplanted tomatoes was significantly greater than on corn. Remarkably, when researchers augmented ladybeetle eggs on both plant species predation of eggs on tomatoes was significantly less than those

placed on corn plants (15-39% survival on corn and 88-100% on tomatoes). Observations described here provide evidence that incorporation of additional plant species into cropfields might make agroecosystems more suitable for natural enemy recruitment, protection, and proliferation. The factors involved in ovipositional preference by biological control agents deserves further research efforts so that this important component of ecologically-based pest management can be considered when designing herbivore-resistant polycultures.

Many agriculturally-dominated landscapes are located in places where seasonal variations in temperature and other abiotic factors can be extreme. Ectothermic species often possess physiological adaptations that can help them weather adverse conditions (Watanabe et al., 2002), but behavior and habitat selection also assists in reducing the impact of unfavorable abiotic circumstances (Landis et al., 2000; May, 1979). If agricultural field conditions are not well suited to allow invertebrate survival during inhospitable periods, then natural enemies may not persist. Recolonization of cropland in such scenarios will likely take place through recruitment from external sources, perhaps not quickly enough to prevent reaching an economic threshold. Increasing vegetative complexity in mixed-cropping systems may provide adequate conditions for natural enemy overwintering (Thomas et al., 1994) or thermoregulation during temperature extremes (Orr et al., 1997). For example, Dennis et al. (1994) described increasing winter abundances of the predatory Staphylinidae beetle, *Tachyporus hypnorum*, in field boarders possessing greater vegetation height. *Tachyporus hypnorum* also had significantly higher winter survival rates in areas with vegetation cover versus bare soil (Dennis et al., 1994).

High temperatures can also adversely affect the survival and services provided by ectothermic invertebrates (Kearney et al., 2009). Temperatures on the surface of bare agricultural soils can be very high in Midwestern crop fields if no residue or living plant matter is present (Teasdale and Mohler, 1993). To buffer the effect of potentially harmful high temperatures many ectotherms require microclimates that provide shade and prevent overheating and desiccation (Kearney et al., 2009; May, 1979). Interseeding CCs into cropfields can have a significant effect on ameliorating extreme abiotic conditions in an agricultural setting (Landis et al., 2000). For instance, when ryegrass (*Lolium multiflorum* Lambert) is established between corn rows the soil's maximum surface temperature is decreased in comparison to bare ground or corn residue alone, allowing for improved egg viability of the parasitoid, *Trichogramma brassicae* Bezdenko (Orr et al., 1997).

Many species of nematodes, fungi, bacteria and viruses infect arthropod hosts and can be a significant contributor in controlling insect populations. An extreme example is through the annually augmented release of *Bacillus thuringiensis* bacteria onto many acres of cropland for control of agronomically-concerning pests (Lacey et al., 2015). However, research detailing the effects of habitat manipulation to increase endemic entomopathogen populations is limited and deserves more attention, but existing work shows that conservation biological control via pathogens can be a successful method and should not to be overlooked (Hajek and Nielsen, 2005). As a measure of potential usefulness of entomopathogens one can look to the work of Hajek and Nielsen (2005), where the researchers observed a soybean aphid "population crash" due to the presence of fungus, *Pandora neoaphidis*, in aphid-infested soybean fields of New York state. Habitat manipulation is of utmost importance in increasing the rate of insect infection by

an entomopathogen. All insect-attacking fungi, for example, possess a portion of their lifecycle where they are free-living in the soil. Through interseeding cover crops a soil's surface would be protected from damaging UV radiation and desiccation in comparison to soil left bare. As maintained high humidity is a necessity for entomopathogenic fungal growth (Pell et al., 2010) interseeding cover crops may be a viable option to improve this biological control agent's habitat requirements. In fact, Pell et al. (2010) explicitly mentioned this phenomenon by stating, "Intercropping with plant species that increase canopy cover may also prove useful by raising ambient humidity". Beyond abiotic condition improvement through interseeding, added plant diversity during the crop growing season would likely increase resource availability for non-infectious saprophytic and rhizosphere-dwelling pathogen life stages (Bruck, 2010; Pell et al., 2010).

5.0 Interseeding to strengthen bottom-up control of invertebrate herbivores

There is more than one way to skin a cat, and there are also ways beyond top-down antagonism by predators for control of pest arthropods. Plant-mediated, bottom-up effects play a vital role in reducing the efficiency of herbivorous arthropod populations, and these effects can be strengthened with increased plant diversity through 'associational resistance' (Barbosa et al., 2009; Prokopy, 2003; Tahvanainen and Root, 1972). Interseeding cover crops may strengthen resistance to pest populations by reducing the concentration of a specialist's food resource ['resource concentration hypothesis' (Grez and Gonzalez, 1995)], in turn confusing visual cues (Smith and McSorley, 2000; Togni et al., 2010) and masking host plant volatiles during host searching behaviors (Eigenbrode et al., 2016; Smith and McSorley, 2000). Additionally,

the augmentation of plant diversity within agroecosystems may alter a crop's volatile profile (Kessler et al., 2006), attract natural enemies via chemical signals from injured plants (Kessler and Baldwin, 2001), and impede a pest's dispersal capabilities (Power, 1987). Together with the effects of natural enemies, reviewed above, these bottom-up forces add another layer of crop protection often lacking in simplified agroecosystems.

Herbivores are known to rely on visual cues when searching for and recognizing adequate habitat (Döring and Chittka, 2007; Powell et al., 2006; Stenberg and Ericson, 2007). Schmid et al. (2017) describe the importance of specific light wavelength within the green color spectrum, and light intensity in attracting an economically important wheat pest, the Hessian fly (*Mayetiola destructor* (Say). In addition to cues derived from plant color, herbivore recognition of plant shape (Degen and Städler, 1996) and leaf orientation (Harris et al., 1993) are also important factors in identifying suitable host plants.

Stark differences in color between a host plant and its background can aid herbivores in host finding. Replicating this effect in a laboratory setting Harris et al. (1993) observed attraction of *M. destructor* to lightly colored targets contrasted with a dark background, whereas when targets and backgrounds were similarly colored no attraction was seen. It may be possible that establishing vegetation between crop rows will hinder visual cues associated with pest host-finding. Simple agricultural landscapes possessing bare ground between crop rows in obvious contrast to green leaf tissue provides an easily navigated environment for searching pests. Field studies examining the effects of interseeding CCs on visual searching by herbivores are in need, but are difficult

to conduct, as added plant diversity affects other factors, such as natural enemies, possibly confounding results (Döring and Chittka, 2007).

Establishment of non-host plants between rows of focal crops may limit movement of pests (Kareiva, 1983) and even disease transmission by herbivorous vectors (Perrin and Phillips, 1978; Theunissen and Schelling, 1996). In Nicaraguan corn fields, researchers examined the abundance and rate of movement of the economically important herbivore and disease vector, maize leafhoppers (*Dalbulus maidis*), in both monocultures and corn fields interseeded with beans (*Phaseolus vulgaris*) (Power, 1987). Interseeding beans not only reduced the overall abundance of leafhoppers, across-row movement of the herbivore was also impeded in comparison to fields where only corn was grown, thus limiting the potential for disease spread (Power, 1987). Farrell (1976) observed a very similar occurrence when interseeding *P. vulgaris* between rows of ground nuts (*Arachis hypogaea*) to control *Aphis craccivora* (Aphididae), a vector of groundnut rosette virus. Interestingly, Ferrell attributed the reduced disease rate to limited locomotion of aphid vectors entrapped by hook-shaped epidermal hairs (trichomes) on *P. vulgaris*.

Crop pests often use olfactory cues when searching for and locating suitable host plants (Bruce and Pickett, 2011). Each plant species possesses a unique profile of volatiles which allows insects to distinguish between host and non-host plants. In simplified agroecosystems where crops are grown monoculturally, crop volatiles are easily recognized by pests because a crop's unique chemical profile is unmasked by other plant species (Togni et al., 2010). In diversified plant communities host plant finding by herbivores can be impeded through the mixing of volatiles from distinct species. *Bemisia tabaci* whiteflies, economically important pests of numerous crops across the globe,

identify their hosts partially via host plant volatiles. However, when Tongi et al. (2010) used a multi-choice test to examine the attractiveness of tomato volatiles, and intermixed volatiles from tomato and coriander, they found that simply mixing volatiles significantly reduced the selection by adult whiteflies in comparison to tomato volatiles alone. In a subsequent field study, tomatoes interseeded with coriander possessed fewer *B. tabaci* compared to tomato monocultures, reaffirming what was observed in the laboratory (Togni et al., 2010).

Natural enemies also search for prey sources by cueing in on herbivore-induced plant volatiles, thus these phytochemicals serve as an important

> "By providing specific and reliable chemical signals, plants may obtain a competitive advantage in the acquisition of herbivore natural enemies".
>
> - De Moraes et al. (1998)

share of plant's defense systems (Ton et al., 2007). For example, Kessler and Baldwin (2001) found that damage from three herbivores, tomato hornworms (*Manduca quinquemaculata*), Dicyphus plant bugs (*Dicyphus minimus*), and tobacco flea beetles (*Epitrix hirtipennis*) altered the volatiles of tobacco (*Nicotiana attenuata*). When isolated volatiles from injured tobacco were artificially emitted from undamaged plants the predator, *Geocoris pallens* (Heteroptera: Geocoridae), successfully located and consumed more sentinel tomato hornworm eggs compared to tobacco where no volatiles were released. In a similar study system, De Moraes et al. (1998) characterized a change in the chemical volatile profile of corn, cotton and tobacco following damage by tobacco budworm (*Heliothis virescens*) caterpillars. *Cardiochiles nigriceps*, a tobacco budworm parasitoid, was successfully recruited to plants with altered volatile profiles, even when caterpillars and damaged plant leaves had been removed.

Plants injured through herbivory can also communicate to undamaged plants via volatile release (Engelberth et al., 2004; Frost et al., 2008). For example, feeding action and deposition of regurgitant by caterpillars on corn plants can elicit a response whereby volatiles from damaged plants induce neighboring undamaged corn plant defensive pathways prior to herbivore attack (Engelberth et al., 2004). This inter-plant communication isn't limited to individuals of the same species. In fact, inter-specific communication via volatile compounds plays an important role in plant community resistance to herbivores (Howe and Jander, 2008). When undamaged tobacco was exposed to mechanically injured sagebrush (*Artemisia tridentata*) a subsequent "priming" effect was observed where defense-related genes were up-regulated and secondary metabolites (proteinase inhibitors) were accumulated more rapidly following attack by the tobacco herbivore, *Manduca sexta*. Rapid accumulation of secondary metabolites resulted in reduced herbivory on primed tobacco plants in comparison to unexposed tobacco (Kessler et al., 2006). To add an additional layer of complexity, inter-plant communication via volatiles can affect multi-trophic interactions between damaged and undamaged plants, and invertebrate biological control agents. Exposure of undamaged plants to plant damage can alter an undamaged plant's volatile profile in a manner which proactively recruits natural enemies. Such a scenario has been described for the parasitoid wasp, *Cotesia marginiventris* in corn (Ton et al., 2007). As was discussed above (banker plants and trap crops), incorporating CCs susceptible to herbivory between crop rows may, through plant mediated release of herbivore-induced plant volatiles, attract predators and parasitoids into farmland for biological control of pests on crops, or prime crops to improve host plant resistance. Results from studies described here underscore the

potential plant volatile-mediated pest management benefits of diversifying agroecosystems through growing multiple species, simultaneously, and in close proximity.

As is the case for natural enemies, crop pests search for habitats conducive to their basic needs: feeding, oviposition, and shelter. The process of searching is aided by an invertebrate's ability to hone in on the volatile and visual cues of specific host species (Couty et al., 2006; Eigenbrode et al., 2016). Crops planted in monoculture provide little physical restriction, and very clear and unmasked visual and olfactory signals allowing herbivores to quickly and easily find host plants in comparison to mixed cropping systems (Couty et al., 2006). Added difficulty of host-finding in mixed cropping systems can increase the time and energy herbivores spend searching for host plants (Finch and Collier, 2000). Any detriment to the ability of an herbivore finding suitable host plants is energetically costly to the individual and may lead to limited reproductive success or even an increased risk of encountering biotic antagonists, a phenomenon termed "the fly paper effect" (Perrin and Phillips, 1978).

6.0 Summary and conclusions

Crop monocultures perpetually maintained in an early secondary successional state are common in Midwestern U.S. agricultural production regiments. This challenges fostering community diversity in ways that resist pest establishment and proliferation. Two ways that diversity resists pests are by 1) encouraging natural enemies and 2) stimulating plant community resistance to herbivores. Human capital employed to curb crop losses typically involves the use of insecticides and GE plants possessing

insecticidal traits as replacements for other effective IPM strategies and naturally-occurring biological control. However, insecticide use, and widespread adoption of GE crops has not been without detrimental effects to non-targeted species and natural habitats. Producers vying to curb pesticide inputs are employing plant diversification techniques within large-scale annual croplands to bolster biological control of herbivores by encouraging persistent natural enemy populations and hindering the efficiency of pests. As was discussed, interseeding alternative plant species within annual crop fields is one method which shows promise for sustainable pest management.

Multiple top-down forces influence the success of pest invertebrate populations in mixed cropping systems. Inclusion of non-prey foods like nectar, pollen and non-crop vegetation, and sources of alternative prey will allow biological control agents to attain necessary nutrition when crop pests are absent. Agricultural habitats made more heterogeneous through plant diversification can provide the structural complexity needed to assist prey capture, escape from intraguild predation, and provide microhabitats for oviposition, overwintering, and amelioration of abiotic extremes.

Reduced efficiency of crop herbivores in polycultures through associational resistance is a result of numerous factors. Confusion of visual and olfactory cues when incorporating plants of different species alongside crops can hinder host-finding capabilities of searching herbivores. Plant volatiles released following injury to associated plants can attract natural enemies or prime the defensive systems of neighboring crops for a possible attack. Finally, mixed cropping systems may physically impede movement and passive dispersal of herbivorous pests, and subsequently, diseases that they vector.

A combination of predator- and plant-mediated factors can result in reduced efficiency by crop pests. Greater amounts of time and energy spent finding suitable habitat for feeding and reproduction may result in a significant reduction in feeding and reproduction while increasing the chance of predation (Perrin and Phillips, 1978).

It is not the aim of ecologically-based pest management to rely solely on any one factor for maintenance of tolerable herbivore populations, but rather a combination, which results in robust and effective control across many growing seasons despite seasonal variability. In other words, ecologically-based pest management does not limit pest population growth via one "silver bullet" method. Such strategies are often ephemerally effective due to rapid evolution in pest communities [as seen in pesticide- and *Bt*-resistant populations (Alyokhin et al., 2008; Gassmann et al., 2011)]. Rather, ecologically-based pest management is more analogous to a "death by a thousand cuts" approach, wherein pests are met by a diverse suite of biotic and abiotic antagonistic factors.

More research examining the effects of field-scale polycultures on invertebrate communities and the ecological services they provide would be advantageous. To further our knowledge of this pest management strategy private, government, and university research infrastructure should be utilized to examine various crop and CC combinations to elucidate synergisms which may increase overall productivity while diversifying the agricultural landscape. Overreliance on insecticides for reactive pest management in crop monocultures is unsustainable and threatens human, animal, and ecosystem health. Research efforts should thus be made to support land managers seeking to create a paradigm shift from chemical use to ecologically-based pest management.

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CHAPTER TWO: INVERTEBRATE GRANIVORY OF CALCIUM CARBONATE-
COATED COVER CROP SEEDS

Abstract

Diversifying cropland plant communities is a prerequisite to restoring ecosystem functions in agricultural habitats. Cover crops are one such way to improve diversity, and broadcasting calcium carbonate-coated seeds can be a viable method for plant establishment. In addition to improving seed-to-soil contact calcium carbonate may also reduce arthropod granivory. Here we examine the effect of this seed coating technology on arthropod granivory for seven cover crop species under field conditions. Carabidae, Gryllidae and Staphylinidae were the three most abundantly-collected granivorous grouped taxa in pitfall samples, and Pterostichus permundus and Gryllus pennsylvanicus represented 60.8% of all individual granivores. Cover crop seed damaged was variable between plant species. Among all plant species the presence of a seed coating significantly reduced granivory by nearly 40%. Individually, hairy vetch and sorghum × sudan seeds were both especially protected by calcium carbonate. No positive correlations were observed between invertebrate groups and the number of seeds consumed. Numerous negative correlations were revealed but were a result of differences in granivory and arthropod abundances between study seasons. Alternative methods for assessing the functionality of granivorous arthropod communities should be pursued, as activity-density measured from pitfall traps failed to reveal important cover crop seed consumers. Protection of cover crop seeds from granivory through a calcium carbonate

coating may allow producers to adjust seeding rates and save on costs, increasing the rate of adoption for this conservation practice.

Key words: Cover crops, seed coating, granivory, granivorous insects, Carabidae, Gryllidae, seed establishment, seed broadcast

1.0 Introduction

A growing number of agricultural producers are planting non-crop vegetation, or cover crops, at some point during their crop rotation (CTIC, 2017). Cover crops are typically grown during a period outside of annual crop growth (i.e. pre-planting, post-harvest, or over winter), but some producers establish cover crops while the cash crop is actively growing (Curran et al., 2018). Regardless of when cover crops are implemented, they are used by farmers to improve farmland performance by restoring or enhancing agroecosystem functions which are lacking or have been lost after a long period of monocultures, simple rotations, and frequent disturbances like tillage and agrichemical use (Blanco-Canqui et al., 2015). Cover crops can have a significant positive effect on the physical properties of soil by decreasing compaction (Chen and Weil, 2010), bulk density (Reeves, 2017) and erosion (Alliaume et al., 2014; De Baets et al., 2011), while increasing a soil's water holding capacity (Basche et al., 2016), organic matter content (Plaza-Bonilla et al., 2016) and ability to allow water infiltration (Kahimba et al., 2008). Cover crops can also influence a soil's chemical properties by altering pH (Fernandez et al., 2016), detoxifying pesticides (Edwards, 1975), ameliorating salinity and sodicity issues (Gabriel et al., 2012), and scavenging for or making critical plant nutrients

available (Grove and Pena-Yewtukhiw, 2017). Concurrently, cover crops can be an important resource for animal conservation on agricultural land. Cover crop-derived refuge and nutrition allows animals including large vertebrate grazers, birds, and members of arthropod guilds to persist in cropland (Landis et al., 2000).

Pest management is one primary reason that producers use cover crops in their rotation (CTIC, 2017). For example, cover crops can suppress weeds by utilizing excess nutrients (Blanco-Canqui et al., 2015), through allelopathic action (Kunz et al., 2016), and by physically restricting weed germination and growth (Teasdale and Mohler, 1993). Additionally, providing non-prey nutrition and favorable abiotic conditions to arthropod biological control agents can improve the management of production-limiting herbivores (Gurr et al., 2017) and weeds (Blubaugh et al., 2016; Davis and Liebman, 2003) without the use of synthetic pesticides. Blubaugh et al. (2016) observed this phenomenon in cover crop plots bearing clover (*Trifolium pratense*), where predation of weed seeds (*Chenopodium album*) by ground beetles (Carabidae) was significantly increased in comparison to plots lacking vegetative cover.

The perceived benefits of cover crops on farms has resulted in increasing adoption rates. Between 2012 and 2017, respondents of the annual Conservation Technology Information Center cover crop survey who were cover cropping on their farms increased the average number of acres where this tool was implemented by nearly 85% (CTIC, 2017). Since the inauguration of the survey, in 2012, cover cropped hectares has invariably increased annually. Another metric that demonstrates this expansion is the increased seed sales. Between the years 2009 and 2018 the amount of seed sold by Greencover Seed ™, of Bladen, Nebraska has increased from enough to cover 400 ha, to

approximately 344,000 ha (personal communication with Keith Burns, owner of Greencover Seed ™, Bladen NE, 68928).

Broadcasting cover crop seeds onto the soil's surface has both benefits and challenges. Deploying seeds like this can be done rather quickly and with relatively inexpensive equipment. Broadcasting seed also opens opportunities for cover crop establishment during the growth of another crop without risk of physically damaging growing plants. Though a simpler method of seeding, broadcasting can result in poorer germination from a lack of seed-to-soil contact (Evert et al., 2009), desiccation of small, shallowly rooted seedlings (Heckman et al., 2002), and loss of viable seed due to animal granivory (Decker et al., 1990; White et al., 2007). Advancements in seed coating technology are continually being developed to ameliorate the negative issues associated with surface-scattering seeds.

On July 3, 1866, U.S. patent number 56,104 was issued to W. Blessing for his invention of the technique for coating cotton seeds with wheat flour paste to improve "plantability" (Blessing, 1866; Porter and Scott, 2016). Since then, seed coating technologies have been developed and used on a regular basis with various types of coating materials, and varying degrees of success (Porter and Scott, 2016; Scott, 1975). In his book, "The One Straw Revolution", author and farmer Masanobu Fukuoka describes coating rice seeds in a homemade blend of clay and water to improve establishment of his hand-broadcasted seeds. In recent years, the use of lime (calcium carbonate, $CaCO_3$) for coating cover crop seeds has been explored to improve seed ballistics (to fly further and penetrate through a dense canopy of vegetation or residue

when broadcasted), and to provide a pseudo seed-to-soil contact that aids water imbibition.

An additional benefit to lime coating, overlooked thus far in the literature, may be a reduction in cover crop seed loss due to granivory. Granivorous arthropods, birds and small mammals can have a significant impact on surface-scattered seeds (Kelt et al., 2004; White et al., 2007), which comes as a benefit when weed seeds are those being consumed (Blubaugh et al., 2016; Kulkarni et al., 2017b), but possibly a detriment when desirable seeds are destroyed. Observations of damage to desirable surface-scattered seeds, not weeds, by arthropods are difficult to find, but examples exist for a few crops. For instance, under laboratory conditions, Lundgren and Rosentrater (2007) observed the destruction of seeds for several crop species, including alfalfa (*Medicago sativa*) and broccoli (*Brassica oleraceae*), by three commonly collected insect granivores in agricultural farmscapes, two Carabidae ground beetles (*Harpalus pensylvanicus* and *Anisodactylus sanctaecrucis*) and one species of cricket (*Gryllus pennsylvanicus*). Coating seeds with substances to deter damage inflicted by animals has proven successful in certain applications. In a study of prairie restorations through re-seeding native species, Pearson et al. (2018) examined the effect of coating seeds in powdered Bhut Jolokia peppers (*Capsicum chinese*) in hopes that capsaicin present in the coating would deter granivory by small mammals. In the laboratory, this seed coating resulted in significantly greater protection from deer mice (*Peromyscus maniculatus*), an important predator of native seeds.

The addition of a lime coating on cover crop seed holds promise for increasing the adoption of this conservation practice by producers because this benign compound

improves water imbibition by surface-scattered seeds. We hypothesize a lime coating on cover crops seeds will provide the additional benefit of deterring arthropod granivory.

2.0 Methods

2.1 Study sites

2.1.1 Site year-2016

Field site location was approximately 10 km North of Bruce, South Dakota, USA (site coordinates: 44.405192, -96.886847). Crops grown during 2015 at the study site were cereal rye (*Secale cereale*), which was chopped for forage, followed by buckwheat (*Fagopyrum esculentum*), harvested for seed. In spring of 2016 corn (*Zea mays*) was no-till planted on May 20 into buckwheat residue at a population of 79,000 seeds/ha (Elk Mound Seed Company, Elk Mound, WI, 54739; variety: EMS 8100; maturity: 80 d) with 76 cm row spacing. Four plots measuring 32×32 m were established with a distance of 16 m between plots. Roundup® (rate: 2338 mL/ha; a.i.: glyphosate; Monsanto™, St. Louis, MO 63167) and Confidence® (rate: 2923 mL/ha; a.i.: acetochlor; WinField™, Arden Hills, MN 55126) were used as preplant herbicides in the field on May 15 while Accent Q™ (rate: 66 mL/ha; a.i.: nicosulfuron; DuPont™, Wilmington, DE 19803) and Status™ (rate: 370 mL/ha; a.i.: sodium salt of diflufenzopyr and sodium salt of dicamba; BASF™, Florham Park, NJ, 07932) were used as post-emergent herbicides on June 16. No fertilizer was applied to the study field during the 2016 growing season.

2.1.2 site year-2018

The same size and number of research plots were used during the 2018 season, but were located approximately 10 km East of Gary, South Dakota (site coordinates: 44.916862, 96.397883). Soybeans (*Glycine max*) were grown during the 2017 season and harvested for grain. The field received tillage following soybean harvest and again in the spring prior to corn planting for seedbed preparation. Organic corn seed (Blue River Organic Seed™; Ames, IA, 50014; variety: P1000684; maturity: 96d) was planted on May 26 at a population of 79,000 seeds/ha with an interrow spacing of 76 cm. A pre-emergent herbicide application of SureStart II™ (rate: 2923 mL/ha; active ingredients: acetochlor, flumetsulam and clopyralid; Dow AgroSciences ™, Indianapolis, IN, 46268) was conducted on May 20. No post-emergent herbicides were applied. Fertilizer was broadcasted into research area at a rate of 157 kg/ha nitrogen as urea, 56 kg/ha phosphorous as diammonium phosphate and 56 kg/ha potassium as potash. Insecticides were not used during either study season.

2.2 Seed cards

Cards bearing seeds coated in lime (1: 1 seed to $CaCO_3$ ratio by weight; seed coating performed by Smith Seed Services™, Halsey, OR 97348) and cards with bare seeds were deployed in research plots to assess granivory. For every seed card, 30 seeds each of cereal rye (*Secale cereale*), flax (*Linum usitatissimum*), Japanese millet (*Echinochloa esculenta*), sorghum × sudan (*Sorghum × drummondii*), hairy vetch (*Vicia villosa*), lentil (*Lens culinaris*) and field pea (*Pisum sativum*) were affixed (in a 3 × 10 orientation) to plastic sheets (12 × 14 cm) cut from three-ring binder separators (Avery™ table of contents dividers; product: 11842; 50 Pointe Drive, Brea, CA, 92821) using

double-sided tape (Scotch™ double sided tape; model number: 237; 3M Center, St. Paul, MN, 55144). Dry, fine-grain sand was sprinkled over seed cards so that arthropods could walk freely without becoming entrapped by tape. Five of each type of seed cards were arranged in an 'X' pattern in each plot, with four cards placed 5 m diagonally in towards the center from four corners and one centrally located. To prevent vertebrate granivory, wire mesh cages (square openings measuring 1.25×1.25 cm; Figure 1) were placed over seed cards and held in place with a small marking flag through the cage. Cage dimensions were 15 cm long, 13 cm wide and 5 cm deep. Metal cages were pressed into the soil until the distance between the cage top and seed card was approximately 3 cm. Seed cards remained in the field for 7 d beginning on July 7 and June 26 during 2016 and 2018, respectively. After collection from the field, each seed was microscopically inspected to determine if arthropod granivory had taken place. A seed was considered to be predated if there was obvious damage from invertebrate mouthparts.

2.3 Insect sampling

In 2016, three sets of barrier-linked cup-in-cup pitfall traps were established in each study plot (Lundgren and Harwood, 2012). Two were located in opposite corners and one located centrally. During the 2018 season, five pitfall traps were established in each plot in the same "X" pattern as seed cards. Two plastic cups (16 oz SOLO™, Lake Forest, IL, 60035) stacked together were buried at a depth where their upper edges were flush with the soil's surface. A 15×90 cm piece of sheet metal standing erect and placed lengthwise was pressed into the soil approximately 2 cm and affixed using a stake so that one end of the metal sheet terminated adjacent to the buried cups. Two more stacked cups

were buried until flush with the soil's surface at the alternate end of the erect metal slat.

Wooden boards were placed over pitfall traps and leaned at approximately 45° against the

metal slat to reduce rainfall from entering traps. To preserve trapped arthropods and

prevent carnivory amongst captives, 50 mL of ethylene glycol antifreeze was poured into

each collection cup. Pitfall traps remained activated during the same period as seed cards.

Contents of collection cups were then collected from the field and transported to the

laboratory where arthropods were placed in 70% ethanol for storage until identification

and tabulation of known granivores.

2.4 Data analysis

Within plots the mean ± SEM damaged seeds per seed card was determined for

each species in both coated and uncoated treatments. A two-way ANOVA ($\alpha = 0.05$) was

conducted to examine within-treatment (plant species) and between-treatment (coated or

bare seeds) differences in rates of seed damage across both field seasons, and if

interactive effects existed. Statistically different means were separated using Tukey's

HSD. A factorial ANOVA with seed species as blocking variable, site year as treatment

variable and number of damaged seeds as dependent variable to determine if seed

damage varied between site years. Mean ± SEM granivorous arthropods per pitfall

(excluding Collembola) were determined for plots across both field seasons. Granivorous

arthropods collected at a rate of ≥ 0.5 individuals per pitfall are included in the results

section. A one-way ANOVA paired with Tukey's HSD all-pairwise comparison was used

to determine differences between abundant granivorous taxa. One-way ANOVAs were

used to examine variability in abundance of pitfall-collected arthropods between site

years. Pearson's correlations ($\alpha = 0.05$) were conducted to test relationships between common granivore groups and rates of seed consumption per seed card, per plot. Statistical analyses were conducted using Statistix™ 10 software (Analytical Software™, 2105 Miller Landing Rd., Tallahassee, FL, 32312).

3.0 Results

3.1 Pitfall-collected arthropods

A total of 3,685 arthropods (excluding Collembola) were collected, with an average of 115.16 ± 13.68 individuals per trap. Three insect families possessing granivorous individuals were commonly collected from pitfalls within research plots (Table 1). Species belonging to Carabidae (ground beetles) were most abundant with an average of 27.16 ± 7.00 individuals per trap, followed by Gryllidae (crickets, 17.35 ± 6.00) and Staphylinidae (rove beetles, 11.79 ± 8.36).

Significant differences were observed among abundant granivore taxa ($F_{7,63} = 3.44$, $P < 0.01$) (Tukey HSD post-hoc groupings are noted in Table 1). Two granivorous species, *Pterostichus permundus* (Coleoptera: Carabidae), and *Gryllus pennsylvanicus* (Orthoptera: Gryllidae) were collected at 13.98 ± 6.18 and 12.16 ± 3.40 individuals per pitfall, respectively. Of commonly collected granivores (≥ 0.5 per pitfall) these two species represented 60.8% of the entire community abundance, and likely have an important impact on granivory of surface-scattered seeds. The remaining frequently collected arthropods comprised two Carabidae [Coleoptera: *Bembidium* sp. (7.12 ± 2.89) and *Stenolophus* sp. (1.77 ± 0.78)], one Cucujidae [Coleoptera: *Pediacus* sp. (1.25 ± 0.49)], one Gryllidae [Orthoptera: *Allonemobius* sp. (5.14 ± 2.34)], one Julida

[unidentified millipede specimen (0.96 ± 0.60)] and one Porcellionidae [Isopoda:

Trachelipus rathkii (0.61 ± 0.34)] (Table 1).

Average abundance of all granivorous invertebrate taxa collected per pitfall did

not differ between site years ($F_{1,7} = 1.42$, $P = 0.27$). However, the abundances of certain

granivore groups differed. Gryllidae were more abundantly collected in 2018 ($F_{1,7} = 8.60$,

$P = 0.03$), with collections of Carabidae also being marginally significantly higher during

that year ($F_{1,7} = 5.42$, $P = 0.06$).

3.2 Cover crop seed granivory

Across all cover crop species, a lime coating on cover crop seeds reduced the

occurrence of damage done by arthropods in comparison to bare seeds ($F_{1,111} = 6.27$, $P =$

0.01). Plant species also had a significant overall effect on the rate of granivory, with

some species being more frequently damaged than others ($F_{1,111} = 9.71$, $P < 0.01$). Lime

coating seeds reduced granivory for two of the species tested: hairy vetch ($F_{1,15} = 6.81$, P

$= 0.02$) and sorghum $\times$ sudan ($F_{1,15} = 7.54$, $P = 0.02$) (Figure 2, Table 2). Cereal rye was

the sole plant species whose mean seed damage tended to be higher for coated seeds in

comparison to bare seeds (3.83 ± 0.92 and 2.88 ± 0.68 per seed card, respectively), but

the difference was not significant ($F_{1,15} = 0.70$, $P = 0.42$).

When comparing the consumption of coated and uncoated seeds separately,

damage to cover crop seeds differed among plant species ($F_{6,111} = 9.71$, $P < 0.01$), with

no interaction effect observed between seed treatment and plant species ($F_{6,111} = 1.32$, $P =$

0.26). Tukey HSD post-hoc comparison rankings within each treatment can be observed

in Table 2.

Among all plant species and treatments, the number of seeds damaged per card varied between site years ($F_{6,111} = 35.61$, $P < 0.01$), with average granivory rates much greater in 2016 (9.68 ± 1.28) than 2018 (3.18 ± 0.52).

No positive correlations were found between abundant arthropod groups and rates of seed damage. However, there were four occurrences where arthropod abundance was negatively correlated with the number of damaged seeds per seed card. These correlations were between lime-coated sorghum × sudan and Gryllidae ($r = -0.78$, $P = 0.02$), bare field pea and Carabidae ($r = -0.73$, $P = 0.04$), bare hairy vetch and Carabidae ($r = -0.80$, $P = 0.02$), and bare hairy vetch and Gryllidae ($r = -0.82$, $P = 0.01$). These negative correlations are likely a result of larger Carabidae and Gryllidae captures and overall decreased seed consumption rates in 2016. When these correlations were assessed via per individual field season the only pairing which remained negatively correlated was between bare hairy vetch and Gryllidae during 2016 ($r = -0.97$, $P = 0.03$).

4.0 Discussion

Many researchers have examined the effects of arthropod granivores on damage and removal of undesirable seeds within agricultural situations (Kulkarni et al., 2017b; Lundgren, 2005). However, few studies have described the effect of otherwise beneficial insects on intentionally surface-scattered seeds such as cover crops. As they have for more than one hundred and fifty years (Blessing, 1866), novel seed coating technologies will likely continue to play an important role in improving plant establishment, in part by protecting cover crop seeds from predation. Conservation practices within agriculture boost invertebrate populations capable of granivory (LaCanne, 2017), and thus methods

of seed protection conducive for habitats hosting beneficial granivores are worth consideration, as these organisms have the potential to affect plant establishment (Honek et al., 2009). As was observed in this study, different cover crop seeds are damaged variably depending on species and, in certain cases, a lime seed coating can reduce granivory by common cornfield invertebrates.

Numerous omnivore arthropods depend on granivory to provide as an important source of nutrition (Lundgren, 2009; Lundgren and Lehman, 2010; Saska et al., 2014). For instance, ground beetles (Family: Carabidae) crickets (Gryllidae) and rove beetles (Staphylinidae), three of the most abundant arthropods collected in this study (Table 1), are well-known seed removers, and are commonly collected in cornfields (LaCanne, 2017; Lundgren and Fergen, 2014; Saska, 2004). In one study, 22% of *Pterostichus permundus*, the ground beetle most frequently captured in pitfall traps here, had consumed dandelion seeds (*Taraxacum officinale*) in an agricultural shelterbelt (Lundgren et al., 2013). In the same study Lundgren et al. observed that > 37% of *Gryllus pennsylvanicus*, and > 3% of all Staphylinidae [both abundant in the present study (Table 1)] had also consumed marked seeds. It is likely that these omnivorous insect populations, recruited by farmers for the ecosystem functions of pest biological control and weed predation, may also have the possible confounding effect of restricting the establishment of intentionally-broadcasted cover crops. Though this may imply that these invertebrates are potential pests to cover crop-producing farmers, an attempt to eliminate these organisms would likely result in a loss of important ecosystem functions such as weed seed granivory (Kulkarni et al., 2017a; White et al., 2007), predation of crop pests

(Douglas et al., 2015; Lundgren et al., 2015) and bioturbation of soil (Garcia and Niell, 1991), to name a few.

As described here (Figure 2, Table 2), seed species were differentially damaged. Coated cover crop seed consumption ranged from as high as 12.45 ± 4.00 per seed card for flax, to a low of 0.03 ± 0.03 per card for hairy vetch, with a comparable range for bare seeds [14.10 ± 3.72 (flax), 1.15 ± 0.43 (hairy vetch)]. Average seed consumption rates for other tested cover crop species fell within this range at varying levels (Figure 2). This same phenomenon has been observed when granivorous arthropods are given a choice between multiple seed types (Honek et al., 2007). For example, in laboratory microcosms White et al. (2007) measured velvetleaf (*Abutilon theophrasti*), redroot pigweed (*Amaranthus retroflexus*) and giant foxtail (*Setaria faberi*) seed consumption by a variety of granivorous invertebrates. Two Carabidae ground beetle species (*Anisodactylus sanctaecrucis* and *H. pensylvanicus*), and the cricket, *G. pennsylvanicus*, preferred consuming pigweed seeds, then giant foxtail and velvetleaf, in decreasing order. Explanations for variability in seed consumption rates may be due to differences between plant species in terms of seed nutritive value (Lundgren, 2009), seed size (Brust and House, 2009; Honek et al., 2007), or physical (Rogers and Kreitner, 1983) or phytochemical deterrents (Hudaib et al., 2017). Such factors might explain why there were significant differences between species within coated seed and bare seed treatments (Table 2).

There were few correlations among granivore abundances and seed removal rates, which may have been an artifact of our methodological approach. Crickets (Gryllidae) and ground beetles (Carabidae) are proven granivores (Lundgren et al., 2013), so it was

unexpected that seed consumption decreased as their abundance increased in the cornfields. This result appears to be an artifact of year-specific patterns in the data, rather than some deeper biological pattern. Other work suggests that pitfall sampling may not always be well correlated with seed removal rates (Kulkarni et al. 2017b). This result supports the idea that multiple sampling methods would be appropriate. A combination of variability in site years and pitfalls being a questionable method for granivore collection makes it difficult to draw conclusions on the ties between arthropod activity-density and cover crop seed damage in this study. Future attempts to correlate the ecosystem function of seed damage through invertebrate granivory in the field could rely on alternate methods such as molecular marking (Hagler and Machtley, 2016; Lundgren et al., 2013) or video recording (Brown et al., 2016; Harrison and Gallandt, 2012) to improve this area of research.

Across all cover crop species, the addition of a lime seed coating significantly ameliorated the effects of arthropod granivory. Though the reduction in granivory was statistically significant for only two of the seven species examined, hairy vetch and sorghum $\times$ sudan, nearly all other cover crops (except for cereal rye) had non-significant reductions in seed removal when a lime coating was present (Figure 2). Though the loss in established plant density as a result of granivory has not been observed under field conditions for cover crops, other work with weed communities may clarify the potential impact granivores have on desired plants in agricultural fields (White et al., 2007). For example, Honek et al. (2009) measured dandelion emergence under field conditions in arenas which were either open to arthropods or totally protected from predation. Allowing access to dandelion seeds by arthropods resulted in a significant reduction in

seedling emergence of up to 40%. Proven plant density reductions due to invertebrate granivory, combined with the quantification of surface-available cover crop seed damage here suggests that arthropod communities do contribute to a reduction in broadcasted cover crop seed germination. Protecting seeds and improving germination by coating them in calcium carbonate has potential to allow farmers to reduce their seeding rates to achieve adequate plant stands and save money on seed costs. Further field research addressing the effect of invertebrates on broadcasted cover crop stand establishment is needed to give producers accurate guidance for adjusting seeding rates depending on if they are or are not using seed-coating technology.

Implementation of cover crops across farming landscapes holds great potential for mitigating the negative environmental effects of agricultural production. In a comprehensive review by Blanco-Canqui et al. (2015) the authors explain how cover crops can elevate the ecosystem service provisioning capabilities of agricultural land on numerous levels, ranging from improving habitat for microbes, to altering the greenhouse gas fluxes of farming operations. The lack of simple, flexible, and effective cover crop establishment methods has been a barrier in the wide-scale adoption of this useful management tool (CTIC, 2017). Research efforts to advance the usability and adoption of cover crops by agricultural producers are worthwhile and should be pursued.

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8.0 Figures

Figure 1. Image of field-deployed seed card possessing uncoated cover crop seeds. A metal mesh cage (15 cm long, 13 cm wide and 5 cm deep square openings of 1.25×1.25 cm) protects seeds from granivory by birds and small mammals. There were 3 cm between cage top and seed card surface. In the lower-left quadrant of the seed card a slug can be observed feeding on Sorghum $\times$ Sudan (*Sorghum $\times$ drummondii*) seeds.
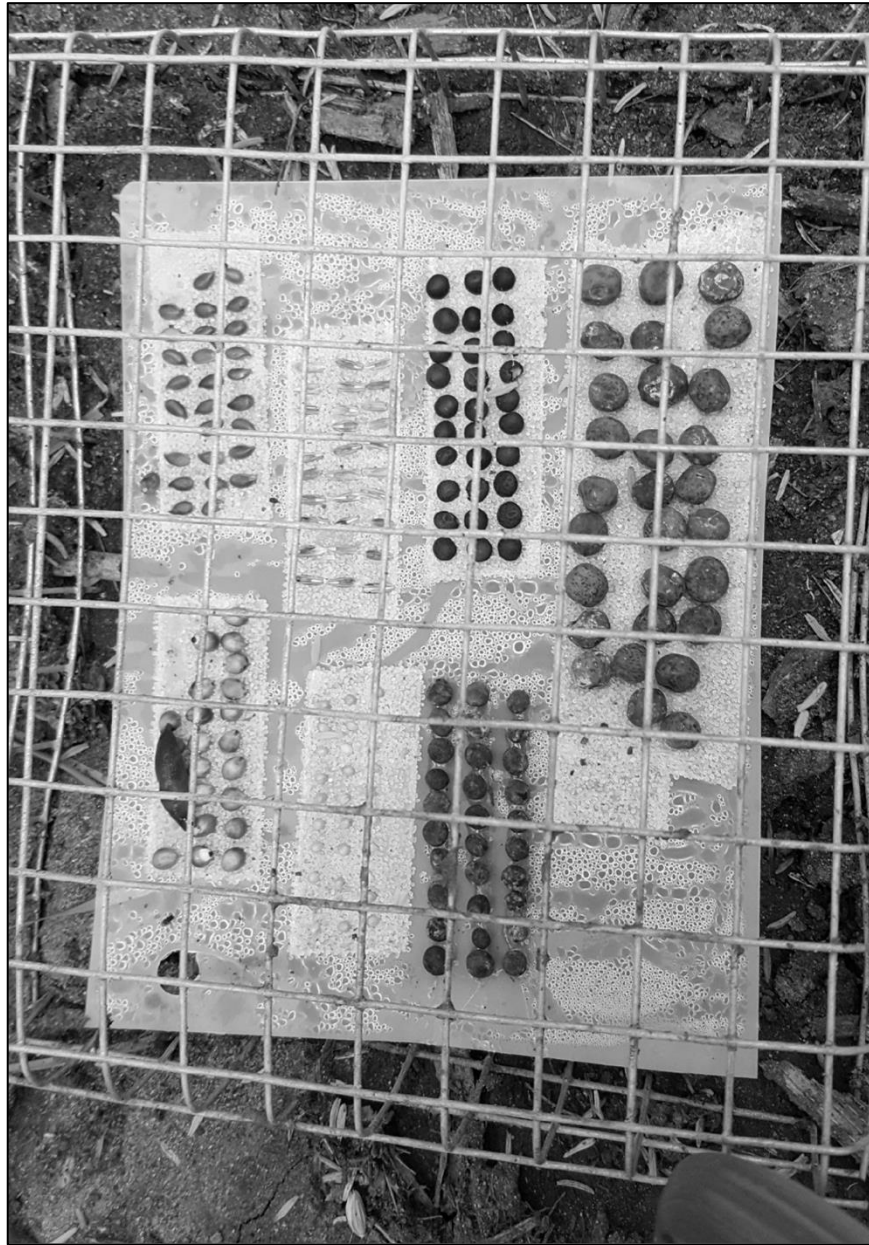
Figure 2. Cover crop seeds per species (mean ± SEM) damaged by arthropods after seeds were exposed to field conditions for 7 d. Bars represent across-plot (n = 8) average coated and bare seeds damaged by invertebrates (five coated and five bare seed cards per plot, each possessing 30 seeds of each species). Asterisks demarcate significant differences (α = 0.05) between coated and bare seed treatments for a given plant species.
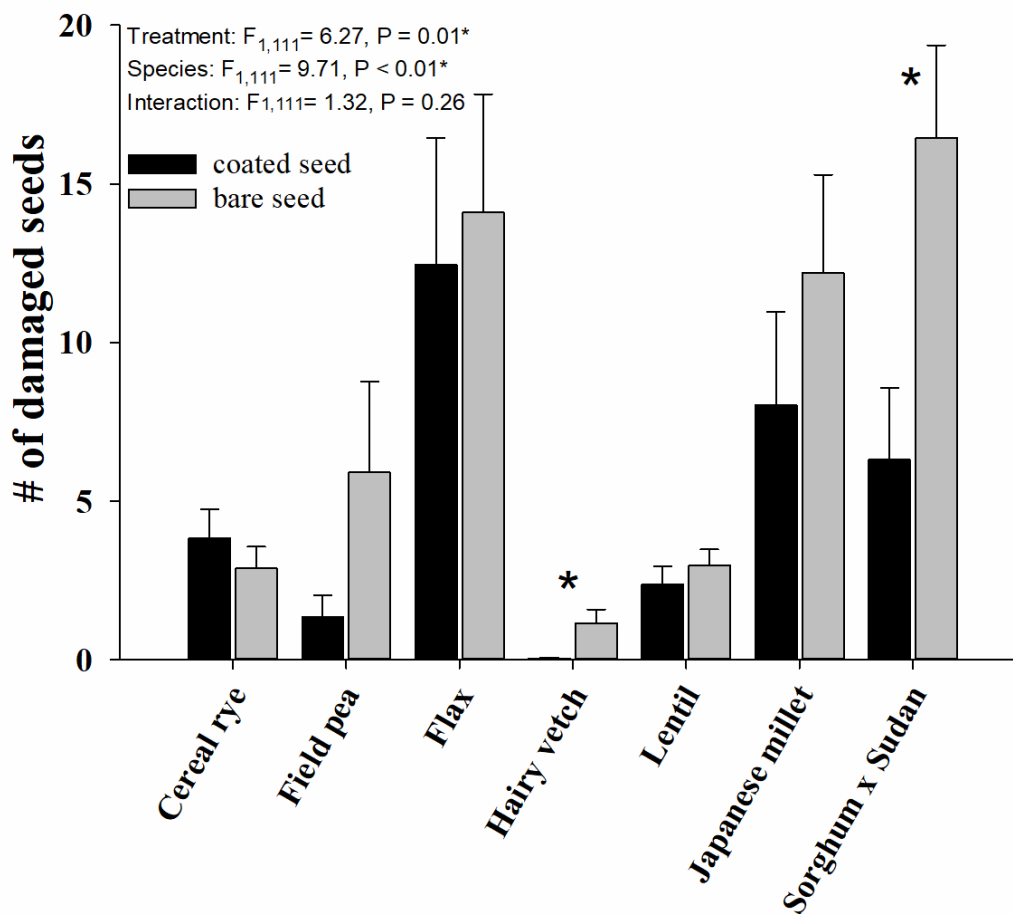
7.0 Tables

Table 1. Mean ± SEM arthropod granivores collected per pitfall in plots possessing cover crop seed cards. Common taxa were included if present at ≥ 0.5 individuals per pitfall (n = 32 slatted, double pitfall traps, total) in research plots (n = 8). Letters written after values represent the Tukey HSD all-pairwise comparison groupings between individual taxa (α = 0.05).

Common taxa, grouped	Insect abundance
Total Carabidae	27.16 ± 7.00
Total Gryllidae	17.35 ± 6.00
Total Staphylinidae	11.79 ± 8.36
Common taxa, individuals	**Arthropod abundance**
Carabidae- *Bembidium* sp.	7.12 ± 2.89 AB
Carabidae- *Pterostichus permundus*	13.98 ± 6.18 A
Carabidae- *Stenolophus* sp.	1.77 ± 0.78 AB
Cucujidae- *Pediacus* sp.	1.25 ± 0.49 B
Gryllidae- *Allonemobius* sp.	5.14 ± 2.34 AB
Gryllidae- *Gryllus pensylvanicus*	12.16 ± 3.40 AB
Julida (millipede order)	0.96 ± 0.60 B
Porcellionidae- *Trachelipus rathkii*	0.61 ± 0.34 B

Table 2. Mean ± SEM lime-coated and bare cover crop seeds damaged by arthropods following 7 d of field exposure. Values represent across-plot (n = 8) average number of coated and bare seeds damaged by invertebrates (five coated and five bare seed cards per plot, each possessing 30 seeds of each species). Asterisks denote significant differences between treatments (α = 0.05). Letters written after values represent the Tukey HSD all-pairwise comparison groupings within respective treatments (α = 0.05).

Cover Crop Species	Damaged coated seed (out of 30)	Damaged bare seed (out of 30)	ANOVA
Cereal rye (*Secale cereale*)	3.83 ± 0.91 AB	2.88 ± 0.68 CD	$P = 0.42$ $F_{1,15} = 0.70$
Field pea (*Pisum sativum*)	1.35 ± 0.67 B	5.90 ± 2.86 BCD	$P = 0.14$ $F_{1,15} = 2.40$
Flax (*Linum usitatissimum*)	12.45 ± 4.00 A	14.10 ± 3.72 AB	$P = 0.77$ $F_{1,15} = 0.09$
Hairy vetch (*Vicia villosa*)	0.03 ± 0.03 B	1.15 ± 0.43 C	$P = 0.02*$ $F_{1,15} = 6.81$
Lentil (*Lens culinaris*)	2.37 ± 0.57 B	2.98 ± 0.49 CD	$P = 0.44$ $F_{1,15} = 0.64$
Japanese millet (*Echinochloa esculenta*)	8.03 ± 2.93 AB	12.2 ± 3.09 ABC	$P = 0.34$ $F_{1,15} = 0.96$
Sorghum × Sudan (*Sorghum × drummondii*)	6.30 ± 2.27 AB	16.45 ± 2.92 A	$P = 0.02*$ $F_{1,15} = 7.54$

CHAPTER THREE: INTERSEEDING COVER CROPS INTO ESTABLISHED CORN
AFFECTS INVERTEBRATE ABUNDANCE AND DIVERSITY AND ELEVATES
THE ACTIVITY OF GENERALIST PREDATORS

Abstract

Corn (*Zea mays*) monocultures provide few ecosystem functions compared to the diverse native prairies they have replaced. Simplified corn fields lack the plant diversity-derived resources necessary for supporting robust beneficial insect populations capable of preventing pest outbreaks. Interseeding cover crops into established corn is a method gaining popularity by farmers for the diversification of cropland plant communities and restoration of ecosystem functions. Here we examine how foliar, epigeic and subterranean arthropod communities and generalist predator activity differ between corn monocultures and corn interseeded with cover crops. Abundances of predators, herbivores, numerous individual taxa and all combined arthropods were greater on the soil surface in interseeded corn fields. Epigeic species richness was also increased as a result of adding cover crops. Within the subterranean environment total arthropod and guild abundances were similar between treatments, and only four commonly collected species abundances differed. In the presence of interseeded cover crops below-ground arthropod diversity increased. There were no individual or grouped taxa differing in abundance between treatments within the corn foliage. Invertebrate species richness and diversity were also unchanged on corn plants. Predation of wax moth (*Galleria mellonella*) sentinel larvae by generalist predators occurred more than twice as frequently in cover-cropped corn (45.5%) than in corn monocultures (22.6%). The effect of cover

crops on corn stand density and yield varied significantly between study locations.

Diversification of the corn agroecosystem by interseeding cover crops had a strong

influence on the surface-dwelling invertebrate fauna and predator activity. This

management tool should be further explored by researchers and farmers as it is a viable

method for improving invertebrate habitat and bolstering biological control of crop pests.

Key words: Biological control, conservation agriculture, cover crops, detritivores,

herbivores, interseeding, predators, prey sentinels

1.0 Introduction

In 2018, 36.4 million hectares of corn were planted in the United States (USDA-

NASS, 2018), 4.7% of the entire landmass of the 48 contiguous states (USDA-ERS,

2019). Nearly all cornfields are planted under monoculture conditions, and these

monocultures have replaced what was historically highly diverse perennial grasslands

(Rashford et al., 2011; Wimberly et al., 2017). Plant-diverse habitats tend to support

greater biodiversity of non-plant species (Schmid, 2014). In addition, diverse habitats

perform numerous ecosystem functions which are lost or severely impaired under

simplified agricultural production (DeFries et al., 2004; Fiedler et al., 2008). One such

ecosystem function provided in plant-diverse environments is the maintenance of

herbivore communities at sub-outbreak populations. When plant diversity is robust

herbivore population growth is curtailed through multiple means including both top-down

and bottom-up antagonisms (Landis et al., 2005; Moreira et al., 2016). Contrarily, in

simplified landscapes, such as those hosting corn in monoculture, herbivores face few

agents of biological control because the habitat does not provide necessary resources to maintain natural enemies. Farmers often resort to chemical control of herbivores in these enemy-free spaces (Fausti et al., 2018; Fausti et al., 2011). Adding plant diversity to would-be monocultures is gaining popularity amongst agricultural producers due to the positive effect of plant diversity on ecosystem functions including crop pest control (Gurr et al., 2017).

Despite widespread use of corn monocultures throughout industrialized agriculture, corn has historically been grown with other plant species, and this is a practice still commonly used in subsistence agriculture (Landon, 2008). Advancements in agricultural technology and research elucidating underlying plant synergisms has led to a recent increase in farmland being planted to multiple plant species simultaneously (CTIC, 2017). Corn producers are accomplishing this in their fields by planting cover crops between establish rows of corn. Cover crop species can be selectively chosen to add benefits to the ecosystem without interfering with crop growth or harvest.

Cover crops growing alongside corn have the potential to alter agroecosystem habitats in ways which make these production areas more suitable for a diversity of arthropod species, not just those whose life histories are supported by corn. Nectar and pollen produced by interseeded cover crops, and alternative prey species consuming cover crop tissues are all resources by which natural enemies can sustain themselves in the absence of crop pests (Lundgren, 2009; Manandhar and Wright, 2016). Adding plant diversity during the corn growing season by incorporating cover crop mixtures may provide a means by which farmers can mimic the diversity and functionality of natural systems row crops have replaced.

Very little work has been done to characterize the effect of added plant diversity through interseeding cover crops on invertebrate community structure and on important functional guilds in corn production systems. The main null hypothesis that we tested was that interseeded cover crops have no effect on insect community structure and the abundances of herbivores, predators, and detritivores. We also tested the null hypothesis that interseeded cover crops in corn do not affect predation rates on the soil surface. Finally, we examined whether these interseeded cover crops affected corn yield and plant density.

2.0 Methods

2.1 Arthropod communities in cornfields with bare soil or cover crops

2.1.1 Field sites

Research was conducted at three locations over two years. The first location was near Estelline, SD (44.58, -96.79), and two locations were near Gary, SD (44.91, -96.40 [Gary-17]; 44.92, -96.40 [Gary-18]). At study locations eight fields measuring 42×42 m each were established in a 2×4 orientation separated by 15 m borders.

At Estelline, corn (Elk Mound Seed Company, Elk Mound, WI, 54739; variety: EMS 8100; maturity: 80 d) was no-till planted on May 26, 2017 at 79,000 seeds/ha in 76 cm wide rows. Roundup™ (rate: 2338 mL/ha; a.i.: glyphosate; Monsanto™, St. Louis, MO, 63167) was applied as a preplant herbicide to eliminate emerged weeds. No post-emergent herbicides or fertilizers were used at Estelline (the farmer intended on using the land for fall animal grazing, not for grain harvest).

At Gary-17, corn (Legend Seeds, Inc.™, De Smet, SD, 57321; variety: A10946, A10258; maturity: 97 d) was planted on May 5, 2017 at the same density and row spacing as at Estelline. The field had been cultivated in the spring prior to planting to prepare the seed bed. SureStart II™ (rate: 2923 mL/ha; active ingredients: acetochlor, flumetsulam and clopyralid; Dow AgroSciences ™, Indianapolis, IN, 46268) was applied on May 1 as a pre-plant herbicide. Fertilizer was broadcasted into research fields at a rate of 157 kg nitrogen/ha as urea, 67 kg phosphorous/ha as MicroEssentials®SZ™ (Mosaic™, Plymouth, MN, 55441) and 56 kg potassium/ha as potash. Neither location was treated with insecticide.

At Gary-18, all plots were cultivated to prepare the seed bed. Corn (Blue River Organic Seed™; Ames, IA, 50014; variety: P1000684; maturity: 96 d) was planted on May 26, 2018 in 76 cm wide rows at a population of 79,000 seeds/ha. Research fields were sized and orientated similarly to those in Gary-17. On May 20, pre-emergent herbicide, SureStart II™ (rate: 2923 mL/ha), was applied in research fields with no further herbicide use. Fertilizer was broadcasted into fields at a rate of 157 kg/ha nitrogen as urea, 56 kg/ha potassium as potash and 56 kg/ha phosphorous as diammonium phosphate. No insecticides were used during 2018.

An eight-species cover crop mixture [coated in calcium carbonate (1: 1, seed: $CaCO_3$, by weight)] of hairy vetch (*Vicia villosa*, 3.5 kg/ha), lentils (*Lens culinaris*, 3.5 kg/ha), mung beans (*Vigna radiata*, 5 kg/ha), oats (*Avena sativa*, 5 kg/ha), flax (*Linum usitatissimum*, 9 kg/ha), cereal rye (*Secale cereale*, 14.6 kg/ha) and field peas (*Pisum sativum*, 14.6 kg/ha) was planted into four of the established plots in an alternating pattern. At Estelline and Gary-17, this seeding was broadcasted immediately following

corn emergence (Gary: May 16; Estelline: June 2). At Gary-18, seeds were planted

following corn emergence using a seven-row homemade tractor-drawn cover crop

interseeder (Figure 1). The device planted a single row of cover crops between each pair

of 76 cm-spaced corn rows.

2.1.2 Insect sampling

Soil-dwelling arthropods were sampled four times in each plot during a given site

year (corn stages: V2, V4, V8 and anthesis) by taking five soil cores (diameter: 11 cm,

depth: 10 cm) within corn rows at random locations within a given plot. This resulted in a

total of 160 cores taken from one site during a single field season, or, 480 cores across all

three site years. Arthropods within collected soil were extracted for 7 d using Berlese

funnels into 70% ethanol. Arthropods were stored in 70% ethanol until identification and

curation.

Epigeic invertebrates were sampled three times (corn stages: V4, V8 and anthesis)

during a growing season at each location. Manual aspirators were used to suck arthropods

off the soil's surface within confined 0.5 × 0.5 m sheet metal quadrats pressed > 2 cm

into the soil to prevent arthropod escape. Four quadrat collections were taken from each

plot on every sampling date for a total of 96 taken from one location over the course of a

field season, and 288 samples across all three site years. Before leaving a sampled plot,

arthropods from each quadrat were placed in 70% ethanol until identification.

Foliar arthropods were surveyed in research plots three times (corn stages: V4, V8

and anthesis) during each season by conducting whole-plant dissections (Lundgren et al.,

2015). On a given sampling date 15 corn plants were collected from random locations

within a plot (> 5 m in from the plot edge) and dissected on white cotton sheets. Invertebrates were identified upon sight to the lowest possible taxonomic unit and recorded. At each location a total of 360 corn plants were examined during a single season, or 1080 plants across all three site years.

2.1.3 Yield

Yield and plant density were measured following corn maturation. Corn ears were collected, and plants were counted within a 3 m row section at four points located 8, 16, 24 and 32 rows from the plot's edge; row series were sampled so the final collection points represented a diagonal pattern across each field. Corn kernels were removed using a hand-sheller (item number: 530065; Premier1Supplies™, Washington, IA, 52353), and weighed (weights were adjusted to 15% moisture for comparison).

2.2 Predation of sentinel prey in corn monocultures and corn interseeded with cover crops

2.2.1 Field sites

Predation experiments were conducted at four separate locations, two in 2016, and two in 2017. The 2017 observations were made at the Estelline and Gary-17 experimental fields that are described above. In 2016, one site was located near Canby, MN (44.81, -96.36) and one site at the Dakota Lakes research farm near Pierre, SD (44.29, -100.00;). At Canby, alfalfa (*Medicago sativa*) had been established for 3 y when it was chisel plowed in the fall of 2015. The seed bed was cultivated in spring, which reduced the alfalfa stand density to approximately 10 plants/m2 growing under the corn

canopy in the interseeded treatment. In corn monoculture fields at Canby alfalfa was terminated by rototilling prior to corn planting. Corn (Blue River Organic Seed™; Ames, IA, 50014; variety: P1000684; maturity: 96d) was planted in similarly sized and oriented fields as in Gary-17 on May 24, 2016 in 76 cm rows at a population of 76,600 seeds/ha. No fertilizer, herbicides or insecticides were used at Canby.

At Pierre, corn (Pioneer™, Johnston, IA, 50131; variety: P0533AM1; maturity: 105d) was planted on May 3, 2016 at a population of 94,000 seeds/ha. Seeds had been treated with the insecticide, clothianidin (0.25mg/seed). Every two corn rows, spaced by 55 cm, were separated by one row of Roundup Ready® alfalfa in interseeded plots. Monoculture and interseeded corn were planted in adjacent blocks where four research fields were established in each (similar size and separation to the other locations). Glyphosate (rate: 2338 mL/ha) was used for control of weeds at corn planting, and Brox™ 2EC (rate: 1169 mL/ha; active ingredient: bromoxynil; AgriStar ™, St Joseph, MO, 64504) was sprayed on June 8 to suppress alfalfa growth. Fertilizer was side-banded at planting as 50.5 kg N/ha as a blend of urea and ammonium sulfate (9: 1, by weight, respectively), and 88 kg/ha of a monoammonium phosphate and potassium chloride blend (8: 2, by weight, respectively). Additional nitrogen was applied at the R1 plant stage through irrigation water based on soil testing to result in a total of 240 kg N/ha throughout the growing season.

2.2.2 Prey sentinels

To measure general epigeic predatory activity, wax moth (*Galleria mellonella*) larvae were used as sentinel baits in both treatments. Although wax moth larvae are not crop

pests, they have been used successfully in previous field experiments for comparing generalist predatory activity between different agricultural habitats (Lundgren et al., 2007; Meehan et al., 2012). Prepupal larvae were individually pinned (#0 black enameled insect pins; model: 01.10; Entochrysis™, Pardubice, 53002, Czech Republic) to 1 cm tall pyramids made of sculpting clay (Sculpey™ original, Polyform Products Co. Inc.™, Elk Grove Village, IL, 60007) through the larvae's posterior segments. Larvae were deployed in corn fields during anthesis within 1 h of being pinned to clay and were only used if obviously alive and active. Clay pyramids were buried in the soil so that pinned larvae were presented flush with the soil surface. Once deployed, sentinels remained undisturbed for 1 h, at which time they were recollected to assess the proportion of sentinels which had been predated. Sentinels were considered predated if there were invertebrates actively feeding, or if the wax moth larva had been partially or wholly consumed. Predators present at sentinels were identified upon sight to the lowest taxonomic rank possible and recorded.

In 2016, 30 sentinel larvae were placed in each research field. Three rows of 10 larvae were placed at the base of corn plants. Within each row, sentinels were spaced 3 m apart, and each row was separated by 4 m. In 2017, 40 larvae were used in each field. The same orientation was used as in 2016, except there was an additional row of 10 sentinels. In total, 1120 wax moth sentinel larvae were deployed to assess predation. Methods for sentinel predation were modeled after those described by Lundgren and Fergen (2011).

2.3 Data analysis

2.3.1 Insect communities

Shannon diversity index was calculated, and species richness reported for the invertebrate communities in each of the three habitats sampled, corn foliage, epigeic, and subterranean for both treatments. Invertebrate species collected from the soil surface and within soil were categorized into three functional groups, predators, herbivores and detritivores. Invertebrates collected on corn foliage were categorized into two groups, predators and herbivores. Mean ± SEM arthropods per plant or per m^2 of soil in the various arthropod guilds from corn monocultures and interseeded corn were determined. Individual taxa were not included in functional groupings if their life histories were unknown. Two-way repeated measures ANOVAs (rm-ANOVAs) coupled with Tukey's HSD all-pairwise comparisons were conducted to examine the effect of treatment (monoculture and interseeded corn), corn stage, or an interaction of both on diversity, species richness, and arthropod abundance in the different cornfield habitats. If an interaction was revealed subsequent one-way ANOVAs were conducted to determine treatment differences for individual corn stages. Individual taxa driving overall trends in abundance for each habitat were determined by performing rm-ANOVAs on taxa representing ≥ 0.5% of the total arthropod community abundance in corn foliage, on the soil surface, or within the soil. To gain a better understanding of treatment effects on the invertebrate community springtails (Collembola) and mites (Acarina) were excluded when determining common epigeic and subterranean taxa due to their disproportionately large abundances. Two-way ANOVAs were conducted on corn yield and density to determine if there was a significant interaction between site year and treatment. If an interaction existed subsequent one-way ANOVAs tested treatment differences at individual sites.

2.3.2 Predation

Mean ± SEM percent sentinel predation per plot was determined for each treatment at individual sites and across all site years. A two-way ANOVA was used to determine treatment and site effects on rates of sentinel predation, and if an interaction existed between the two. Subsequent one-way ANOVAs were used to examine the levels of significance between treatments at individual study locations. Linear regressions were conducted to examine if correlations existed between diversity (Shannon H) or epigeic predators/m2 (during anthesis) and predation rate at Gary-17 and Estelline as invertebrate community assessments and sentinel predation experiments were done at these locations. Data analyses were conducted using Statistix® 10 software (Analytical Software, Tallahassee, FL, U.S.A.).

3.0 Results

3.1 Invertebrate communities

A grand total of 63,868 invertebrates were collected from epigeic, subterranean and foliar cornfield habitats. The invertebrate community consisted of 516 species from 22 orders, including: Araneae, Acarina, Cephalostigmata, Coleoptera, Collembola, Diplura, Diptera, Ephemeroptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, Odonata, Opiliones, Orthoptera, Protura, Pseudoscorpiones, Psocoptera, Stylommatophora, Thysanura and two unidentified orders, one each from classes Chilopoda and Diplopoda.

3.1.1 Epigeic invertebrates

A total of 8098 invertebrates were collected from the soil's surface using quadrats. Across all site years and sampling dates, more than twice as many invertebrates were collected from the epigeic environment in interseeded corn (n = 5722) compared to corn monocultures (n = 2376) ($F_{1,71}$ = 10.36, P < 0.01; Figure 2A).

The five most abundantly collected taxa from the soil surface were Collembola (all springtails, 24.46%), *Rophalosiphum padi* (Aphididae) (12.07%), *Stenolophus comma* (Carabidae) (7.26%) Acarina (all mites, 6.74%) and *Elaphropus* sp. (Carabidae) (3.52%). Based on known life histories, 165 species were designated as predators, 52 species were classified as herbivores, and 16 were detritivores. Throughout the growing season, both predators and herbivores were more abundant in interseeded plots compared to monocultures (predators: $F_{1,71}$ = 17.90, P < 0.01, herbivores: $F_{1,71}$ = 9.93, P < 0.01; Figures 2B-C), but detritivore abundance was statistically similar between treatments ($F_{1,71}$ = 1.87, P = 0.19; Figure 2D). Increased predator abundance on the soil surface in interseeded plots was largely driven by three predatory groups and four commonly collected individual taxa. Ground beetle (Carabidae: $F_{1,71}$ = 5.42, P = 0.03), rove beetle (Staphylinidae: $F_{1,71}$ = 4.53, P = 0.04) and spider (Araneae: $F_{1,71}$ = 6.90, P = 0.02) abundances were higher in cover-cropped corn. Individually, predatory *Bembidion* sp. ($F_{1,71}$ = 8.89, P = 0.01), *Nabis americoferus* ($F_{1,71}$ = 5.50, P < 0.01), a Tetragnathid spider species ($F_{1,71}$ = 4.57, P = 0.04) and larval Coccinellidae ($F_{1,71}$ = 16.69, P < 0.01) were more abundant when cover crops were present. For herbivores, significantly more bird cherry oat aphids (*Rhopalosiphum padi*: $F_{1,71}$ = 4.43, P = 0.05) and plant bugs (Miridae:

$F_{1,71} = 6.10$, $P = 0.02$) were found in the interseeded fields compared with the monoculture cornfields (Table 1).

Twenty-seven species individually represented $\geq 0.5\%$ of all surface-dwelling arthropods (excluding the abundances of mites and Collembola). Of these 27, the abundance of six species, two herbivorous and four predatory, were significantly increased in interseeded corn (Table 1). There were no species whose abundance was lower in interseeded corn compared to corn in monoculture.

An interaction existed between treatment and corn stage for surface-dwelling invertebrate abundance, and for the abundance of predatory and herbivorous guilds (Figures 2A-C). Total arthropod abundances did not differ during V4 and V8 plant stages, but during anthesis more invertebrates were collected in interseeded plots ($F_{1,23} = 19.23$, $P < 0.01$). Predators were more abundant in interseeded plots during all of the sampled corn stages (V4: $F_{1,23} = 14.06$, $P < 0.01$, V8: $F_{1,23} = 6.75$, $P = 0.02$, anthesis: $F_{1,23} = 4.15$, $P = 0.05$), whereas herbivores were more numerous in interseeded plots during V4 and V8 ($F_{1,23} = 7.89$, $P = 0.01$ and $F_{1,23} = 4.42$, $P = 0.05$, respectively), but not anthesis.

There were 298 epigeic invertebrate species collected over the season (in both treatments), with greater species richness in interseeded corn fields than in monocultures ($F_{1,71} = 13.07$, $P < 0.01$; monoculture: 39.50 ± 3.04, interseeded 61.25 ± 4.03 species). Across treatments, invertebrate richness increased incrementally among sampled corn stages ($F_{2,71} = 30.70$, $P < 0.01$). Interseeding cover crops did not affect the diversity (Shannon H) of surface-dwelling arthropods ($F_{1,71} = 0.00$, $P = 0.99$), but invertebrate

diversity did vary among corn stages ($F_{2,71} = 14.16$, $P < 0.01$), with V4 corn being less diverse than corn at the V8 and anthesis stages.

3.1.2 Subterranean invertebrates

A total of 45,797 invertebrates were extracted from soil cores throughout the duration of the experiment, with 19,254 from corn monocultures and 26,543 from corn interseeded with cover corps. Despite collecting 7289 more invertebrates from interseeded plots total arthropods per square meter was not significantly different between treatments ($F_{1,95} = 0.72$, $P = 0.41$, Figure 3A). When the community was separated into functional guilds (predators: $n = 167$ species; herbivores: $n = 38$ species; detritivores: $n = 41$ species) an interseeded cover crop did not affect invertebrate abundance for predators ($F_{1,95} = 1.03$, $P = 0.32$), herbivores ($F_{1,95} = 1.20$, $P = 0.29$) or detritivores ($F_{1,95} = 0.73$, $P = 0.40$) (Figures 3B-D).

Mites and collembola dominated the subterranean community, representing 56.83% and 33.01% of all individuals collected, respectively. Diplurans were next most abundant, representing 0.68%. Abundances of taxa fell sharply following diplurans, without distinctly abundant individuals. 27 species each represented $\geq 0.5\%$ of all collected subterranean arthropods, and although an overall treatment effect did not exist for the abundances of subterranean invertebrates, the abundances of four individual taxa differed between interseeded and monoculture corn. One Lycosidae spider species, a detritivorous Cryptophagidae beetle and all Thripidae (herbivorous) were increased when cover crops were present, whereas Beetle Larvae 15 (see Table 1 footnote for specimen description) was found less frequently in cover-cropped fields (Table 1).

Interseeded corn possessed a greater diversity (Shannon H) of subterranean invertebrates than corn monocultures ($F_{1,95} = 4.17$, $P = 0.05$). There was a total of 361 species collected throughout the growing seasons in both treatments with no difference in terms of species richness between monoculture and interseeded fields ($F_{1,95} = 2.00$, $P = 0.17$; monoculture: 55.25 ± 5.32, interseeded 64.50 ± 6.39 species). Across treatments, both species richness and diversity varied between corn stages ($F_{3,95} = 12.08$, $P < 0.01$ and $F_{3,95} = 4.17$, $P < 0.01$, respectively), with corn having the greatest number of species and diversity during anthesis compared to all earlier sampled stages.

3.1.3 Foliar invertebrates

A total of 9973 invertebrates were collected from plant samples, with 4681 from interseeded corn and 5292 from corn monocultures. The abundance of total invertebrates per dissected corn plant did not vary between treatments ($F_{1,71} = 0.53$, $P = 0.47$, Figure 4A). Likewise, interseeding corn with cover crops did not affect the number of predators ($n = 29$ species) or herbivores ($n = 18$ species) per corn plant ($F_{1,71} < 0.01$, $P = 0.99$ and $F_{1,71} = 0.89$, $P = 0.36$, respectively, Figures 4B-C). Across treatments, abundance varied among corn stages for total arthropods, predators and herbivores, with corn plants during anthesis possessing more invertebrates than V4 or V8 plants (Figure 4).

Only six individual or grouped taxa represented $\geq 0.5\%$ of total invertebrate abundance in corn foliage. Herbivorous thrips (Thripidae), corn leaf aphids (Aphididae: *Rhopalosiphum maidis*) and mites (Acarina), represented 34.44%, 32.61 and 1.81% of the total foliar community abundance, respectively. Predatory minute pirate bugs (*Orius insidiosus*), spiders and ladybeetle larvae (Coccinellidae), represented 5.17%, 3.15% and

0.53% of the total foliar community, respectively. None of these taxa significantly differed in population size between interseeded and monoculture corn plots.

Corn earworms (*Helicoverpa zea*), European corn borers (*Ostrinia nubilalis*) and northern corn rootworms (*Diabrotica barberi*) are particular pests of concern for corn producers (SDSU, 2019), but in this study only 10 individuals of each species were collected from a total 1080 plants. Seven *H. zea* were collected from interseeded plots and three from monoculture, two *O. nubilalis* from interseeded and seven from monoculture, and eight *D. barberi* from interseeded and two from monoculture plots.

In both interseeded and monoculture fields there was a total of 66 species collected throughout the growing season. Species richness did not differ between treatments ($F_{1,71} = 0.13$, $P = 0.73$; monoculture: 17.00 ± 1.24, interseeded 18.50 ± 1.40 species), but differed among corn stages ($F_{2,71} = 36.58$, $P < 0.01$), with greater richness measured at V8 and anthesis than at V4. Similarly, foliar arthropod diversity did not differ between corn monocultures and interseeded corn ($F_{1,71} = 0.07$, $P = 0.79$), but diversity differed among plant stages ($F_{2,71} = 35.55$, $P < 0.01$) with arthropods being more diverse during V8 and anthesis compared to V4 corn.

3.2 Predation experiments

Across all site years, $45.5 \pm 7.7\%$ of sentinels were consumed in interseeded corn compared to only $22.6 \pm 3.2\%$ in corn monocultures ($F_{1,31} = 27.6$, $P < 0.01$, Figure 5). Variability in treatment effects at different study locations resulted in a significant interaction effect between treatment and site ($F_{3,31} = 18.2$, $P < 0.01$). At Canby and Gary-17 sentinels were consumed at a significantly higher rate in interseeded corn than corn

monocultures (Canby: $F_{1,7} = 201.9$, $P < 0.01$; Gary-17: $F_{1,7} = 7.01$, $P = 0.04$), whereas at Pierre and Estelline sentinel predation was not different between treatments (Pierre: $F_{1,7} = 1.65$, $P = 0.25$; Estelline: $F_{1,7} = 0.37$, $P = 0.57$) (Table 3).

Of the 369 total predation events, we observed active feeding by 547 invertebrates on 211 sentinels at the conclusion of the one-hour deployment period. Crickets (Orthoptera: Gryllidae) were most commonly observed consuming sentinels (n = 330, 60.3%), followed by ants (Hymenoptera: Formicidae; n = 119, 21.8%), harvestmen (Opiliones: Phalangiidae; n = 37, 6.8%) and ground beetles (Carabidae; n = 31, 5.7%) (remaining grouped taxa presented in Table 4). When comparing sentinel predation rates among both treatments at Estelline and Gary-17 to epigeic predator abundance and species diversity (Shannon H) during anthesis no significant correlations were revealed (predator abundance: $F_{1,16} = 1.81$, $P = 0.20$, community diversity: $F_{1,16} = 0.50$, $P = 0.49$).

3.3 Corn yield and density

Significant interactions between treatment and site year existed for both corn yield ($F_{2,23} = 44.26$, $P < 0.01$) and corn density ($F_{2,23} = 14.47$, $P < 0.01$), owing to the uniquely large treatment effect observed in both measurement types at Estelline. Neither yield nor density differed between interseeded and monoculture corn plots at Gary-18. At Gary-17 corn density was also unchanged between treatments, but yield was marginally-significantly greater in cover-cropped corn ($F_{1,7} = 4.74$, $P = 0.07$). At Estelline corn plant density was greater in interseeded plots ($F_{1,7} = 58.93$, $P < 0.01$), but corn yield was reduced in the presence of cover crops ($F_{1,7} = 117.97$, $P < 0.01$). (Table 2)

4.0 Discussion

Over 63,000 specimens collected across several plant stages and from multiple cornfield strata resulted in a robust bioinventory of cornfield-dwelling invertebrates, making this the most comprehensive bioinventory of cornfields yet compiled. Demographics of this community were relatively consistent with other cornfield bioinventories described previously (LaCanne, 2017; Lundgren et al., 2015; Stevenson et al., 2002). Cornfields hosted thousands of invertebrates per m^2, representing hundreds of species from a total of 22 orders. By far, most invertebrates (approximately 70% of specimens and 70% of species) were collected in the top 10 cm of the soil column. These numbers are particularly stark when one considers the relative spatial area sampled over the study; soil communities were assessed for approximately 15 m^2, whereas the epigeal and foliar communities were collected from 72 and 137 m^2, respectively. Within this community in the soil column, Collembola and mites dominated the community, and diversity of the mites would have added even more species to our tally. Biomass of invertebrates in the soil represents a significant source of nutrients and ecosystem function within the soil, and one that can be readily managed by farmers (Altieri, 1999; Landis et al., 2000; Pearsons and Tooker, 2017). The diverse community revealed in this study provides many services to farmers, but the diversity found in cornfields still pales in comparison to the invertebrate communities found in ancestral habitats that cornfields have replaced (Nemec et al., 2014; Schmid et al., 2015; Standen, 2000; Wimberly et al., 2017). Species of this community that receive the most attention from land managers are those considered pests. Despite the lack of insecticides used in fields assessed for community characteristics, corn pests were never found at actionable levels, and foliar

(corn earworms and European corn borers) and root pests (corn rootworms) of special concern were particularly scarce (fewer than one per 100 dissected corn plants for foliar pests, and corn rootworm larvae were not found). These results call into question the need for prophylactic insecticidal products meant to control these arthropods, which has been pointed out by previous researchers (Bredeson and Lundgren, 2015; Hutchison et al., 2010). Clearly, a broader view of biodiversity that transcends managing the handful of problematic species to managing the function of insect communities seems justified by the current study (Coll and Wajnberg, 2017).

Interseeding covers into standing corn increased the community complexity of invertebrates over corn planted in bare soil. Foliar communities were largely unaffected by the cover crop. The reason for this may have been that the low-growing cover crops attracted specialists to this habitat stratum. This pattern has been seen in orchard systems as well, where enhanced ground level community structure did not move into the orchard canopy (Altieri and Schmidt, 1986; Horton et al., 2009). Within the soil column, invertebrate abundances were consistent between the two systems. We imagine that the rate of dispersal of invertebrates throughout this habitat is curtailed relative to the soil surface and foliar habitats (Ojala and Huhta, 2001). It is feasible that communities within annual, ephemeral cropping systems simply don't have the time to respond to the added plant diversity. The observation that species diversity (Shannon H) increased may be the initial rebalancing of the successional community in the soil in response to the cover-crop mediated habitat change (Longcore, 2003). We hypothesize that most niches in the soil column in this disturbed habitat were occupied by early successional species that are adapted for cropland, and as plant diversity increased, it began to change the relative

abundances of the species that were there. If this is true, then we might expect richness and diversity to increase in the interseeded cropland over monocultures over time (Siemann et al., 1999; Suding and Gross, 2006).

The epigeic community was most strongly influenced by the addition of interseeded cover crops. More than twice as many specimens were collected on the soil surface of interseeded corn fields, and all functional guilds (except for detritivores) were increased significantly relative to corn planted in bare soil. Conditions on the soil surface within plant-diverse fields also resulted in a greater number of species inhabiting this environment. We expected that this community would be the most affected, as this community is most proximate to the resources made available by the cover crops [similarly shown by (Horton et al., 2009)]. These resources might be most simply categorized as habitat and trophic in nature. Diversifying plant assemblages directly affect invertebrate communities by ameliorating harsh abiotic conditions (Orr et al., 1997), increasing habitat structural complexity and niche partitioning (Langellotto and Denno, 2004; Letourneau et al., 2011) and providing a variety of nutritional sources (Lundgren, 2009; Venzon et al., 2006), to name a few. Exactly how diversification of plant communities alters habitat suitability for invertebrates is often categorized into these simplified cause-and-effect relationships. In truth, multi-trophic ecological interactions within even the most simplified agroecosystem become nearly infinitely complex (Lundgren and Fausti, 2015), and in some ways, it is impossible to predict why invertebrate communities respond the way that they do to habitat manipulations except for in broad patterns.

Interseeding cover crops increased predation on the soil surface in corn. Adding plant diversity into simplified agricultural production systems often has the resulting effect of elevating biological control (Altieri and Schmidt, 1986; Bickerton and Hamilton, 2012; Lundgren and Fergen, 2010). One explanation is that community structure and function are linked; that changes in predator community structure in response to plant diversification is responsible for increasing predation rates. This was not what we observed. Predator abundance and diversity were not correlated with consumption rates per field. Factors known to influence predation include relative prey and non-prey food sources in a habitat (Nomikou et al., 2010), intraguild interactions (Frank et al., 2010), structural complexity of the habitat that may impede or enhance foraging by predators (Finke and Denno, 2002), etc. Within interseeded fields it is likely that a combination of altered behavioral factors, not predator community structure, interacted to increase predation by the natural enemy community.

The response of crop yield to undersown cover crops is highly variable depending on a host of factors such as nutrient availability, weed pressure, soil moisture, cover crop species and sowing times (Abdin et al., 1998; Curran et al., 2018). It is possible that reduced yield in cover-cropped fields at Estelline was a result of competition between corn and cover crops for nutrients in fields not receiving fertilizer. Interseeded fields at this location also possessed a challenging weed population, further depleting available soil fertility. Alternatively, marginally increased corn yield in cover-cropped fields at Gary-17 reflects the results of studies identifying synergisms in mixed-cropping systems. For example, Rerkasem and Rerkasem (1988) recorded an increase in corn yield and leaf tissue nitrogen when ricebean (*Vigna umbellata*) was grown in close proximity. Meng et

al. (2015) help explain this synergistic phenomenon by observing the transfer of legume-fixed nitrogen to corn via mycorrhizal fungi. Within published literature the synergistic effects of intercropping typically leads to a yield advantage in comparison to monocultures (Yu et al., 2015). Crop establishment and density also benefit from companion plants by protecting young seedlings from adverse abiotic conditions such as damaging wind and erosion (Rinehart, 2006), or biotic factors such as stand-reducing herbivores (Frank et al., 2010). These protective effects may have contributed to improved corn stand density in interseeded fields at Estelline.

5.0 Acknowledgements

6.0 Literature cited

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7.0 Figures

Figure 1. Interseeder built to plant cover crops between corn rows in 2018. One pair

of double-disc openers planted a single row of cover crops equidistantly between corn

rows spaced 76 cm; the planter is capable of planting 7 rows in a single pass.

Figure 2. Total invertebrates (A), predators (B), herbivores (C) and detritivores (D) captured on the soil surface from corn monocultures (open circles) and corn interseeded with cover crops (dark circles). Data presented are mean ± SEM, generated from four fields at three locations (n = 12). Samples were collected per m^2 at three plant stages throughout the growing season. Results of rm-ANOVAs are presented (α = 0.05) with significant differences between treatments denoted with an asterisk. Note the differences in scale on the y-axis among the different trophic groups.

A. Total invertebrates/m^2

Treatment: $F_{1,71}$: 10.36, P < 0.01*
Plant stage: $F_{2,71}$: 9.60, P < 0.01*
Interaction: $F_{2,71}$: 10.74, P < 0.01*

Corn + Cover Crop
Corn Monoculture

B. Predators/m^2

Treatment: $F_{1,71}$: 17.90, P < 0.01*
Plant stage: $F_{2,71}$: 10.51, P < 0.01*
Interaction: $F_{2,71}$: 4.75, P = 0.01*

C. Herbivores/m^2

Treatment: $F_{1,71}$: 9.93, P < 0.01*
Plant stage: $F_{2,71}$: 9.86, P < 0.01*
Interaction: $F_{2,71}$: 7.61, P < 0.01*

D. Detritivores/m^2

Treatment: $F_{1,71}$: 1.87, P = 0.19
Plant stage: $F_{2,71}$: 6.38, P < 0.01*
Interaction: $F_{2,71}$: 2.68, P = 0.08

Plant stage: V4, V8, Anthesis

Figure 3. Total invertebrates (A), predators (B), herbivores (C) and detritivores (D) in the soil column of corn monocultures (open circles) and corn interseeded with cover crops (dark circles). Data presented are mean ± SEM arthropods collected in the top 10 cm of the soil column from four fields at three locations (n = 12). Samples were taken during four corn stages throughout the growing season. Results of rm-ANOVAs are also presented (α = 0.05) with significant differences between treatments denoted with an asterisk.

A — Total invertebrates/m^2

Treatment: $F_{1, 95}$ = 0.72, P = 0.41
Plant stage: $F_{3, 95}$ = 3.72, P = 0.02*
Interaction: $F_{3, 95}$ = 0.46, P = 0.71

● Corn + Cover Crop
○ Corn Monoculture

B — Predators/m^2

Treatment: $F_{1, 95}$ = 1.03, P = 0.32
Plant stage: $F_{3, 95}$ = 20.35, P < 0.01*
Interaction: $F_{3, 95}$ = 1.0, P = 0.46

C — Herbivores/m^2

Treatment: $F_{1, 95}$ = 1.20, P = 0.29
Plant stage: $F_{3, 95}$ = 4.28, P = 0.01*
Interaction: $F_{3, 95}$ = 0.50, P = 0.69

D — Detritivores/m^2

Treatment: $F_{1, 95}$ = 0.73, P = 0.40
Plant stage: $F_{3, 95}$ = 3.59, P = 0.02*
Interaction: $F_{3, 95}$ = 0.38, P = 0.77

Plant stage: V2, V4, V8, Anthesis
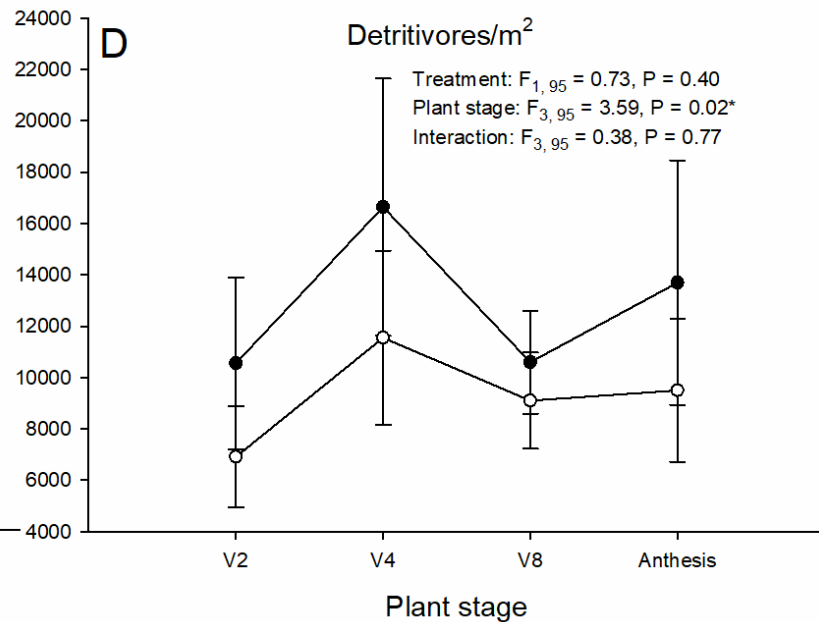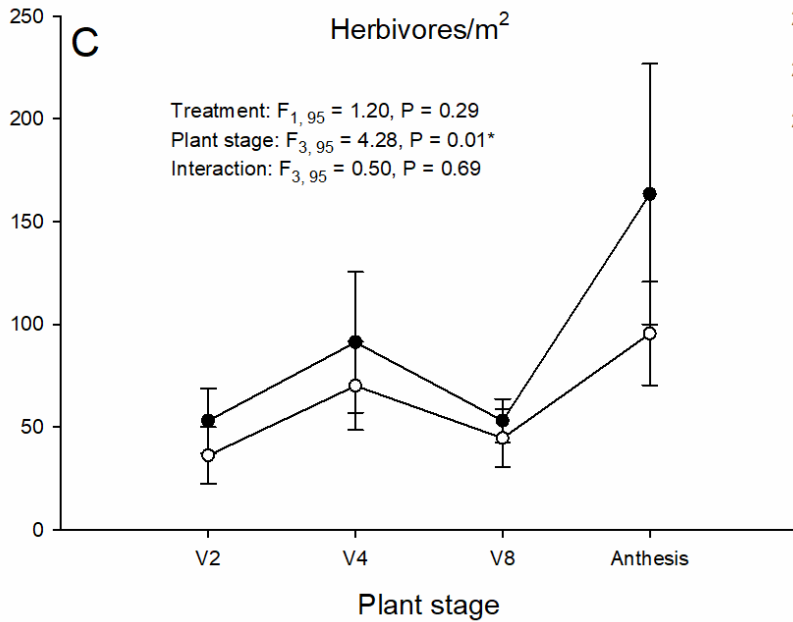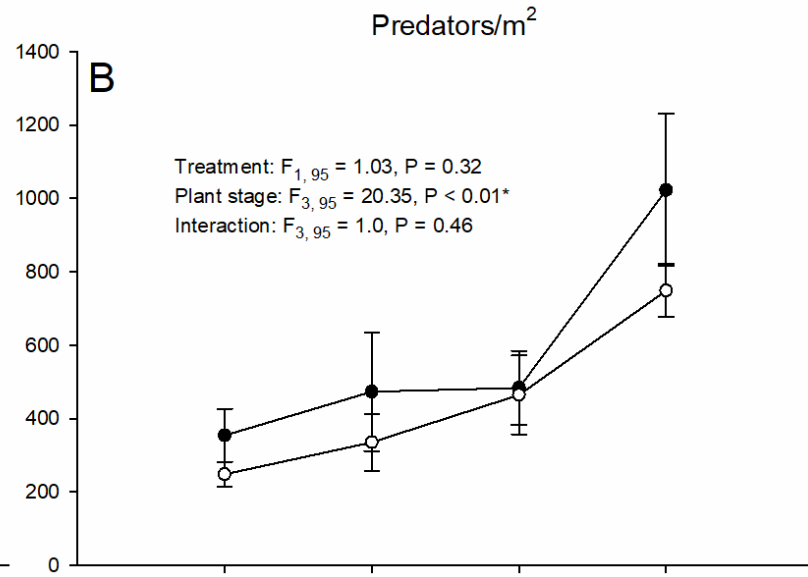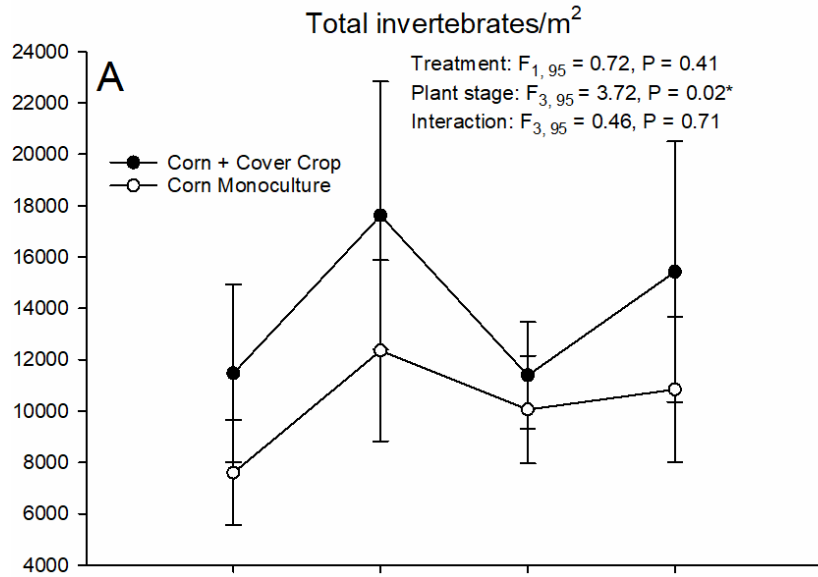
Figure 4. Total invertebrates (A), predators (B) and herbivores (C) collected from corn foliage at three plant stages from corn monocultures (open circles) and corn interseeded with cover crops (dark circles). Data presented are mean ± SEM arthropods collected per corn plant from four fields at three locations (n = 12) Results of rm-ANOVAs are also presented (α = 0.05) with significant findings denoted with an asterisk.

Figure A. Total invertebrates/plant

Treatment: $F_{1,71}$: 0.53, P = 0.47
Plant stage: $F_{2,71}$: 12.28, P < 0.01*
Interaction: $F_{2,71}$: 0.38, P = 0.69

Corn + Cover Crop
Corn Monoculture

Figure B. Predators/plant

Treatment: $F_{1,71}$: 0.00, P = 0.99
Plant stage: $F_{2,71}$: 26.94, P < 0.01*
Interaction: $F_{2,71}$: 0.51, P = 0.60

Figure C. Herbivores/plant

Treatment: $F_{1,71}$: 0.89, P = 0.36
Plant stage: $F_{2,71}$: 9.46, P < 0.01*
Interaction: $F_{2,71}$: 0.42, P = 0.66

Plant stage

V4   V8   Anthesis

Figure 5. The 4 site-year percentage of sentinel wax moth (*Galleria mellonella*) larvae consumed during a 1-h field exposure in corn monocultures and cornfields interseeded with cover crops. Each bar (mean ± SEM) represents a total of 560 observations averaged across 16 research plots.
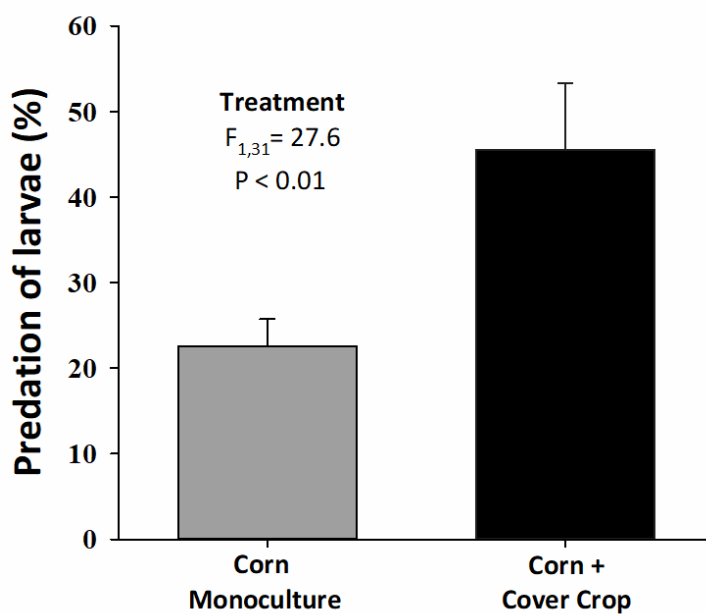
8.0 Tables

Table 1. Mean ± SEM invertebrates in corn plots interseeded with cover crops and in corn monocultures summed across sampled corn stages in the corn foliage, on the soil surface, and in the soil column. Data are presented this way to give relative abundances of invertebrate taxa throughout the growing season. The plant stages sampled were: soil surface and corn foliage: V4, V8 and anthesis; Subterranean: V2, V4, V8 and anthesis. Individual and grouped taxa included in the table represent those comprising ≥ 0.5% of the community abundance in their respective habitats and differed significantly between treatments. Collembola and mite abundances were excluded when determining commonly collected epigeic and subterranean taxa due to these species being a disproportionately large percentage of the total soil-dwelling community abundance. Results of repeated measures-ANOVAs ($\alpha = 0.05$) are presented for treatment effects with significant differences denoted with an asterisk

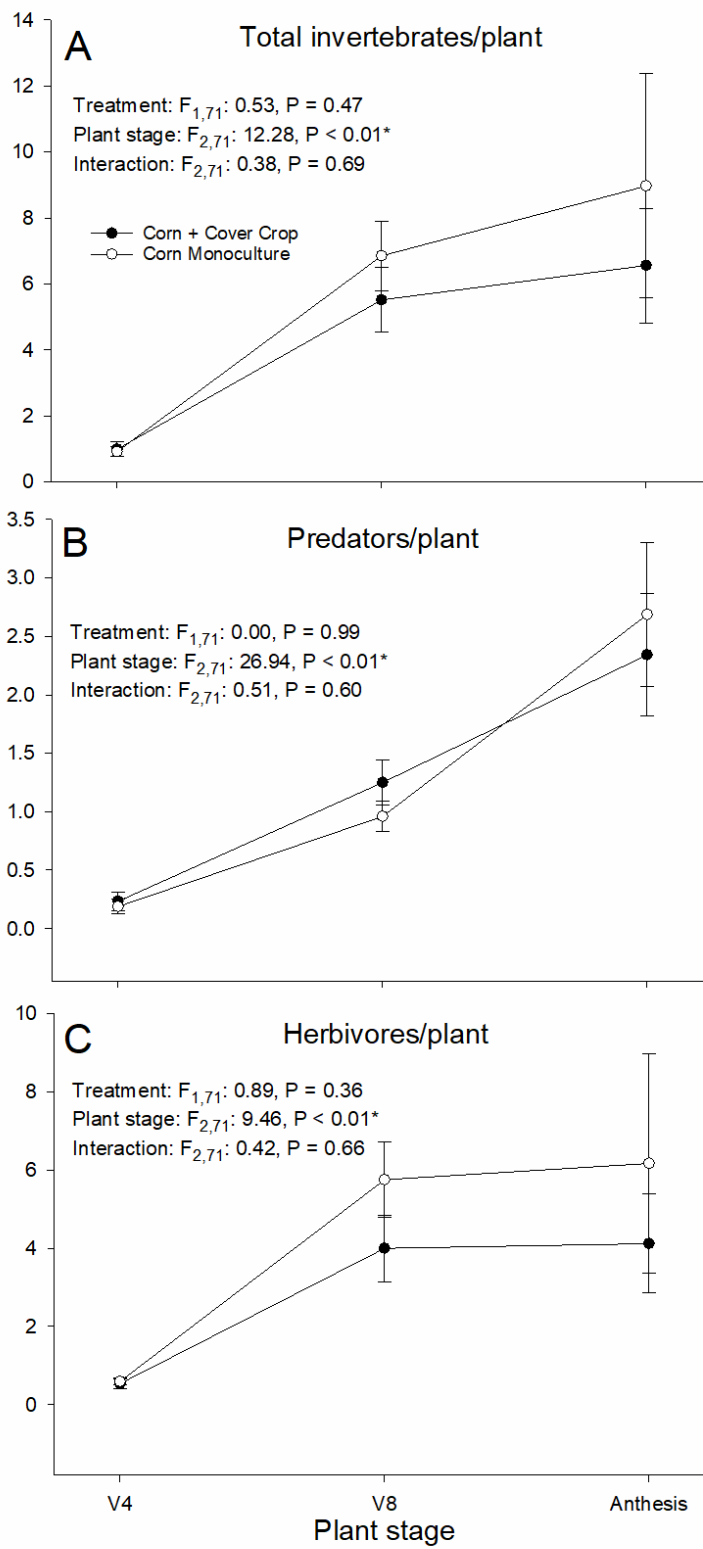| Habitat	Taxa	Mean ± SEM Interseeded corn	Mean ± SEM Monoculture corn	Rm-ANOVA
	Taxa	**Per m2**	**Per m2**	**Rm-ANOVA**
	Invertebrates (total)	476.83 ± 80.49	198.00 ± 32.00	$F_{1,71} = 10.36$, $P < 0.01$*
	Predators (total)	207.91 ± 23.63	93.41 ± 13.19	$F_{1,71} = 17.90$, $P < 0.01$*
	Herbivores (total)	113.33 ± 31.01	14.58 ± 4.54	$F_{1,71} = 9.93$, $P < 0.01$*
	Detritivores (total)	145.67 ± 40.12	84.42 ± 19.82	$F_{1,71} = 1.87$, $P = 0.19$
	Araneae (total)	66.25 ± 13.22	28.00 ± 6.11	$F_{1,71} = 6.90$, $P = 0.02$*
	Coleoptera Carabidae (total)	76.17 ± 19.19	29.17 ± 6.25	$F_{1,71} = 5.42$, $P = 0.03$*
	Hemiptera Miridae (total)	22.92 ± 8.04	2.83 ± 1.22	$F_{1,71} = 6.10$, $P = 0.03$*
	Coleoptera Staphylinidae (total)	5.17 ± 1.06	2.58 ± 0.60	$F_{1,71} = 4.53$, $P = 0.05$*
	Coleoptera Tetragnathidae sp.	2.50 ± 0.57	1.08 ± 0.34	$F_{1,71} = 4.57$, $P = 0.04$*
Soil surface	Hemiptera Nabidae *Nabis americoferus*	6.42 ± 1.80	1.83 ± 0.77	$F_{1,71} = 5.50$, $P = 0.03$*
	Coleoptera Coccinellidae (larval)	4.50 ± 0.88	0.75 ± 0.25	$F_{1,71} = 16.69$, $P < 0.01$*
	Coleoptera Carabidae *Bembidion* sp.	14.33 ± 2.49	6.25 ± 1.03	$F_{1,71} = 8.89$, $P = 0.01$*
	Hemiptera Miridae *Lygus* sp.	16.50 ± 5.53	1.08 ± 0.47	$F_{1,71} = 7.72$, $P = 0.01$*
	Hemiptera Aphididae *Ropalosiphum padi*	75.00 ± 32.28	6.42 ± 4.35	$F_{1,71} = 4.43$, $P = 0.05$*

	Taxa	Per m2	Per m2	Rm-ANOVA
Subterranean	Invertebrates (total)	55905.85 ± 14908.85	40858.27 ± 9681.05	$F_{1,95} = 0.72, P = 0.41$
	Predators (total)	2334.27 ± 469.24	1797.39 ± 245.26	$F_{1,95} = 1.03, P = 0.32$
	Herbivores (total)	360.75 ± 88.59	246.16 ± 55.75	$F_{1,95} = 1.20, P = 0.29$
	Detritivores (total)	51491.95 ± 14204.70	37066.14 ± 9169.67	$F_{1,95} = 0.73, P = 0.40$
	Araneae Lycosidae sp.	118.84 ± 39.07	29.71 ± 13.24	$F_{1,95} = 4.67, P = 0.04*$
	Coleoptera Cryptophagidae sp.	50.93 ± 15.36	14.85 ± 4.91	$F_{1,95} = 5.01, P = 0.04*$
	Beetle larva 15[‡]	6.37 ± 6.37	48.81 ± 19.69	$F_{1,95} = 4.21, P = 0.05*$
	Thysanoptera Thripidae sp.	25.47 ± 10.86	4.24 ± 2.86	$F_{1,95} = 5.77, P = 0.03*$
	Taxa	Per plant	Per plant	Rm-ANOVA
Corn foliage	Invertebrates (total)	13.06 ± 2.71	16.73 ± 4.24	$F_{1,71} = 0.53, P = 0.47$
	Predators (total)	3.83 ± 0.64	3.84 ± 0.74	$F_{1,71} = 0.00, P = 0.99$
	Herbivores (total)	8.65 ± 2.05	12.51 ± 3.54	$F_{1,71} = 0.89, P = 0.36$

[‡]Footnote:

Unidentified "Beetle larva 15" is approximately 3.5 mm long and possesses three pairs of thoracic legs, prognathous mouthparts

(reduced) and two distinct posterior dorsally-pointing hook-like urogomphi.

1

Table 2. Corn grain yields and plant densities from corn monocultures and cover crop-interseeded plots. Data presented are mean ± SEM values. Significant differences between treatments are indicated with an asterisk ($\alpha = 0.5$).

Site year	Interseeded corn	Monoculture corn	ANOVA
Yield (kg/Ha)			
Gary-18	12184.65 ± 284.19	12483.99 ± 333.61	$F_{1,7} = 0.45$, P = 0.53
Gary-17	13416.37 ± 647.57	11898.21 ± 250.61	$F_{1,7} = 4.74$, P = 0.07
Estelline	1481.61 ± 359.16	7171.66 ± 381.94	$F_{1,7} = 117.97$, P < 0.01*
Density (plants/Ha)			
Gary-18	72794.33 ± 1567.88	71674.42 ± 895.93	$F_{1,7} = 0.37$, P = 0.56
Gary-17	71674.42 ± 5151.60	67194.77 ± 1567.88	$F_{1,7} = 0.17$, P = 0.69
Estelline	68897.04 ± 2060.64	41705.55 ± 2866.98	$F_{1,7} = 58.93$, P < 0.01*

Table 3. Predation on wax moth (*Galleria mellonella*) sentinels in corn monocultures and in corn possessing a mix of interseeded cover crops. Data represent mean ± SEM percent consumed. Asterisks next to one-way ANOVA results represent significant treatment differences (ɑ = 0.05).

| Site year | Wax moth sentinels eaten | | ANOVA |
	Cover Crops	Monoculture	
Canby	93.3 ± 4.1	17.5 ± 3.4	$F_{1,7} = 201.9$, P < 0.01*
Pierre	22.5 ± 8.1	33.3 ± 2.4	$F_{1,7} = 1.65$, P = 0.25
Estelline	31.3 ± 7.7	23.8 ± 9.7	$F_{1,7} = 0.37$, P = 0.57
Gary-17	35.0 ± 5.3	15.6 ± 5.0	$F_{1,7} = 7.01$, P = 0.04*
Across site years	45.5 ± 7.7	22.6 ± 3.2	$F_{1,31} = 27.6$, P < 0.01*

Table 4. Invertebrate taxa observed actively eating sentinel wax moth larvae (*Galleria mellonella*) presented on the soil's surface in corn. In total, 1120 sentinels were deployed and after one hour 211 larvae were actively being eaten by one or more invertebrates.

Class	Order	Family	n
Insecta	Orthoptera	Gryllidae	330
	Hymenoptera	Formicidae	119
	Coleoptera	Carabidae	31
		Coccinellidae	1
	Hemiptera	Geocoridae	2
Arachnida	Opiliones	Phalangiidae	37
	Araneae	Lycosidae	4
		Linyphiidae	1
Gastropoda	Stylommatophora	No I.D.	20
Chilopoda	Lithobiomorpha	No I.D.	1
Diplopoda	Julida	No I.D.	1
		Total	**547**

CHAPTER FOUR: NEONICOTINOID INSECTICIDAL SEED-TREATMENT ON CORN CONTAMINATES INTERSEEDED COVER CROPS INTENDED AS HABITAT FOR BENEFICIAL INSECTS

Abstract

Neonicotinoid seed treatments are extensively used to systemically protect corn from invertebrate herbivory. Interseeding cover crops can promote beneficial insect communities and their ecosystem services such as predation on pests, and this practice is gaining interest from farmers. In this study, cereal rye (*Secale cereale*) and hairy vetch (*Vicia villosa*) were planted between rows of early vegetative corn that had been seed-treated with thiamethoxam. Thiamethoxam and its insecticidal metabolite, clothianidin were quantified in cover crop leaves throughout the growing season. Thiamethoxam was present in cereal rye at concentrations ranging from 0 to 0.33 ± 0.09 ng/g of leaf tissue and was detected on six out of seven collection dates. Cereal rye leaves contained clothianidin at concentrations from 1.05 ± 0.22 to 2.61 ± 0.24 ng/g and was present on all sampling dates. Both thiamethoxam and clothianidin were detected in hairy vetch on all sampling dates at rates ranging from 0.10 ± 0.05 to 0.51 ± 0.11 ng/g and 0.56 ± 0.15 ng/g to 9.73 ± 5.04 ng/g of leaf tissue, respectively. Clothianidin was measured at a higher concentration than its precursor, thiamethoxam, in both plant species on every sampling date. Neonicotinoids entering interseeded cover crops from adjacent treated plants is a newly discovered route of exposure and potential hazard for non-target beneficial invertebrates. Future research efforts should examine the effects of systemic insecticides

on biological communities in agroecosystems whose goal is to diversify plant communities using methods such as cover cropping.

Key words: clothianidin, insecticide, thiamethoxam, non-targets, risk assessment, *Zea mays*

1.0 Introduction

Under conventional agricultural management, corn grown as a monoculture is damaged by numerous invertebrate pests (SDSU, 2018), which annually cost US farmers > \$3 billion in control measures (Lundgren and Fausti 2015). Farmers typically react to corn pests once they reach a certain population threshold by applying insecticides (Bode and Calvin 1990). Alternatively, prophylactic control methods can be employed which typically involve use of genetically modified plants producing insecticidal proteins (Tabashnik 2010), or insecticidal seed treatments such as neonicotinoids (Douglas and Tooker 2015).

Neonicotinoid seed treatments are commonly used by agricultural producers worldwide (>120 countries) on a large number of crops (Jeschke et al. 2011). It is estimated that from 70 to 100% of corn seed planted in the U.S. (37.1 million ha) in 2011 were treated with neonicotinoids (NASS, 2011; Douglas and Tooker 2015). Neonicotinoids are highly water soluble and enter a plant's root system, eventually becoming systemic throughout all tissues and exudates (Bredeson and Lundgren 2018; Cowles and Eitzer 2017; Laurent and Rathahao 2003). The aim of systemically treating crops with insecticides is to kill herbivorous pests while limiting insecticide contact to non-pests. However, there are numerous cases describing detrimental effects of

neonicotinoid seed treatments on non-target invertebrate species (Douglas et al. 2015; Gontijo et al. 2014, 2015; Mogren and Lundgren 2016; Moser and Obrycki 2009; Pisa et al. 2017) and communities (Bredeson and Lundgren 2018; Seagraves and Lundgren 2012). When exposed to systemic neonicotinoids through any of several possible routes (contaminated nectar (Bredeson and Lundgren 2018), pollen (Krupke et al. 2012), prey (Bredeson et al. 2015; Douglas et al. 2015), vegetation (Bredeson and Lundgren 2015), dust (Krupke et al. 2012), etc.), susceptible non-targeted insects can experience direct mortality (Douglas et al. 2015) or altered behavior and possible indirect mortality if sublethal concentrations are encountered (Henry et al. 2012).

Cover crops are one way that agricultural producers can reduce their input costs, lower the environmental impact of their operations, and reduce pesticide costs. In fact, US producers increased the number of hectares planted to cover crops on their farms by over 84% between 2012 and 2016 (CTIC, 2017). Typically, cover crops are established on farmland outside the period when cash crops are growing, but some farmers incorporate cover crops onto agricultural land while row crops such as corn are actively growing. Cover crops can be employed as a versatile tool to assist in meeting several agronomic goals (including, but not limited to: nutrient uptake (Li et al. 2014), nitrogen fixing (Ashworth et al. 2017), weed suppression (Khan et al. 2006), amelioration of microclimates (Orr et al. 1997), water infiltration (Haruna et al. 2018), erosion prevention (Alliaume et al. 2014), providing wildlife habitat (Wilcoxen et al. 2018), stimulation of microbial communities (Schmidt et al. 2018), and building organic matter (LaCanne and Lundgren 2018)).

An emerging practice among these producers is the use of cover crops to improve habitat suitability for insect diversity, predator communities, and improve the biological control of pest arthropods. (Barbosa 1998; LaCanne and Lundgren 2018; Landis et al. 2000; Lundgren and Fergen 2014). This works in part by providing habitat for natural enemies and non-prey foods (Lundgren 2009), alternative prey or hosts (Manandhar and Wright 2016), and a variety of microclimates (Orr et al. 1997). Within interseeded cover crops, Manandahar and Wright (2016) observed an increase in parasitization of *Helicoverpa zea* eggs by *Trichogramma* spp. when cowpeas (*Vigna unguiculata*) and sunnhemp (*Crotalaria juncea*) were established between rows of sweetcorn. The same study also revealed that interseeded buckwheat (*Fagopyrum esculentum*) increased predator *Orius* spp. populations in relation to pest abundance compared to sweetcorn monocultures, likely due to the provisioning of nectar by flowering plants. Similarly, in corn fields where the stem borer, *Chilo partellus* (Swinhoe) is of economic concern, interseeding lablab (*Lablab purpurens*) effectively reduces pest infestations (Maluleke et al. 2005).

Systemic neonicotinoids unintentionally present in the leaves and flowers of cover crop species may increase the likelihood that beneficial invertebrates attracted to cover crops are exposed to the toxins. Scenarios have recently been described where the neonicotinoid clothianidin was detected in untreated milkweed (*Asclepias syriaca*) (Pecenka and Lundgren 2015) and dandelion (*Taraxacum officinale*) (Krupke et al. 2012) tissue growing along the margins of seed-treated cornfields. The purpose of the present study was to quantify neonicotinoids thiamethoxam and metabolite, clothianidin, in

cereal rye (*Secale cereal* L.) and hairy vetch (*Vicia villosa* Roth) leaf tissue growing between rows of seed-treated corn under field conditions.

2.0 Methods

2.1 Treated cornfield establishment

Experimental plots were established on a farm located near Toronto, South Dakota, U. S. A. (44.585211, -96.579910; latitude, longitude). In an eight-hectare field corn (*Zea mays*) (Legend Seeds Inc.; variety mixture: A10946, A10258; 95% Bt, 5% refuge seed; Maturity: 97d) was planted on May 5, 2017 at a rate of 79,074 seeds per ha with 76-cm between rows. The field was planted to untreated spring wheat in 2016. Corn seed was pre-treated with 0.25 mg of thiamethoxam per seed (CruiserMaxx® 250, Syngenta, Greensborough, NC, U. S. A.).

Plots were fertilized prior to corn planting according to soil test results. Urea, monoammonium phosphate, potash, ammonium sulfate and zinc were applied at rates of 162.6, 84.0, 84.0, 11.1, and 3.5 kg/ha, respectively. A pre-emergent herbicide mixture was applied on May 6, 2017 following corn planting. The mixture consisted of acetochlor, mesotrione, clopyralid MEA salt (3 L/ha; Resicore®, Dow AgroSciences, Indianapolis, IN, U.S.A.), atrazine (1.2 L/ha; Syngenta), glyphosate (1.2 L/ha; Roundup PROMAX® glyphosate; Monsanto Company, St. Louis, MO, U.S.A.) and a surfactant containing ammonium sulfate, corn syrup, and alkyl polyglucoside (1.2 L/ha; Class Act®, Winfield, St. Paul, MN, U.S.A.). Urea was broadcasted into plots on June 17, 2017 at a rate of 51.6 kg N/ha. No post-emergent herbicides were used.

2.2 Interseeded cover crops

Six, single-row plots (10 m long) of interseeded cover crops were planted between corn rows on June 6, 2017; corn was in the two-leaf stage of development. Each plot was separated by 2.3 m of corn and bare soil. Cover crop species used in the mixture were hairy vetch (*Vicia villosa*, 3.5 kg/ha), lentil (*Lens culinaris*, 3.5 kg/ha), Japanese millet (*Echinochloa esculenta*, 3.5 kg/ha), sorghum x sudangrass (*Sorghum bicolor* ssp. *Drummondii*, 5.7 kg/ha), cereal rye (*Secale cereale* 14.6 kg/ha), field pea (*Pisum sativum* 14.6 kg/ha) and flax (*Linum usitatissimum* 8.9 kg/ha) (rate recommendations advised by Greencover Seed®, Bladen, NE, U.S.A.). Cover crop seeds were dispersed by hand into a 3-cm deep furrow that was created directly in between the corn rows.

Plant tissues were collected from thiamethoxam seed-treated fields on June 19, 28, July 6, 14, 25, August 17, and October 6. On each collection date, three hairy vetch and three cereal rye samples were taken from each of the six plots for a total of 18 samples of each species on each collection date. For consistent tissue collection the most recently developed hairy vetch trifoliates were removed, and approximately 8 cm of rye tissue from the most apical leaves were taken for analysis. Forceps sterilized in 70% ethanol were used to remove leaves from individual plants, and all leaf tissues were weighed. Samples were frozen at -20° C until insecticide quantification.

2.3 Greenhouse-grown control plants

Untreated plants and soils in nature are routinely contaminated with neonicotinoids in the field (Ainsley et al. 2014; Botías et al. 2015; Krupke et al. 2012; Mogren and Lundgren 2016; Pecenka and Lundgren 2015), and so we produced cover

crop plants in the greenhouse to create untreated controls. Plastic flower pots (n = 5, 150 mm tall × 105 mm wide at the base, Kord Traditional Std.; Brantford, Ontario, Canada) were filled to a depth of 10 cm with potting soil (Master Garden Premium Garden Soil: Flower and Vegetable; Premier Horticulture Ltd.; Riviere-du-Loup, Quebec, Canada). Cover crop seed mixture (15 mL) was spread onto the soil surface and covered with 1 cm of soil. Pots were watered to soil saturation every other day. Greenhouse conditions were set to 16 h: 8 h (light: dark) photoperiod (300W; Viparspectra V300 LED lights; Shenzhen Bailuo Technology Co., Ltd., 638 Block C Baoyuan, Shenzhen, China), 24°C, with variable humidity. Samples of hairy vetch and cereal rye leaf tissue were harvested from greenhouse-grown plants after 5 wk. Forceps sterilized in 70% ethanol were used to remove leaves from individual plants, and all leaf tissue was weighed. Samples were frozen at -20° C until analysis.

2.4 Insecticide analysis

Leaf tissue was homogenized using a plastic pestle in distilled water at a ratio of 600 µL water/0.1 g tissue. Samples were agitated for 1 h at room temperature using an orbital shaker set to 210 rpm (orbit diameter: 22 mm). Samples were vortexed for 10 s, and then centrifuged at 10,000 × g for 5 min. Supernatant was separated from solid materials, diluted to 20% using distilled water and placed into a new 1.5 mL centrifuge tube.

Standard curves (n = 3) of known insecticide concentrations were run on each ELISA plate. Supernatant from untreated control plants was mixed with distilled water to achieve a 40% concentration (4: 6, v:v; supernatant: water). Thiamethoxam

(Thiamethoxam PESTANAL®, Sigma-Aldrich®, Product number: 37924, St. Louis,

MO, U.S.A.) and clothianidin (Sigma-Aldrich®, Product number: 33589) dilutions were

established in distilled water at 2X the final desired concentrations (0, 0.0625, 0.125,

0.25, 0.5, 1.0, 2.0 and 4.0 ng/mL). Final standard concentrations were made by mixing

equal parts 40% control supernatant and 2X thiamethoxam or clothianidin mixtures,

yielding standards used directly in ELISA analyses composed of 20% supernatant at 0,

0.03, 0.06, 0.13, 0.25, 0.5, 1.0 and 2.0 ng/mL thiamethoxam or clothianidin, depending

on which insecticide was being quantified.

Insecticide analysis via ELISA was conducted based on kit instructions

(Thiamethoxam HS plate kit, lot no. 10031; Beacon Analytical Systems Inc., Saco, ME,

U.S.A., and clothianidin, Product No. 500800, Abraxis LLC®, 54 Steamwhistle Drive,

Warminster, PA, U.S.A.) and closely followed the procedures described previously

(Bredeson and Lundgren 2018; Bredeson et al. 2015). Sample absorbances (at 450nm)

were read using a spectrophotometer (SpectraMAXX®, Molecular Devices, LLC. San

Jose, CA, U.S.A.). Quantities of thiamethoxam or clothianidin were calculated based on

the plate-specific standard curve series.

2.5 Data analysis

Sample absorbances were deemed outliers if they fell above or below established

bounds. To establish upper and lower bounds the first and third quartiles and interquartile

range (IQR) were found for a plant species on a given sampling date (n = 18 samples).

An upper bound was set at $1.5 \times$ IQR above quartile three, while a lower bound was $1.5 \times$

IQR below quartile one. Sample sizes used to calculate mean neonicotinoid

concentrations are noted in Figures 1 and 2. To avoid reporting false-positives, a sample

was considered to have no detectable insecticide if its absorbance reading was a greater

value than one standard deviation below the mean of negative control samples (n=5 per

ELISA plate) (sample absorbance in direct competitive ELISA is inversely correlated

with insecticide concentration). Kruskal-Wallis one-way analysis of variance was used to

determine differences between mean insecticide levels across sampling dates for each

plant species and neonicotinoid ($\alpha = 0.05$). If insecticide concentrations varied across

sampling dates Dunn's all-pairwise comparison tests were used for post-hoc analysis

between sampling dates ($\alpha = 0.05$). Kruskal-Wallis and Dunn's tests were conducted

using Statistix® 10 software (Analytical Software, Tallahassee, FL, U.S.A.).

3.0 Results

3.1 Cereal Rye

Thiamethoxam was present in interseeded cereal rye leaf tissue on all sampling

dates except for August 17. Thiamethoxam concentrations per date ranged from 0 to 0.33

± 0.09 ng/g tissue with the highest being in samples collected on June 28 (Table 1). Mean

concentrations of thiamethoxam varied among sampling dates ($\chi^2_6 < 0.01$, $P = 0.03$).

Thiamethoxam's primary insecticidal metabolite, clothianidin, was found in cereal rye

tissue on all collection dates and varied in mean concentration among dates ($\chi^2_6 < 0.01$, P

< 0.01). Levels of clothianidin were found at higher levels than that of its precursor on all

dates, ranging from 1.05 ± 0.22 to 2.61 ± 0.24 ng/g tissue (Table 1), with the highest

level of clothianidin collected on July 14. Both neonicotinoids in cereal rye leaf tissue

tended to follow a pattern of higher concentrations for the first five sampling dates,

followed by the lowest amount being recorded on the sixth date (August 17), and then a subsequent increase on the final sampling date (October 6) (Figures 1A and 1B).

3.2 Hairy Vetch

Thiamethoxam varied throughout the growing season ($\chi^2_6 < 0.01$, $P < 0.01$), but was present in hairy vetch leaf tissue from all sampling dates ranging from 0.10 ± 0.05 to 0.51 ± 0.11 ng/g tissue, with the lowest concentration recorded from August 17, and the highest from June 28 (Table 1). Clothianidin within hairy vetch leaf tissue was measurable in samples from all collection dates and accounted for the three highest neonicotinoid concentrations across both plant species (Table 1). Levels of clothianidin varied across sampling dates ($\chi^2_6 < 0.01$, $P < 0.01$) and ranged from a low of 0.56 ± 0.15 ng/g (August 17) to as high as 9.73 ± 5.04 ng/g collected during the earliest sampling date (June 16). Neonicotinoid concentrations in hairy vetch leaf tissue tended to be higher toward the beginning of the growing season, gradually tapering to a season low on the sixth sampling (August 17), followed by a slight increase on the final collection date (Figures 2A and 2B).

4.0 Discussion

Risk associated with a toxicant is characterized by hazard and exposure to a particular species. Here we document that untreated cover crops are contaminated with neonicotinoid insecticides, which represents a potential risk to non-target species. Despite using the lowest commercially available rate of seed-treatment, CruiserMaxx®, and planting cover crops in a single row at a maximum distance from adjacent corn rows,

neonicotinoids thiamethoxam and clothianidin were present in both cover crop species on all sampling dates except for one (Figure 1A, Table 1). Additional research will need to substantiate the degree to which non-target beneficial insects are exposed via cover crops and whether the concentrations documented here are harmful. An additional risk factor requiring further attention which is not addressed in this research is the potential for additive and synergistic effects between neonicotinoids and other types of pesticides often applied concurrently (David et al. 2016).

Concentrations of neonicotinoid insecticides observed in cover crop leaf tissue are near to, or exceed amounts previously found in contaminated plant tissues (Goulson 2013; Krupke et al. 2012; Pecenka and Lundgren 2015), and are at levels that can cause direct harm to beneficial insects (Prabhaker et al. 2017). For example, a recent laboratory study of monarch butterfly (*Danaus plexippus*) larval susceptibility to neonicotinoids revealed LC_{10} and LC_{20} values for clothianidin to be 7.72 and 9.89 parts per billion (ppb), respectively (Pecenka and Lundgren 2015). The same study also found that first instar monarchs were shorter, weighed less, and were slower to develop compared to control larvae at clothianidin concentrations as low as 0.5 ppb (Pecenka and Lundgren 2015). When Sandrock et al. (2014) exposed solitary red mason bees (*Osmia bicornis*) to artificial nectar spiked with sub-lethal dosages of thiamethoxam (2.78 ng/g) and clothianidin (0.45 ng/g) reproductive success was significantly altered. Specifically, neonicotinoid-exposed bees produced 47.7% fewer offspring, and at a male dominated sex ratio, in comparison to unexposed bees (Sandrock et al. 2014). Susceptibility to these insecticides varies among insects (Pisa et al. 2017), and additional toxicological work on the majority of important species in the corn system remain to be quantified.

The observed pattern of steadily decreasing insecticide concentration in systemically-treated plant tissue throughout the growing season (Figures 1 & 2) represents a fluctuating exposure scenario that should be reflected in a risk analysis. This diminishing insecticide content has been documented in previous studies on neonicotinoid fate (Bredeson and Lundgren 2015). Interestingly, interseeded cereal rye and hairy vetch possessed the smallest amount of thiamethoxam and clothianidin within their tissues on the penultimate sampling date (August 17,) before again trending upward for the final collection (October 6) (Figures 1 & 2). It is possible that an increase in insecticide concentration could have occurred because of factors related to corn maturity. By the final sampling date corn leaves had desiccated, and the previously dense canopy created by corn leaves had senesced allowing light necessary for cover crop growth to reach interrow spaces (den Hollander et al. 2007). Additional light penetration resulted in noticeable late-season cover crop growth which may have also increased cover crop transpiration (McNaughton and Jarvis 1991) and uptake of dissolved neonicotinoids.

Though thiamethoxam was treated to corn seeds in this study the toxic metabolite, clothianidin, was ubiquitous in both cereal rye and hairy vetch on all sampling dates and was always measured at a higher concentration compared to thiamethoxam on the same date (Figures 1 & 2). Special attention must be given to such metabolites when performing environmental risk assessments and when considering agrichemical usage where non-targets may become exposed. Pesticides metabolized into additional compounds through largely unknown processes in plants and soils can show similar or elevated toxicity (Nauen et al. 2003; Simon-Delso et al. 2015) and even persist for extended periods (Goulson 2013) when compared to their parent molecules. For example,

under field conditions thiamethoxam seed-treated sunflowers possess clothianidin within leaf tissue even after thiamethoxam is no longer measurable, possibly contributing to reductions in pollinator and predatory populations in treated fields (Bredeson and Lundgren 2015; Bredeson and Lundgren 2018). Uptake and persistence of neonicotinoids and their metabolites by interseeded cover crops pose a risk to beneficial organisms attracted to the resources provided by additional plant diversity.

5.0 Acknowledgements

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7.0 Figures

Figure 1. Thiamethoxam and Clothianidin within cereal rye tissue. Seasonal pattern of mean ± SEM nanograms thiamethoxam and clothianidin per gram of cereal rye (*Secale cereale*) leaf tissue interseeded between two-leaf corn treated with CruiserMaxx 250® (Syngenta, US) seed treatment. Corn and cereal rye were planted on the 125 and 157 days of the year, respectively. Numbers above error bars represent sample sizes used to calculate mean ± SEM
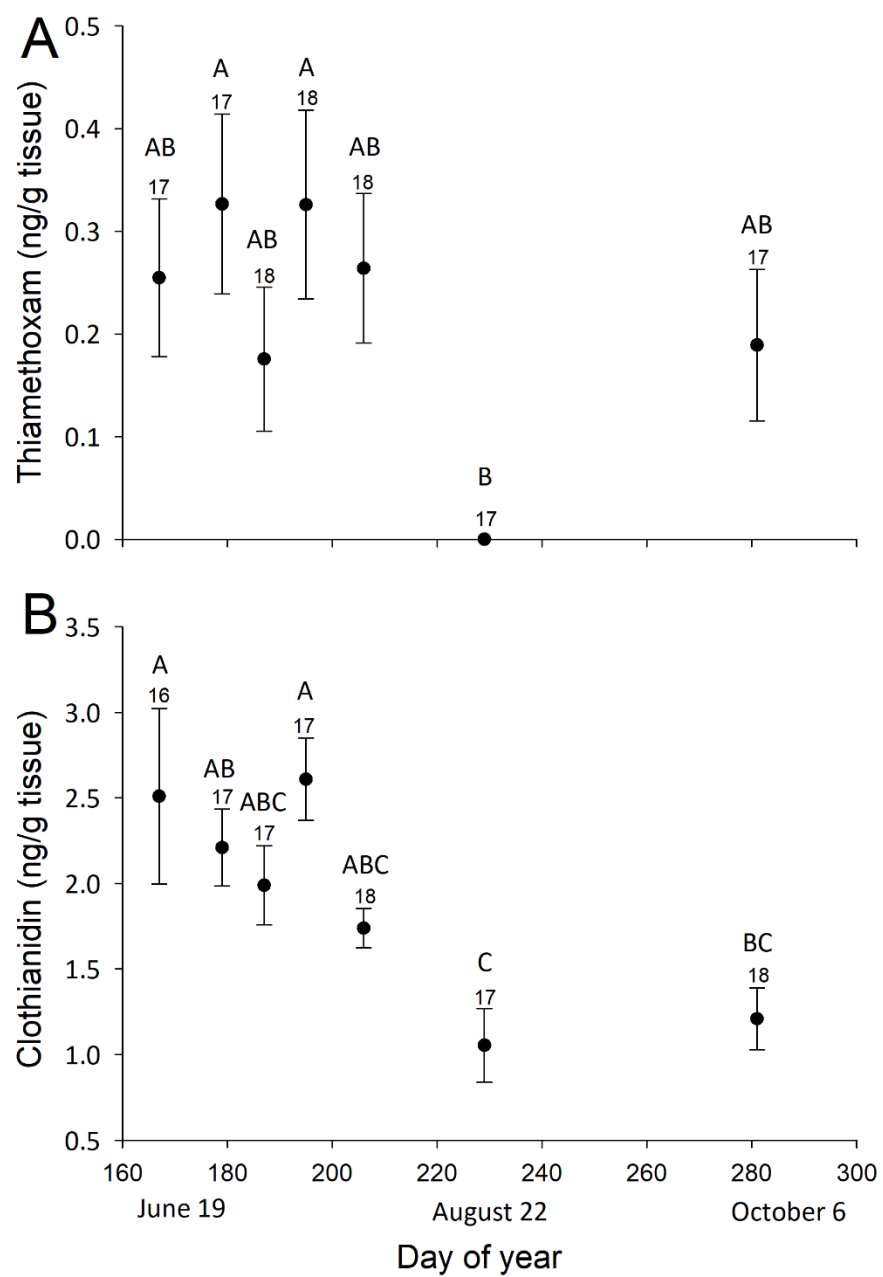
Figure 1.

Figure 2. Thiamethoxam and Clothianidin within hairy vetch tissue. Seasonal pattern of mean ± SEM nanograms thiamethoxam and clothianidin per gram of hairy vetch (*Vicia villosa*) leaf tissue interseeded between two-leaf corn treated with CruiserMaxx 250® (Syngenta, US) seed treatment. Corn and hairy vetch were planted on the 125 and 157 days of the year, respectively. Numbers above error bars represent sample sizes used to calculate mean ± SEM

Figure 2.
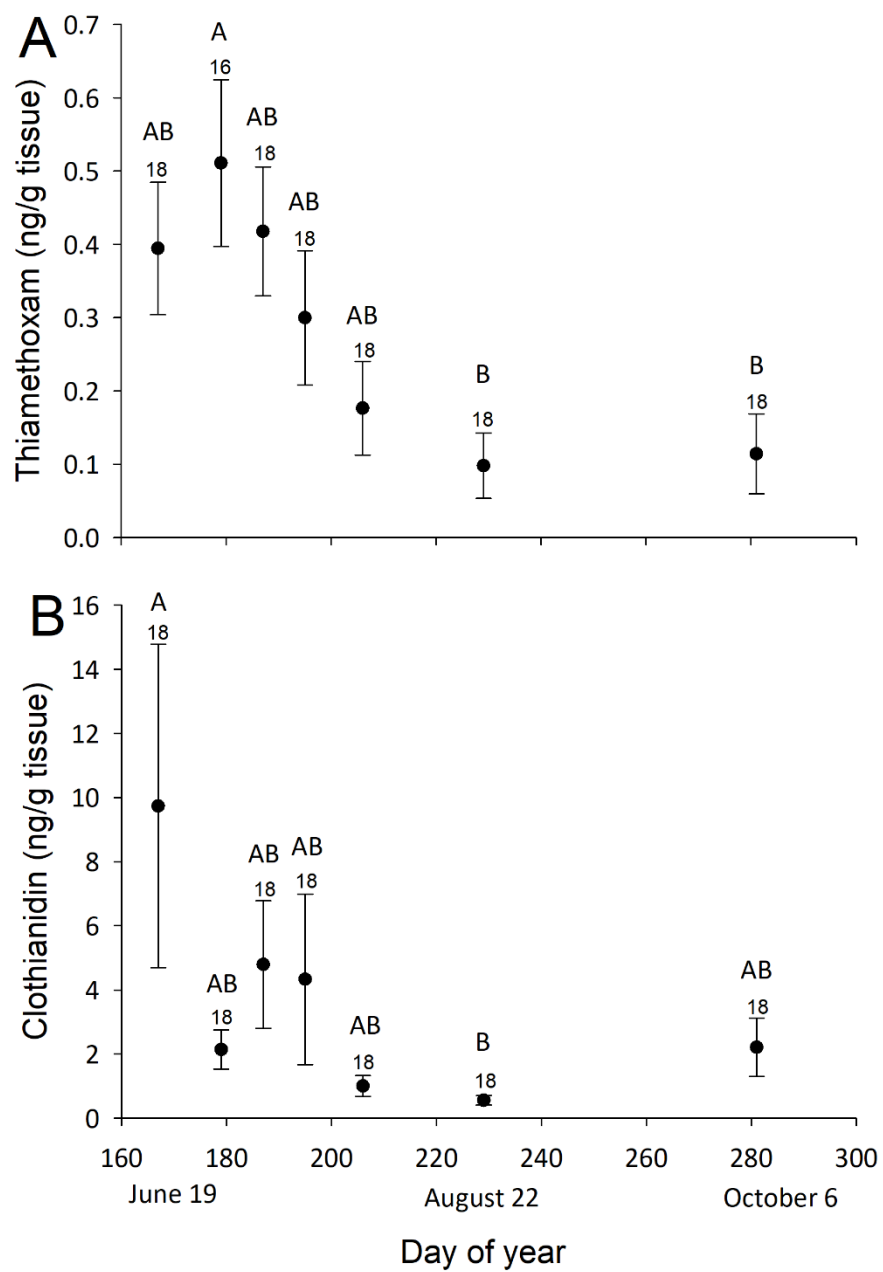
8.0 Tables

Table 1. Neonicotinoid levels in cover crop tissue. Mean ± SEM thiamethoxam and clothianidin concentrations in cover crops cereal rye and hairy vetch planted between rows of V2 corn seed-treated with CruiserMaxx 250® (Syngenta, US).

Collection date	Julian Date 2017	Cereal Rye		Hairy Vetch	
		Thiamethoxam (ng/g leaf tissue)	Clothianidin (ng/g leaf tissue)	Thiamethoxam (ng/g leaf tissue)	Clothianidin (ng/g leaf tissue)
June 19	167	0.254 ± 0.077	2.510 ± 0.514	0.394 ± 0.090	9.731 ± 5.040
June 28	179	0.327 ± 0.088	2.209 ± 0.225	0.511 ± 0.114	2.143 ± 0.609
July 6	187	0.176 ± 0.070	1.989 ± 0.231	0.418 ± 0.088	4.793 ± 1.991
July 14	195	0.326 ± 0.092	2.609 ± 0.241	0.300 ± 0.092	4.330 ± 2.658
July 25	206	0.264 ± 0.073	1.739 ± 0.116	0.177 ± 0.064	1.003 ± 0.326
August 17	229	0.000 ± 0.000	1.054 ± 0.216	0.098 ± 0.045	0.559 ± 0.152
October 6	281	0.189 ± 0.074	1.209 ± 0.181	0.114 ± 0.055	2.210 ± 0.913

CONCLUSIONS

Despite possessing the knowledge that diverse plant communities provide critical ecosystem services and habitat resilience the modern agricultural paradigm supports the use of advanced equipment, specialized chemical formulas and genetically engineered plants to maintain species in monoculture. Simple agroecosystems do little to support wildlife, store carbon, prevent soil loss, infiltrate water and detoxify pollutants, to name just a few of the shortfalls. A significant effect of undiverse farmscapes is their contribution to the global decrease in arthropod diversity and biomass. If we are to reverse some of the severe global environmental issues facing our culture the crop production sector must adapt. A prerequisite to improving the service-provisioning of farmland is by incorporating diverse and persistent plant communities. Research unveiling methods to diversify the farmscape will be increasingly relevant as agricultural producers further realize the importance of low-input, mixed-cropping systems.

For guidance in developing efficient and productive mixed-cropping systems we can look to the prairie for inspiration and a rough blueprint. If left to their own devices, many croplands would eventually revert to the landscape that was present before conversion to crop monocultures. Fortunately for us, there are a few remnant and restored prairies scattered throughout farm country to learn from in our quest to mimic the functionality of these habitats in our food production system.

At a glance, we notice that prairies possess a great diversity of plant species. Plants are at various stages of development, and additionally, every stage of decomposition. Both living and dead plants are providing nutrition to organisms above

and below the soil surface and protecting the soil structure from abiotic perturbations. Structural complexity and food resources provide habitat for wildlife including pollinators and arthropod natural enemies. Herbivores are maintained through biological control agents such as diseases and predators. This is not the place to list all of the known ways prairies regulate environmental systems, but suffice it to say, these natural habitats are highly efficient despite receiving few inputs.

Though agricultural production systems will doubtfully look exactly like the prairies they have replaced, the principles of diversity and reduced disturbance are transferrable and can result in improved ecosystem service provisioning when implemented on working land. Some of the more well-studied cropland diversification techniques were mentioned in chapter one of this dissertation, and many of those methods can lead to improved farmland functionality. Mixed-cropping systems with multiple plant species grown simultaneously is the next step some farmers are implementing to further mimic the conditions of natural prairies while boosting overall agland productivity.

Throughout this dissertation I attempted to fill fundamental knowledge gaps which will direct researchers and farmers in their future studies and management plans involving mixed-plant crop communities. Designing efficient mixed-cropping systems that producers will adopt requires much trial and error to determine suitable companion crop species, strategies for plant establishment, and recognition of incompatibilities between polycultures and existing pest management techniques (i.e. herbicides and insecticides).

Knowledge of ecological mechanisms driving pest and natural enemy populations is critical in making well-informed farm management decisions. In chapter one this

subject matter was important to review as it gave context and weight to the subsequent primary research chapters. Once a land manager is equipped with information on bottom-up and top-down effects on pest populations their farm planning can be founded in ecological theory and not solely the recommendations of stakeholders with possible economic incentives.

In chapter two, an assessment of calcium carbonate seed coating revealed that this method for seed broadcasting improvement may deter arthropod granivory of cover crop seeds. As was discovered in chapter three, increasing the number of acres possessing an interseeded cover crop will result in more abundant, diverse and specious invertebrate communities within agricultural landscapes capable of performing biological control. Such a transition could have a profound effect on the amount of insecticide applied to the environment.

Finally, in chapter four it was revealed that producers with intentions of bolstering beneficial insect populations by interseeding cover crops are often dealing with the confounding factor of their main cash crop possessing a neonicotinoid seed treatment. Pesticide which doesn't enter the treated crop can be translocated via soil water to adjacent plants where considerable amounts of toxin can be taken up through the roots. This novel route of pesticide exposure deserves much attention in further research studies as it risks the wellbeing of non-targeted organisms inhabiting the agroenvironment.

As a stand-alone practice interseeding cover crops will not solve all the negative environmental issues related to food production. However, in collaboration with many other effective farmland conservation methods interseeding can be an important tool to diversify the plant community during a time typically depauperate.

Agricultural production can be performed without destruction of natural resources. In fact, farmers are restoring functions to their land by using well-established methods and seeking out new practices to further improve the land they steward. It is increasingly important for trained agroecologists to translate scientific research, develop educational materials, and most importantly, develop the trusting relationships with farmers that leads to conservation of natural resources across the agricultural landscape.