WILD BEE COMMUNITIES IN CENTRAL WISCONSIN VEGETABLE CROPS

by

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A thesis submitted in partial fulfillment

of the requirements for the degree of

Master of Science

(Agroecology & Entomology)

at the

UNIVERSITY OF WISCONSIN-MADISON

2016

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Acknowledgements

I would first and foremost like to thank my advisor, Dr. Russ Groves, for taking me on as a student. Being in Russ' lab has been a great experience and I am grateful for the opportunity to venture into the world of insects and agriculture. I would also like to thank my committee members, Dr. Richard Lindroth and Dr. Claudio Gratton, for their feedback and support in developing this work. I am grateful to my funding sources for this project: The Midwest Food Processors Association and the USDA Specialty Crop Research Initiative provided support for the processing vegetables component of this research, while the Ceres Trust provided support for the organic component. I thank Don Caine and Matthew Badtke of Del Monte Foods, Inc. for their accommodation of the space, seeds, maps, and fields that were necessary for this project to take place. I also thank Tony Whitefeather of Whitefeather Organics, LLC along with Adam Flyte of Flyte Family Farms for their accommodation of this project on their organic farms. I'd like to thank Nick Keuler for providing life-saving statistical consulting, as well as Dr. Jason Gibbs of Michigan State University for identifying for the majority of bee specimens, especially the many difficult to distinguish *Lasioglossum* species. I'd like to thank my departmental colleagues and the fantastic staff and faculty of the entomology and agroecology departments. I'd especially like to thank Dr. Ned Rubert-Nason for helping me to formulate a tissue analysis procedure and for running all the plant tissue samples. Furthermore, I'd like to thank my field assistants, Katie Chipman and Coralie Deniot, for their help and insight during fieldwork, as well as for their company and map-reading prowess as we struggled to navigate back-country roads. A thank you to my fellow and former Groves Lab members –Ben Bradford, Emily Duerr, Chris Bloomingdale, Linda

Chapter 1: Literature Review

The importance of bees

Plants are pollinated by many diverse animals. Familiar pollinators like birds and flower flies are active during the day, while nocturnal animals such as bats and moths take the night shift. But the most efficient pollinators – and thus most economically important – are bees (Greenleaf et al 2007, Michener 2007). Bees are nectar and pollen-eating members of the superfamily *Apoidea* within the insect order *Hymenoptera.* In North America there are more than 3,300 species of bees (Borror & White 1970). Some bees live socially in colonies founded by a queen, such as honey bees (genus *Apis*) and bumble bees (genus *Bombus*). These social genera are perhaps the best-known by the average person, but most bee species are solitary, with females creating individual nest cells that they provision with nectar and pollen (Michener 1967). Still others are nest parasites that lay their eggs in a host species' nest (Wolf & Ascher 2008).

Approximately 35% of the world's food crop production depends on pollinators like bees (Klein et al 2007), amounting to a current annual value of more than $160 billion worldwide (Potts et al 2010, Gallai et al 2009). Of all bee species, the agricultural contributions of the European honey bee *Apis mellifera* are among the most important. Honey bees are a common sight in crops and backyards alike, and the primary managed pollinator used for 90% of commercial pollination (Genersch 2010). In addition to their valuable pollination services, the honey they produce generates over 300 million dollars in annual sales within the United States (USDA NASS 2016a).

During the twentieth century, additional bee species began to be domesticated for use in agriculture. Bumble bees have proven particularly adept at pollinating greenhouse tomatoes (Velthius & van Doorn 2006, Goulson 2013b), as well as surviving the cold climes

of northern growing regions. Bumble bees are large enough to shake pollen loose from tomato flowers by rapidly vibrating their wing muscles in a phenomenon called *buzz pollination* (Harder & Barclay 1994). Pollination by bumble bees can also increase the quality and yield of other greenhouse crops like peppers and melons (Guerra-Sanz 2008). The European species *Bombus terrestris* has experienced an increase in use –and exportation- as greenhouse agriculture has grown in popularity (Guerra-Sanz 2008). In North American greenhouses the native bumble bee *Bombus impatiens* is the dominant domesticated species (Guerra-Sanz 2008). Solitary bee species like the alfalfa leafcutter bee (*Megachile rotundata*) and the alkali bee (*Nomia melanderi*) have also begun commercial use in several countries, where they are deployed to pollinate fruit trees and alfalfa (Velthius & van Doorn 2006).

Pollination by wild bee species can supplement or surpass honey bee pollination (Mallinger & Gratton 2015, Vaughan et al 2015, Winfree et al 2008). Even crops that are not dependent on insect pollination may experience an increased yield in the presence of high bee diversity (Greenleaf & Kremen 2006). Self-pollinating plants like sweet peppers (*Capsicum annuum*) and tomatoes (*Solanum lycopersicum*) experience greater fruit set and better fruit quality when visited by pollinating insects (Delaplane & Mayer 2000).

In addition to their agricultural services, domesticated and wild bees alike contribute to the pollination and reproduction of flowering plants in natural ecosystems (Winfree et al 2009). Bees pre-date humans on the planet; the first bees appeared more than 130 million years ago (Goulson 2013b). These early bee species coevolved with the plants that they pollinate, resulting in a unique diversity of floral displays and nectar-seeking strategies between bees and flowers that can be seen around the world today. These include *Tibouchina* flowers that change color after they're pollinated to appear less attractive (Pereira et al 2011),

and orchids in the *Ophrys* genus, whose flowers' remarkable resemblance to female bees serves to lure male bees nearer (Streinzer et al 2009).

The global mix of generalist and specialist bees helps ensure that Earth's pollinator-dependent flowers receive pollination each year (Moisset & Buchmann 2011). Yet after surviving the extinction of the dinosaurs in the late Cretaceous period and the radical temperature fluctuations during the millennia that followed, these enduring creatures now face a new threat: human activity.

Many insects have been experiencing accelerated losses over the last century, and particularly over the last several decades (van Lexmond et al 2015). Already three species of bumble bees are presumed extinct (Goulson 2013b), and one American bumble bee, *Bombus affinis*, is being considered for endangered species status by the U.S. Fish and Wildlife Service (Federal Register 2015). The population trends of most of the world's bee species have not been evaluated, so the conservation status of the vast majority of bee species remains unknown.

The loss of bees would pose a terrible problem for modern food production and natural ecosystem function as well as human culture, which has developed alongside apiculture for thousands of years across ancient Europe, Africa, and the Middle East (Bloch et al 2010). Given these stakes, the ongoing decline of both wild and domestic bee species throughout the world (National Academic Press 2007, Potts et al 2010) has received increased attention from the scientific community. Research efforts are presently underway to understand the set of interactive factors contributing to pollinator disappearance and honey bee colony failure as losses of these insects continue.

Challenges facing domestic pollinators

Domesticated bee species, especially honey bees, have undergone intensive changes in management practices over the last several decades. The global demand for pollination is higher than ever before, and managed honey bee populations have been unable to keep up (Aizen & Harder 2009). In the United States, commercial honey bee keepers must now transport their hives across long distances to meet the high pollination demand of crops across the country (Oldroyd 2007). Such honey bee colonies typically begin the year by travelling to California in February to pollinate almond trees, moving on to stone fruit in the Pacific Northwest in April and May, then hopping from crop to crop across the continental U.S. This lifestyle can lead to physiological and nutritional stress (Ahn et al 2012). Furthermore, the close quarters shared by honey bees when visiting a region's flowering crops en masse can exacerbate disease and pathogen spread. Pathogens circulating in domestic hives can spill over into wild bee populations, as has been found to occur with wild and domesticated bumble bees (Graystock et al 2014, Otterstatter & Thomson 2008).

The spread of disease is not often observed in natural bee populations, in part because diseased wild bees are hard to find; most of what is known about bee disease has come from the study of domesticated honey bees, bumble bees (especially *B. terrestris*), and alfalfa leafcutter bees (James 2008). When bees succumb to disease they are difficult to cure. As such, in the case of colony-wide outbreaks the most effective course of action is often to destroy contaminated colonies and their associated equipment (James 2008). Among honey bees, there are a variety of viral, fungal, bacterial, and metazoan pathogens that cause heavy colony mortality, including parasitic varroa mites, nosema fungus, and Israeli acute paralysis virus (Genersch 2010). Colony Collapse Disorder, or CCD, is another problem for honey bee

keepers in the United States. Colonies affected by CCD lose foragers until the colony dwindles to a number too low to support itself and dies off. A single root cause for this phenomenon has not been found.

Land use change increases pollinator stress

Approximately 40% of Earth's land surface has been converted to agricultural production or pastureland (Foley et al 2005), and with the human population expected to reach nine billion by the year 2050 (Godfray et al 2010) the fraction of global land devoted to agriculture will likely increase. Since bees need access to land that can provide continuity in floral and nesting resources, agricultural areas will likely play center stage in the upcoming struggle to maintain pollinator diversity (and consequently the productivity of many pollinator-dependent crops). There are several major obstacles that must be overcome if high levels of bee diversity are to be maintained in agroecosystems that are becoming increasingly intensive.

First, bees must be able to find enough food: widespread habitat loss and a lack of landscape connectivity have made it harder for bees to forage successfully (Kennedy et al 2013, Kremen et al 2007, Ricketts et al 2008, Winfree et al 2009) Most bees are central place foragers that make foraging trips to and from a nest location where gathered resources are stored. Because bees must return to their nests, the flowers they are able to pollinate are limited by proximity to the nest site. The distance a bee can fly is determined by its size (Gathmann & Tscharntke 2002). Large bees are able to cover a large foraging radius of up to 15 km, while smaller bees may be restricted to a radius of less than a few hundred meters (Greenleaf et al 2007, Godfray et al 2014).

Crop management practices may be exacerbating the lack of floral resources available for bees. Alfalfa, for example, is frequently grown as a cover crop for corn and soy and can be an excellent source of both pollen and nectar for bees (USDA 2015). However, alfalfa cover crops are usually harvested just as they begin to flower, making them useless as a pollinator resource. Fruit and vegetable crops can bear prolific flowers, but many only do so for a brief period of the growing season. For most of the year these crops are also unable to support foraging bees.

In addition to the disappearance of floral resources, habitat loss has reduced suitable nesting sites for bees. Ground-nesting bees, which compose the majority of wild species, are limited in their nest-building by soil preference. Many favor sandy soil, which tends to have larger particles that are easier to excavate than other types of soil such as clay or silt loam (Cane 1991). Bees that nest in tree cavities, hollow stems, and abandoned rodent dens are also reliant upon the presence of necessary habitat features for nesting (Potts et al 2005, Klein et al 2007).

A farm field's nesting suitability is affected by its management. A lack of habitat in the uncultivated edges of fields will not contribute to sustaining a pollinator community. Tillage, a common practice that breaks up soil to allow increased water and air permeability, can occur at depths of up to 45 centimeters. Since many soil-nesting bee species are found within 20 cm of the surface (Cane & Neff 2011), tilling a field at standard depth could destroy bees that may have nested there. Even in untilled fields, compaction from heavy farm machinery can compress otherwise suitable soil, making it too densely packed for bees to excavate.

Agricultural expansion has contributed to a reduction in both foraging habitat and nesting habitat for bees (Lonsdorf et al 2009). Establishing connectivity between land parcels could prove critical to recovering and maintaining pollinator habitat across agricultural landscapes. Some federal programs exist that facilitate wildlife habitat protection in agricultural areas, such as the Conservation Reserve Program and Environmental Quality Incentives Program (Feng et al 2005). Growers who participate in such programs receive stipends in exchange for keeping land out of production and converting it to a buffer of diverse vegetation on the edges of their fields. Many fields bordered by federally funded conservation buffers contain crops grown to feed animals, like maize and soy beans. The market price of these crops can fluctuate dramatically over time. If the value of a participating grower's crops suddenly increases, he or she may choose to forfeit enrollment in the conservation program, pay the opt-out penalty, and expand crop production into buffer zones and previously un-farmed areas to maximize profitable acreage. Increased demand for maize in the United States has led to the agricultural conversion of millions of acres of land since 2008, much of it grassland (Lark et al 2015).

To increase the abundance of flowers available for pollinators when crops are not in bloom, beneficial insect habitat like native plant field borders and insectary strips can be incorporated as a permanent fixture of working farms (Lee-Mäder et al 2014). Small-scale, diversified agricultural practices can serve as a refuge for pollinators by providing the foraging and nesting needs that are missing from the expansive monocultures typical of large-scale, conventional farms (Klein et al 2007, Hole et al 2005). As of 2012, certified organic farmers are required to provide resources like beneficial insect habitat that foster biodiversity on their farms (USDA NOP 2016, Lee-Mäder et al 2014).

Simply being an organic-certified farm, however, is not enough to boost biodiversity (Brittain et al 2010a). Rather, the benefits non-conventional agriculture can provide for biodiversity are a function of practices on a local farm level as well as on a landscape level (Brittain et al 2010a, Gabriel et al 2010, Andersson et al 2014, Tscharntke et al 2005). A combination of pollinator-friendly farm practices and suitable habitat in the surrounding landscape can dramatically boost a farm's yield via increased pollination (Andersson et al 2014) as well as via other ecosystem services like pest predation by beneficial insects (Lee-Mäder et al 2014) and weed suppression (Tscharntke et al 2005). Organic farms may also benefit pollinators by offering diminished pesticide exposure (Hole et al 2005).

Pesticide exposure risks for beneficial insects

In addition to the problems associated with habitat loss, the acute and chronic effects of pesticide exposure are considered another major contributor to the decline of bees (Larson et al 2014, Brittain et al 2010b, Hopwood et al 2013). The neonicotinoid class of insecticides has come under particular scrutiny as a pollinator risk factor. These insecticides are neurotoxins that act selectively on the insect central nervous system, causing neurons in susceptible animals to over-fire, leading to paralysis and death (Maienfisch et al 2001, Bonmatin et al 2015, Simon-Delso et al 2015, Jeschke & Nauen 2008). The neonicotinoids have become the most widely used insecticide class, comprising approximately a third of the global market (Simon-Delso et al 2015). The compounds are applied in the United States at a rate of millions of pounds of formulated product per year and rising (USGS 2016). Neonicotinoids are also commonly used outside of agriculture –often at higher application

rates– and have been detected in the tissue of plants sold in garden centers throughout North America (Brown et al 2014).

Globally, the three major neonicotinoid compounds used in agriculture are thiamethoxam, clothianidin, and imidacloprid (Elbert et al 2008). The compounds can be sprayed directly onto leaves, soaked into soil where seeds are planted, or applied onto seeds as a seed coat, which is the most popular agricultural method (Hopwood et al 2013). As the treated plant grows, the neonicotinoid becomes systemically mobile, moving through the root and shoot to provide protection against damage from tissue- and fluid-feeding insects like aphids, thrips, and leaf hoppers (Goulson 2013a) and in doing so simultaneously protecting against the pathogens these species can transmit (Simon-Delso et al 2015). Neonicotinoids' combination of high toxicity at low doses, rapid whole-plant translocation, low mammalian toxicity, and a lack of cross-resistance to other pesticide classes has led them to be used in a wide variety of crops (Tomizawa & Casida 2005). Applications of neonicotinoids can provide long-lasting pest control and eliminate the need for further applications of toxic insecticides like carbamates, chlorinated hydrocarbons, and organophosphates (Jeschke & Nauen 2008). Unfortunately, the lack of target specificity that makes neonicotinoids so effective against many different crop pests has also made them a danger to beneficial insects like bees.

Bees come into contact with neonicotinoids by ingesting contaminated nectar and pollen. For this reason, the United States Environmental Protection Agency (EPA) updated neonicotinoid labeling in 2013 to prevent the compounds from being sprayed during a crop's flowering period (EPA 2014). Even with these precautions in place, concentrations of up to 11 ppb have been found in pollen and nectar of flowering crops that were planted with a

neonicotinoid seed coating (Hopwood et al 2013). Dust clouds that form when neonicotinoid-coated seeds are planted pose an additional route of exposure for bees, both in the planted field and in surrounding uncultivated areas (Krupke et al 2012).

Bees can also be exposed to neonicotinoids by consuming a plant's guttation fluid (Girolami et al 2009). Guttation often appears in the early morning during humid conditions. Although similar to dew in appearance, guttation fluid is actually a natural excretion of a plant's xylem sap from the leaf tips. If a foraging bee were to consume a treated crop plant's guttation fluid, especially from a young plant, the bee could be exposed to a neonicotinoid dose as much as 100,000 times higher than that found in nectar (Godfray et al 2004). For example, corn plants grown for three weeks from neonicotinoid-treated seeds have been found to contain up to 100 ppm of thiamethoxam or up to 200 ppm of imidacloprid in their guttation fluid (Girolami et al 2009). These concentrations are well within the range that can harm bees (Hopwood et al 2013, Godfray et al 2014, Iwasa et al 2004).

A bee that has come into contact with an acutely toxic dose of neonicotinoids will die (Hopwood et al 2013). In honey bees the median lethal dose (LD_{50}) of oral neonicotinoid exposure occurs as low as 3.7 ng/ bee with imidacloprid, and the contact toxicity LD_{50} occurs between 24-81 ng/bee for the three major neonicotinoid compounds (Godfray et al 2014). One study found contact doses of imidacloprid as low as 0.0179 µg/bee could cause mortality (Iwasa et al 2004). The exact dose that will prove lethal to a bee differs dependent upon which neonicotinoid compound, which bee species, and which route of exposure. For most wild bee species, a lethal dose level has not been determined. Lethal and sub-lethal doses for bees can also change if neonicotinoids are mixed with other compounds and applied as a chemical combination (van Lexmond et al 2015, Camargo Gil 2016). Even lower, non-lethal

doses of neonicotinoids may cause bees to exhibit sub-lethal effects such as inhibited larval growth, disrupted foraging and navigation, and reduced queen production (Hopwood et al 2013).

Although neonicotinoids degrade quickly in the surface layers of soil, they persist in deeper, colder, and high-clay soils (Bonmatin et al 2015, Júnior et al 2004). The compounds are highly water soluble and have been found in wetlands and groundwater in areas of high agricultural activity (Main et al 2015, Huseth & Groves 2014). This neonicotinoid-contaminated groundwater may be drawn up and sprayed onto crops while they're in flower, representing a further potential route of exposure for pollinators in areas of irrigated agriculture (Huseth & Groves 2014).

Pollinator conservation in central Wisconsin

The state of Wisconsin is home to 68,900 farms (USDA NASS 2016b), with thousands of hectares devoted to irrigated specialty crops such as processing vegetables and potatoes grown within an ecological region known as the "Central Sands" (Huseth & Groves 2014). Major pollinator-dependent specialty crops cultivated in the Central Sands include cucumbers, cranberries, and tree fruit that together account for more than 55 million dollars of annual within-state revenue (WI DATCP 2016). Many of these crops are pollinated more efficiently by wild bees than by honey bees (Delaplane & Mayer 2000, Mallinger & Gratton 2015), so protecting wild pollinator habitat can help improve pollination rates while reducing reliance on our already overtaxed domestic pollinators.

Specialty agriculture also includes crops that are not pollinator-dependent such as sweet corn, snap beans, and peas. In Wisconsin many of these crops are grown to be

processed via canning or freezing rather than sold as fresh produce. Even though pollinating insects are not required for the production of certain processing vegetables, the crops nonetheless provide floral resources that pollinators may visit during the blooming period (USDA 2015). Because the presence of bees is not an economic necessity, the benefits and risks of processing crops for pollinator communities are largely absent from the present body of scientific literature. Bees living within the nine-county Central Sands region have been overlooked within the state of Wisconsin (Wolf & Ascher 2008), with only recent investigations occurring in pickling cucumbers (Lowenstein et al 2012) and cranberries (Gaines Day 2013).

Vegetables grown in the Central Sands region benefit from sandy, fast-draining soil and a water table that lies very close to the surface (WI DNR, 2014). But the benefits also bear costs: large-scale growers must install groundwater-fed irrigation systems to supply crops with sufficient water to survive the dry summer months. As a result, the Sands region has one of the highest concentrations of irrigated vegetable fields in the state, receiving 170 million liters of water per year from more than 1,300 high-capacity wells (Huseth & Groves 2014). These large, center-pivot irrigation systems have been a key factor in allowing the Central Sands to become the major vegetable production region that it is today.

Trace amounts of insecticides, including neonicotinoids, have recently been detected in the surface and groundwater in areas of high agricultural intensity, including Wisconsin's Central Sands (Huseth & Groves 2014, Carnemark et al 2015). It is this contaminated groundwater that is withdrawn as irrigation water for vegetable crops, including at times when crops are in bloom and pollinators may be present and foraging.

In Wisconsin, the neonicotinoid thiamethoxam is applied for early season pest control in most annually-grown processing vegetables (Colquhoun et al 2016). Thiamethoxam has been on the market since 1992, and is applied as a foliar, soil, or seed treatment (Jeschke et al 2010). The United States Environmental Protection Agency (EPA) has already released a preliminary risk assessment of imidacloprid that recommends a concentration of less than 25 ppb in nectar (EPA 2016), and the agency plans to fully review the registrations of imidacloprid along with neonicotinoids clothianidin, thiamethoxam, and dinotefuran between 2017 and 2018. In the meantime, research is needed to inform regulators and insecticide registrants about the concentrations of these insecticides that are present in processing vegetable crops, and to learn more about the seasonal sequence and diversity of pollinators present in this important eco-region of Wisconsin.

Research rationale

Pollinators are put at risk by land use change and pesticide exposure. The Central Sands ecological region is a place where pollinator risk factors come together, especially landscape fragmentation by agriculture and heavy neonicotinoid use. Given these circumstances, central Wisconsin is an ideal study area to analyze specific aspects of the pollinator community's relationship with vegetable agriculture.

The purpose of this study was to determine which bees are present in predominant central Wisconsin vegetable crops, and when these crops may bear peak in-plant neonicotinoid concentrations. We had three goals. First, we sought to obtain temporal data describing the region's bee community within the bounds of processing vegetable crops that have not previously been surveyed for pollinators. Second, we quantified changing

neonicotinoid concentrations in the flowers and leaves of three processing vegetable crops that occur commonly in the Central Sands: snap beans (*Phaseolus vulgaris*), peas (*Pisum sativum*), and sweet corn (*Zea mays*). Finally, we examined whether the predominant land use surrounding agricultural sites within the same central Wisconsin growing region had an effect on pollinator communities.

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Chapter 2: The Bee Community of Central Wisconsin Vegetables

Abstract

Pollinator insects like bees are in decline throughout the United States, in part due to habitat loss from expanding agriculture. This study's goal was to examine the suitability of the heterogeneous agricultural region of central Wisconsin for wild bees. We assessed the composition and distribution of bee species in central Wisconsin vegetable agriculture using pan trap captures and observational data taken within conventionally grown processing vegetable fields and in the semi-natural field edges of conventional and organic vegetable farms. Although many bees were observed foraging at flowers in conventional and organic field margins, almost none were seen foraging within conventional fields. Eighty-six bee species were captured and identified within the study region, including one new state record. The majority of bees captured from all sites were species belonging to the family *Halictidae* and the genus *Lasioglossum*, but the majority of bees captured from within vegetable fields were honey bees (*Apis mellifera*). There was significantly greater diversity, abundance, and species richness in field margins than in fields. Bee communities collected from within crop fields were negatively influenced by surrounding agricultural land use and positively influenced by surrounding natural land use at scales of one to two kilometers, but were largely unaffected by the proportion of surrounding land use within smaller radii of 250 and 500 meters. These results suggest that distances of one to two kilometers are within the foraging range of the bee species found in the central Wisconsin area, and that field edges may be important habitat features for pollinators in central Wisconsin. Since many types of vegetable crops share space and rotation across areas where bees are known to travel, cooperation across property lines is imperative to ensure that crops and bees alike continue to thrive in this area.

Introduction

The role of landscape in bee conservation

Bees are responsible for pollinating 35% of the world's food crops (Klein et al 2007). Though honey bees are a common sight in North America (indeed, honey bees are the state insect of seventeen of the United States), they are a non-native species that has been imported to pollinate the vast majority of pollinator-reliant crops (Genersch 2010), many of which are also non-native. In recent years, faced with the rapid expansion of pollinator-dependent agriculture, honey bee populations have begun to lag behind pollination demand (Aizen & Harder 2009).

In addition to honey bees there exist approximately 3,600 species of wild bees in North America (Ascher & Pickering 2016). The wild bees display a great diversity of life histories and adaptations that allow them to pollinate a variety of flowering plants. Wild bee species can complement or surpass honey bees' pollination performance in many food crops, such as tree fruit, blueberries, tomatoes, and squash (Mallinger & Gratton 2015, Gibbs et al 2016, Greenleaf & Kremen 2006, Vaughan et al 2015, Garibaldi et al 2013).

From the mid-twentieth century onwards, many insect populations, including bees, have been declining (van Lexmond et al 2015). The factors contributing to global bee declines include Colony Collapse Disorder in honey bees (Winfree et al 2011), between- and within-species pathogen transmission (James 2008, Genersch 2010, Graystock et al 2014, Otterstatter & Thomson 2008), restricted genetic diversity (Oldroyd 2007, Cameron et al 2011), and pesticide exposure (Brittain et al 2010b, Hopwood et al 2013, Krupke et al 2012, Fischer et al 2014, Larson et al 2014, Samson-Robert et al 2014). Overall, however, habitat loss may be the most important contributor to bee decline (Potts et al 2010). Widespread

habitat loss caused by agricultural expansion and intensification during the last several

decades has made it harder for bees to forage and establish nests (Kennedy et al 2013,

Kremen et al 2007, Ricketts et al 2008, Winfree et al 2009, Lonsdorf et al 2009). Most bees

are central place foragers that make trips to and from a central nest location where gathered

resources are stored. Thus, a bee's ability to visit flowers is limited by proximity to the nest

site. Maximum foraging distances are determined by a bee's size; larger bees may traverse a

radius as long as 15 km while very small bees are restricted to a few hundred meters

(Greenleaf et al 2007, Godfray et al 2014). The average foraging distance of North

American bees ranges from 100 to 2,000 meters (Koh et al 2016, Gathman & Tscharntke

2002, Ricketts et al 2008, Greenleaf et al 2007).

Because bees are mobile within the environment, their conservation depends on both

local level land management as well as landscape level land use (Kremen et al 2007). At the

local level, pollinator-conscious farm management practices can serve to mitigate some

negative effects from the surrounding landscape (Albrecht et al 2007). Organic farms, for

example, often have more robust bee communities than conventional farms due in part to

decreased pesticide use and the resultant increase of floral diversity, often from weed species

(Hole et al 2005, Andersson et al 2014, Bengtsson et al 2005, Gabriel et al 2010). However,

the benefits derived from organic practices may be offset when the farm is situated in a

homogeneous agricultural area (Brittain et al 2010a). Furthermore, the creation of on-farm

floral habitat that is not sufficiently diverse may only benefit certain groups of bees, to the

detriment of specialists and non-corbiculate species (Wood et al 2015).

At the landscape scale, previous studies have found that the amount of natural and

semi-natural land surrounding farm fields is positively correlated with pollinator richness and

abundance (Ricketts et al 2008, Kennedy et al 2013, Gibbs et al 2016). Land use change resulting in habitat fragmentation is negatively correlated with pollinator presence, particularly in landscapes where little suitable habitat remains (Winfree et al 2009). Predicted declines in pollinator abundance are most pronounced in states in the Midwest and Mississippi river valley where agricultural land use predominates, with the most intensively cultivated areas having the lowest predicted abundance of wild bees (Koh et al 2016).

The Central Sands: Wisconsin's processing vegetable growing region

Rotations of soy and maize, which do not require insect pollination, are predominant across Wisconsin (Haines et al 2010), but the state also has a high reliance upon bees for agricultural pollination. Wisconsin produces nearly a million dollars' worth of specialty crops annually, including cranberries, tree fruit, and vegetables for the fresh market (USDA NASS 2015). Many of these crops are heavily dependent upon insect pollination (USDA 2015a). Wisconsin also ranks second in the nation in the production of major processing vegetables grown to be canned or frozen (USDA NASS 2015). The dominant processing vegetables include sweet corn (*Zea mays*), snap beans (*Phaseolus vulgaris*) and field peas (*Pisum sativum*) (Haines et al 2010). These three processing crops are not dependent upon insect pollination; snap beans and peas are self-pollinating, while sweet corn is wind-pollinated. Despite their respective adaptations to produce viable fruit without insect pollination, bees do visit these crops while foraging for pollen and all three of these crops are visited to varying extents by honey bees, bumble bees, and other wild bees (USDA 2015a).

In Wisconsin, the majority of these and other high-value specialty crops are grown within a region known as the Central Sands (Haines 2010). The Central Sands, located in the

center of the state, is unique in that it is heavily cultivated but also heterogeneous when compared with other agriculturally intensive regions. Central Wisconsin's soil is sandy with poor water retention (WI DNR 2014), but extensive irrigation has made the region well-suited for growing high-value specialty crops like cranberry, potatoes, sweet corn, and other vegetables (Haines et al 2010). The region annually supports approximately 80,000 hectares of irrigated specialty crops (Haines et al 2010, WICCI 2011). Fields growing pollinator-dependent crops share both space and rotation with non-pollinator dependent fields, resulting in a mosaic of diverse crops and management regimes across the landscape.

The suitability of the Central Sands as pollinator habitat remains unexplored in several respects. The bees of the Central Sands are regionally the least-studied in the state (Wolf & Ascher 2008). Furthermore, the overall effect of surrounding land use types on bees found within processing vegetables in this unique region has been explored in pollinator-dependent crops such as cucumbers (Lowenstein et al 2012, Lowenstein 2011) and cranberries (Gaines Day 2013), but not in processing vegetables without pollinator dependence. Since these processing vegetable crops comprise as much as 35% of the total cropland in Central Sands counties (Haines et al 2010), their prevalence in the area may make them an important component of local pollinator habitat. Although organic agriculture is not prevalent in the Central Sands - there are only four certified organic vegetable farms in the most intensively farmed central three counties (Portage, Waushara, and Adams Counties) (Farm Fresh Atlas 2016), the sector is rapidly expanding. The number of organic farms in Wisconsin has continued to grow even as the number of total farms diminishes (Haines et al 2010). If trends in expanding organic prevalence occur in the Central Sands, such farms may become an important component of wild bee habitat in the area. As goals are set to conserve

declining pollinator populations at both federal (USDA 2015b) and state (WI DATCP 2016) levels, the role of the Central Sands' unique agricultural landscape should be further explored as it applies to pollinator conservation.

Research rationale

This project had two main objectives. First, we examined the seasonal bee community in fields of three dominant processing crops grown within Wisconsin's Central Sands region that do not require pollination. We related our findings to the large-scale effects of surrounding land uses to understand whether these crops are utilized by bees living in the Central Sands, and whether the dominance of conventionally grown vegetable crops in the surrounding landscape affects their prevalence and diversity.

Second, we examined the seasonal bee community present in semi-natural marginal lands adjacent to these conventional vegetable crops and compared our findings to conventional in-field bee communities, as well as to the bee communities in small-scale and large-scale organic vegetable field margins within the same region, to understand how farm management practices might influence the suitability of field edge habitat.

Materials and Methods

Conventional processing vegetable fields

This study took place during the growing seasons of 2014 and 2015, from early June through mid-August. Fields of sweet corn (*Zea mays*), snap beans (*Phaseolus vulgaris*), and field peas (*Pisum sativum*) were selected for sampling due to their prevalence within Wisconsin's Central Sands region. In total, 53 conventionally grown, irrigated vegetable

fields of sweet corn (19 fields) snap beans (18 fields) and peas (16 fields) were visited during each of their respective blooming periods (**Fig. 1**), all of which were located within the region's three most intensively farmed counties: Portage, Waushara, and Adams. Each field was privately owned by a grower contracting their irrigated land with the Del Monte Corporation (Walnut Ridge, CA). Field sizes ranged between 10.5 and 121 ha, with the average field size being 47.8 ha.

At each field site, the bee community was sampled using pan traps made with 150 mL plastic bowls (Chinet Co, Desoto KS). The bowls were either left white or painted blue or yellow with fluorescent paint (Ace Glo Spray) to attract foraging bees based on Droege (2008). Each bowl was filled with a mixture of water and a small amount of Dawn® blue dish soap (Procter & Gamble, Cincinnati OH), which served to prevent landing bees from escaping by changing the water's surface tension. The pan traps were arranged at or near the center of each field in 100 m^2 grids of nine bowls in alternating colors. Depending on the size of the field, the distance from the central trap location to the nearest field edge ranged from 31 to 410 meters (mean = 187 m). Each trap was attached to a stake and adjusted to the height of surrounding flowers. The pan trap grids were placed near the center of each conventional vegetable field while the crop was at or beyond 75% bloom, then left in the field for 24-48 hours to capture the full temporal range of bee activity. At the end of each sampling period any bees caught were collected from the traps, mounted, and identified to the species level with the assistance of Dr. Jason Gibbs, Michigan State University.

To assess whether bees were engaging in foraging behavior at study sites, observational data were collected in 2015 within all snap bean fields (n = 9) and sweet corn fields (n = 9) for a total observational period of 10 minutes per site. Visual transects were

arranged as two parallel 10 meter lengths within each pan trap grid. Observation points were spaced 2 meters apart on each transect line, making a total of five observation points on each transect line and ten observation points in each trap grid. At every observation point, the observer watched flowers within a visual radius for one minute and recorded any honey bees or wild bees seen visiting flowers. Bees observed during the observational sampling period were categorized and recorded (**Table 1**).

Conventional and organic field margins

Additional components were added to the study during the 2015 field season to examine the role of semi-natural field margins as pollinator habitat at the local level. Thirteen field edge sites located in a patch of untilled land in an edge directly adjacent to one of the flowering crop fields (two pea field margins, six bean field margins, and five sweet corn field margins; **Fig. 2**) were sampled using the same 100 m^2 pan trap grid method used in conventional fields. These edge sites were located in the triangular corners of fields where the irrigator does not reach. Observational data of foraging bees in eleven of the sampled field edges (6 snap bean edges and 5 sweet corn edges) was recorded with the same ten-minute visual transect procedure used in conventional fields.

Along with the thirteen conventionally grown vegetable field margins, the bee community of two certified organic farms was also sampled over the course of the 2015 field season (**Fig. 2**). Consistent with this study's conventional fields and conventional edge sites, the organic farms selected for this study were also located within the three most heavily farmed vegetable growing counties of central Wisconsin (Portage, Adams, and Waushara Counties). Flyte Family Farms of Coloma, WI (Waushara County) annually produces more

than two hundred ha of organic vegetables. A 103.2 ha portion of land being used to grow three organic crops (potato, sweet potato, and alfalfa) was selected as a study site to represent large-scale organic cultivation. The second organic site was Whitefeather Organics LLC of Custer, WI (Portage County), a 9.3 ha diversified vegetable and livestock operation that represented this study's small-scale organic farm. The field margins of both the small-scale and large-scale organic farms were sampled for bees weekly during the summer, with each visit being paired with a visit to a conventional edge site on the same date. Bee community data were taken from field margins directly adjacent to organic crops using the same pan trap grid method described in conventional field margins. Observational data of foraging bees were recorded weekly (n = 10 for both farms) within each pan trap grid, just as in conventional field edges.

Data analysis

The abundance, species richness, and diversity of bees were calculated using the individuals captured from each site's pan trap grid. Total bee abundance was calculated as a rate of bees per hour by dividing the total number of individual bees captured per site by the number of hours in the corresponding sampling period (the average sampling period was 33.2 hr, with a minimum of 20.6 hr and a maximum of 49.0 hr). Since honey bees (species *Apis mellifera*) made up a disproportionately large amount of the individual bee species captured within conventional vegetable fields, a separate wild bee abundance value, which excluded this species, was calculated for bee community comparisons within conventional vegetable fields. Bee species richness was calculated as the total number of bee species captured during

each sampling period. The diversity value of each site was calculated using the Shannon's

Diversity Index H-value formula as follows:

$$H = -\sum [p_i \cdot \ln(p_i)]$$

Where p_i = the proportion of each site's population comprised by species i.

The factors examined for their individual and interactive influences on bee

community metrics included time (measured in days since April first), year, crop type, field

size (ha), and the land use types surrounding field sites. Land use classifications were

obtained using the National Agricultural Statistics Service Cropland Data Layer, or

Cropscape (CDL: USDA NASS 2015, https://nassgeodata.gmu.edu/CropScape/), which is a

rasterized digital rendering of land cover obtained via remote-sensing at a 30m pixel

resolution. Land cover buffers were generated using Arc Map 10.3 (ESRI 2015) at radii of

250, 500, 1000, and 2000 meters from each sampling point. Buffers at or below 2000 meters

were chosen based on data from a meta-study comparing bee foraging trends in relation to

distance from habitat (Ricketts et al 2008). At each buffer size, classified CDL land cover

pixels were converted into percentages based on categories of "natural," "agricultural,"

"developed," or "surface water." Land categorized as natural included forest, wetlands,

grasslands, and fallow or idle cropland, while the agricultural land use category comprised of

any crops under managed cultivation, including Christmas tree farms. Developed land was

pre-classified by CDL to describe paved surfaces and buildings at varying intensities.

Because surface water was either absent or near zero in all buffers, this land use classification was not included in data analysis.

All data were analyzed using R software, version 3.3.0 (R Core Team 2016) and SAS 9.4 (SAS Institute Inc., Cary NC). In conventional processing fields, each of the four community metrics (total abundance, wild abundance, diversity, and species richness) were analyzed with separate models. The presence or absence of bees as a function of all combinations of factors was analyzed using a binary generalized linear model to account for the high number of zero values in the community metrics. Following these analyses, all zero values were removed and the remaining sample points yielding bee community data were analyzed using a multiway ANOVA with linear models. Significant models were chosen using the criteria of a *p*-value less than 0.05 and an R^2_{adj} of greater than 15 percent. The effect of year was ruled out as a significant predictor variable, and thus was not included in the models. Diversity was analyzed using untransformed values, while wild and total abundance were converted to logarithmic values to fit assumptions of normality and equal variance. Species richness was converted to logarithmic values as necessary. Pairwise comparisons of bee community metrics in conventional fields versus conventional field margins, small-scale organic margins versus large-scale organic margins, and organic margins as a whole versus conventional field margins were analyzed using paired *t*-tests.

Results

Conventional processing vegetable fields

A total of 1,233 bees representing 86 different species and five families were collected from all field sites in this study (**Table 5**). Of these, 347 individual bees (28% of all

individuals captured) were domesticated honey bees, *Apis mellifera* (**Fig. 3**). Overall, the majority of wild bee species captured in this study were members of the genus *Lasioglossum* in the family Halictidae (**Table 5**). Of the halictids, most were soil-nesting eusocial species. The least prevalent wild species were members of the families Megachilidae (16 individuals captured representing 10 species), Andrenidae (13 individuals captured representing eight species), and Colletidae (12 individuals captured representing four species). Only one individual megachilid bee, *Hoplitis producta*, was captured from within a conventional vegetable field; all other megachilids were collected from field margins. Members of the family Melittidae, which are among the rarest bees in North America, were not captured at all.

Within the 53 conventional vegetable fields sampled in 2014 and 2015, 498 individual bees were collected representing 28 species. Of these, 294 honey bees were captured, representing 59% of all individual bees collected from conventional vegetable fields (**Fig. 3**). The presence of bees within crop fields (interpreted here as the presence or absence of a diversity H-value at a sampling site) was not significantly correlated with time, surrounding land use, or field size. However, the presence of multiple bee species in crop fields was significantly affected by field size, with larger fields tending to have more bee species ($Z = 2.01$, $p = 0.044$, AIC = 62.08) (**Fig. 4**).

The proportion of developed land, including roads, buildings, and other non-natural structures, had no observed effect on the bee community in the Central Sands. The study sites were by nature all rural; developed land never comprised more than 10% of the surrounding landscape, so it is likely that no effects were observed because the proportion of developed

land was too small. The effect of surrounding land use at a smaller scale of 250 -500 m also tended to have little effect on bees in Central Sands vegetable crops.

Crop type and time had an interactive effect on the log-transformed total abundance of bees in conventional processing vegetables. As time progressed, the total abundance of bees significantly increased in bean fields but not in pea or corn fields (**Fig. 5**). With honey bees excluded, the log-transformed abundance of wild bees within vegetable fields was positively correlated to the proportion of natural land and negatively correlated to the proportion of agricultural land occurring within a 2,000-meter radius (**Fig. 6; Table 2**).

Across conventional fields as a whole, bee diversity was positively correlated with the proportion of natural land at radii of 500 meters, 1,000 meters, and 2,000 meters and was negatively correlated to the proportion of agricultural land at radii of 1,000 meters and 2,000 meters (**Table 2**). There was a significant interactive effect of crop at all these buffer sizes (**Fig. 7; Table 3**). The bee diversity within corn fields was not significantly affected by the amount of natural area within 500 meters, 1,000 meters, or 2,000 meters, nor by the amount of agricultural land within 1,000 meters or 2,000 meters. In bean fields, conversely, the diversity of bees was significantly positively affected by the amount of natural area within 500 meters, 1,000 meters, and 2,000 meters and was significantly negatively affected by the amount of agricultural area within 1,000 meters and 2,000 meters (**Table 3**). The diversity of bees in pea fields was also significantly positively correlated with the amount of natural area within 500 meters, 1,000 meters and 2,000 meters while being significantly negatively correlated with the amount of agricultural land 2,000 meters and weakly negatively correlated with the amount of agricultural land at 1,000 meters (**Table 3**).

Conventional and organic field margins

Halictid bees comprised the majority of individuals captured in organic and conventional field margins (**Fig. 3**). Of the total 1,233 individual bees captured across all study sites, 226 were collected from the margins of thirteen different conventionally grown vegetable fields in 2015, including 31 honey bees (14% of individuals captured). In the same year, 489 individual bees were collected from a large-scale and small-scale organic farm across ten weeks, including 22 honey bees (4% of individuals) (**Fig. 3**). Fourteen bee species were unique to the large-scale farm, five were unique to the small-scale farm, and one was unique to organic margins (**Table 5**). None of the bee species were uniquely found in the semi-natural edge sites bordering conventional fields. One species, *Lassioglossum semicaeruleum*, was a new record east of the Mississippi river.

The bee communities of paired (n = 13) conventionally grown vegetable fields and conventional vegetable field edges varied significantly (**Fig. 8; Table 4**). The diversity of bees in conventional was significantly lower than the diversity of field edges. The natural log of wild bee abundance within fields was also significantly lower than in margins. Likewise, the natural log of total bee abundance was significantly lower in conventional fields than in margins. The natural log of bee species richness was significantly lower in conventional vegetable fields than in margins as well.

The bee communities of paired small- and large-scale organic farms (n = 10) did not significantly differ from each other (**Fig. 9; Table 4**) in diversity value, species richness, or the total abundance of bees. When the organic farm margins were compared to semi-natural conventional field margins sampled on the same date (n = 9), the organic margins harbored

significantly higher species richness than conventional margins (**Fig. 9**), though there was once again no difference in diversity or total abundance.

Discussion

Bees in conventionally grown vegetable fields

Only two bees were recorded actually foraging within 18 conventional vegetable fields surveyed in 2015, whereas in the same year 61 bees were recorded foraging in 11 conventional margins and 48 bees were recorded foraging in organic margins at 20 time points (**Table 1**). These results support existing data (USDA 2015a, Woodcock 2012) reporting that honey bees, bumble bees, and some wild bees collect pollen from corn, snap beans, and peas but do not visit preferentially.

The proportion of bees captured within conventional fields was dominated by honey bees. Interestingly, in conventional and organic field margins, where floral diversity tends to be greater, the proportion of honey bees captured dropped dramatically (**Fig. 3**). Honey bees foraging in the study region may have focused their foraging primarily upon the neighboring crops that they were stocked to pollinate rather than venturing into field margins offering more sporadic floral rewards than what a crop in full bloom would offer. Previous research in agricultural areas has also found that honey bees display a foraging preference for mass-flowering crops whereas wild bees are most closely associated with woody or herbaceous semi-natural habitats (Rollin et al 2013).

In all fields sampled, the presence of more than one bee species in a given processing vegetable field was significantly, positively correlated with the field's size (**Fig. 4**). It is possible that larger fields were more likely to have bees because foraging bees were forced to

fly through these larger fields rather than around them in search of foraging patches. Among fields where bees were captured, the proportion of surrounding land use at large buffer sizes tended to best predict wild bee abundance (**Fig. 6**), and diversity (**Fig. 7**). Previous research by Ricketts et al (2008) found that bee diversity decreases by half once the distance from natural habitat exceeds 1,500 meters, so it is logical that land use within radii nearing this size would be a reflection of wild bee diversity in this study as well.

Whereas the abundance of wild bees was most affected by the land cover within 1,000 to 2,000 meters, total bee abundance within conventional vegetable fields was most affected by time, but only in bean fields (**Fig. 5**). This discrepancy is likely due to the fact that total abundance included honey bees, which were by far the predominant species in conventional snap bean fields (75% of individuals; **Table 5**). Honey bees are stocked in fields of pickling cucumber (Lowenstein et al 2012), which is often grown in proximity to this study's processing vegetable crops and under irrigation in the Central Sands (CDL: USDA NASS 2015). Indeed, half of all cucumber land cover pixels detected within 2,000 m of this study's conventional field sites were found around bean fields. Since bean fields were positively correlated with increased total abundance as time progressed and other crop types were not (**Fig. 5**), it is likely that the bean fields were within the flight path of honey bees visiting pollinator-dependent crops, and that this correlation described a hive stocking event rather than a bean-specific phenological pattern.

The land use within 250-500 meters of capture sites did not tend to exert an effect on the bees captured in vegetable fields or edges. These distances are smaller than the average foraging range of North American bees (Koh et al 2016), so the lack of observed effects could be due to the fact that these ranges are not limiting to foraging bees. It is also possible

that these radii did not encompass enough of the surrounding landscape for an effect to be observable, since some capture points were as far as 410 meters from the nearest field edge.

There was an interactive effect of surrounding land use and crop type on diversity such that bean and pea fields were significantly affected by surrounding land usage but corn fields were not (**Fig. 7**). Time was ruled out as an interactive effect during model selection, so it's possible that the three crops' differential trends were a result of some bees' reluctance to enter these fields. Previous research has indicated that bees prefer to take the path of least resistance when entering a crop field, favoring row orientation that's perpendicular to habitat in field edges rather than parallel (Gibbs et al 2016). Corn is a much taller crop than snap beans and peas, so it may have attracted a less diverse group of bees that were willing to fly at a higher altitude.

Bees in field edges

The semi-natural edges of conventional processing vegetable crops dominant within the Central Sands could be important habitat for the region's bees. Conventional field margins yielded significantly higher bee diversity and abundance as compared to conventional fields themselves. When edge sites within the bounds of organic farms were compared to these conventional edge sites, the organic edges harbored significantly higher species richness, though there was no difference in diversity or abundance. Thus, the total number of different species tended to be higher in organic sites but did not amount to a significant increase in overall bee activity, nor a more diverse bee community (**Fig. 9**). The bee communities of this study's small- and large-scale organic farms did not significantly differ. These data imply that, regardless of the size or management regime of representative

organic growers in central Wisconsin, the number of unique bee species and those species'
relative abundances within agricultural edge sites will likely be much the same. It is possible
that organic farm margins and conventional farm margins in central Wisconsin may be
equally favorable for wild bees. However, due to the dearth of organic vegetable agriculture
within the Central Sands region, the only source of replication for organic sites was across
time. The p-values comparing organic field edges with conventional field edges were much
lower than the p-values of the large-scale and small-scale organic farms compared to each
other, for example, so it is reasonable to assume that a study encompassing more organic
farms over a longer time period with greater sampling replication could result in a more
pronounced difference in the bee community among edge types. If the community of organic
vegetable growers in central Wisconsin follows the same increasing trend as the total number
of organic growers in the state, further replication in the region may become possible.

Comparisons of conventional and organic edge conditions were only added in 2015,
and subsequently did not have replication across seasons. Long-term data encompassing the
history of field margins may reveal clearer patterns, since older field margins tend to provide
better habitat for pollinators (Denys & Tscharntke 2002). Long-term, species-level data is
also desperately needed to inform population trends of lesser-known bee species, since
almost all conservation listings in North America are bumble bees (IUCN 2016). This
problem is paradoxical, however, because many wild bee species can only be accurately
identified to species as pinned specimens.

Summary

Of more than 400 bee species found in Wisconsin (Wolf & Ascher 2008), 86 were captured from the Central Sands agricultural region, and 49 of these species were found within conventional processing vegetable fields. In-field bee species were most affected by the surrounding land uses at scales of 1,000 and 2,000 m, particularly the proportion of natural land, which suggests that lesser distances are within the average foraging range for the bee species found in the Central Sands. Many bees were recorded actively foraging in field margins, but few if any foraged within conventional fields in full flower. It seems likely that field pea, snap bean, and sweet corn processing crops are not favored floral resources among bees foraging in the Central Sands, but that bees do visit these fields on foraging trips.

The season-long suitability of field edges on a small-scale and a large-scale organic farm were compared to each other and to conventional margins within the Central Sands. Field margins yielded more observed foraging and a more robust bee community than that found within vegetable fields themselves, and bee communities from organic field edges were relatively equivalent to conventional edges, regardless of organic growers' scale. Future pollinator conservation efforts in the region would do well to consider the potential importance or marginal lands as bee habitat.

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Figure 1. The location of fifty-three conventional fields of processing vegetables sampled in 2014 and 2015. All fields were located within Portage, Adams, and Waushara Counties in the Central Sands growing region of Wisconsin. Fields of vegetable cultivation are indicated in dark grey and study sites are indicated in color.
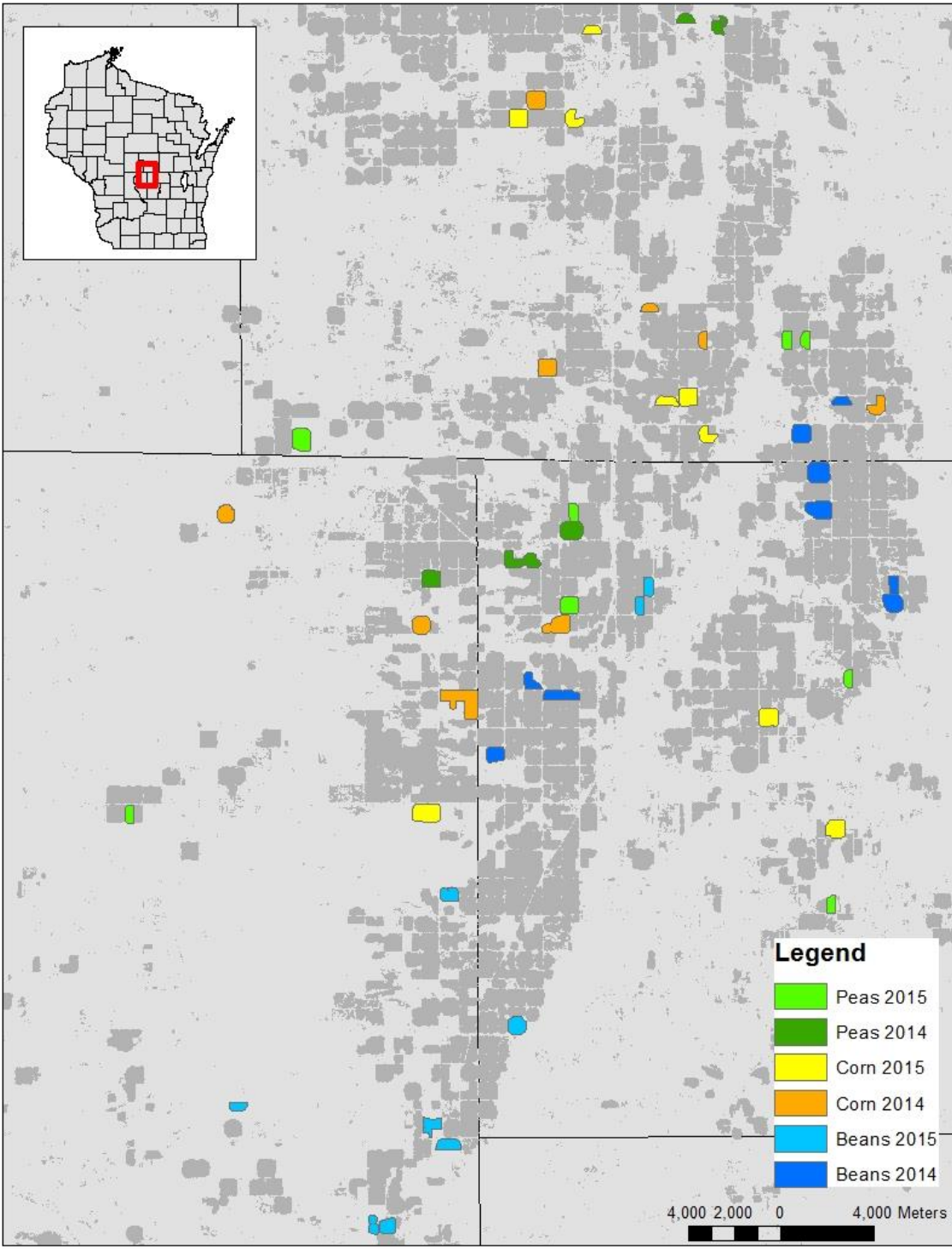
Figure 2. The locations of small- and large-scale organic farm sites along with 13 uncultivated conventional edge sites from the vegetable growing study region (total vegetable fields are indicated in grey) located in Portage, Waushara, and Adams Counties within the Central Sands region of Wisconsin.
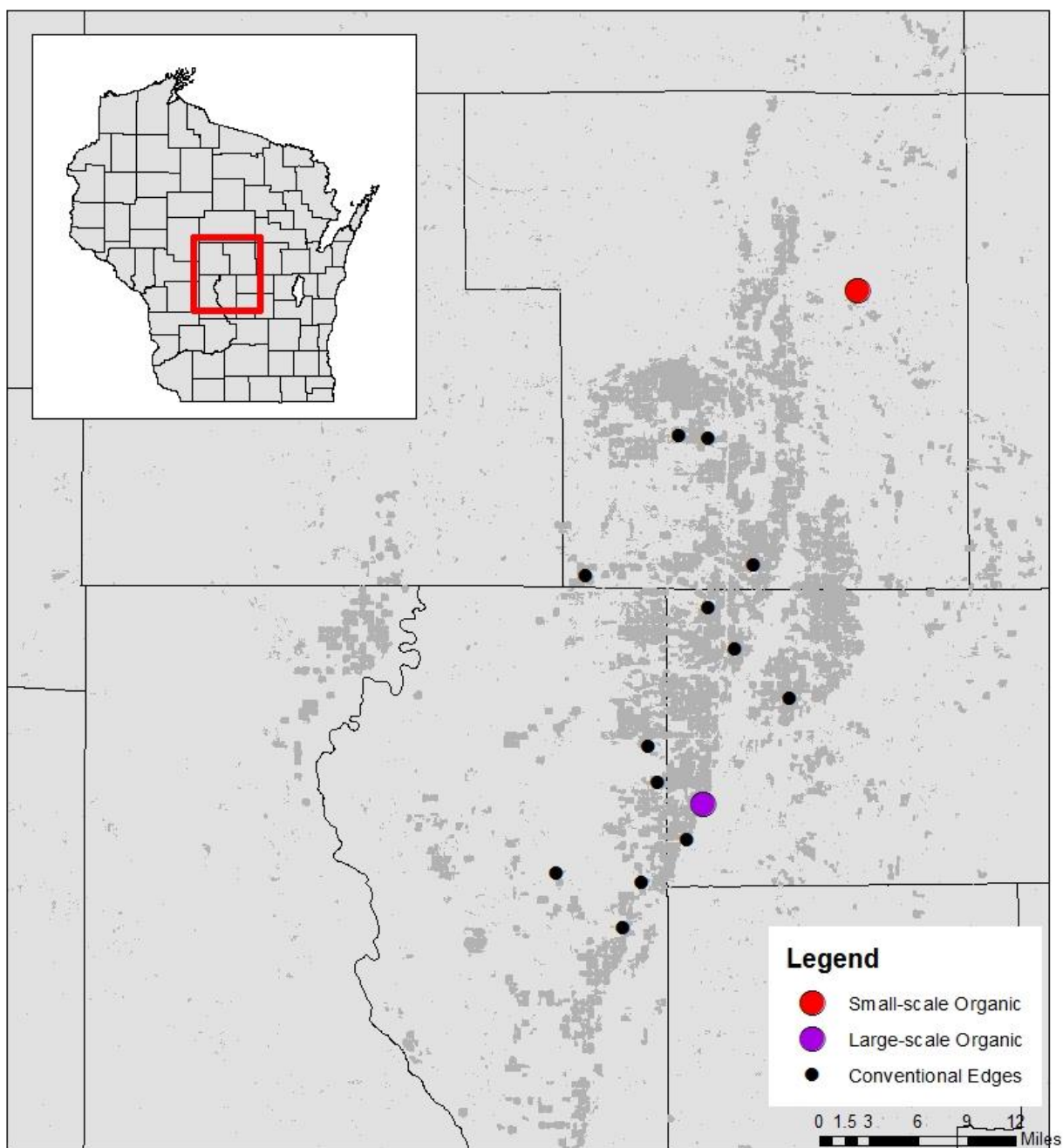
Figure 3. Composition of cumulative captures of honey bees (*Apis mellifera*) and wild bees representing five different families collected in conventional processing vegetable fields (n = 53), conventional processing vegetable field margins (n = 13), two organic farms, and the summation of all sites. Bees obtained from each of the organic farms were replicated across time (n = 10). All collection sites were located within the Central Sands region of Wisconsin.
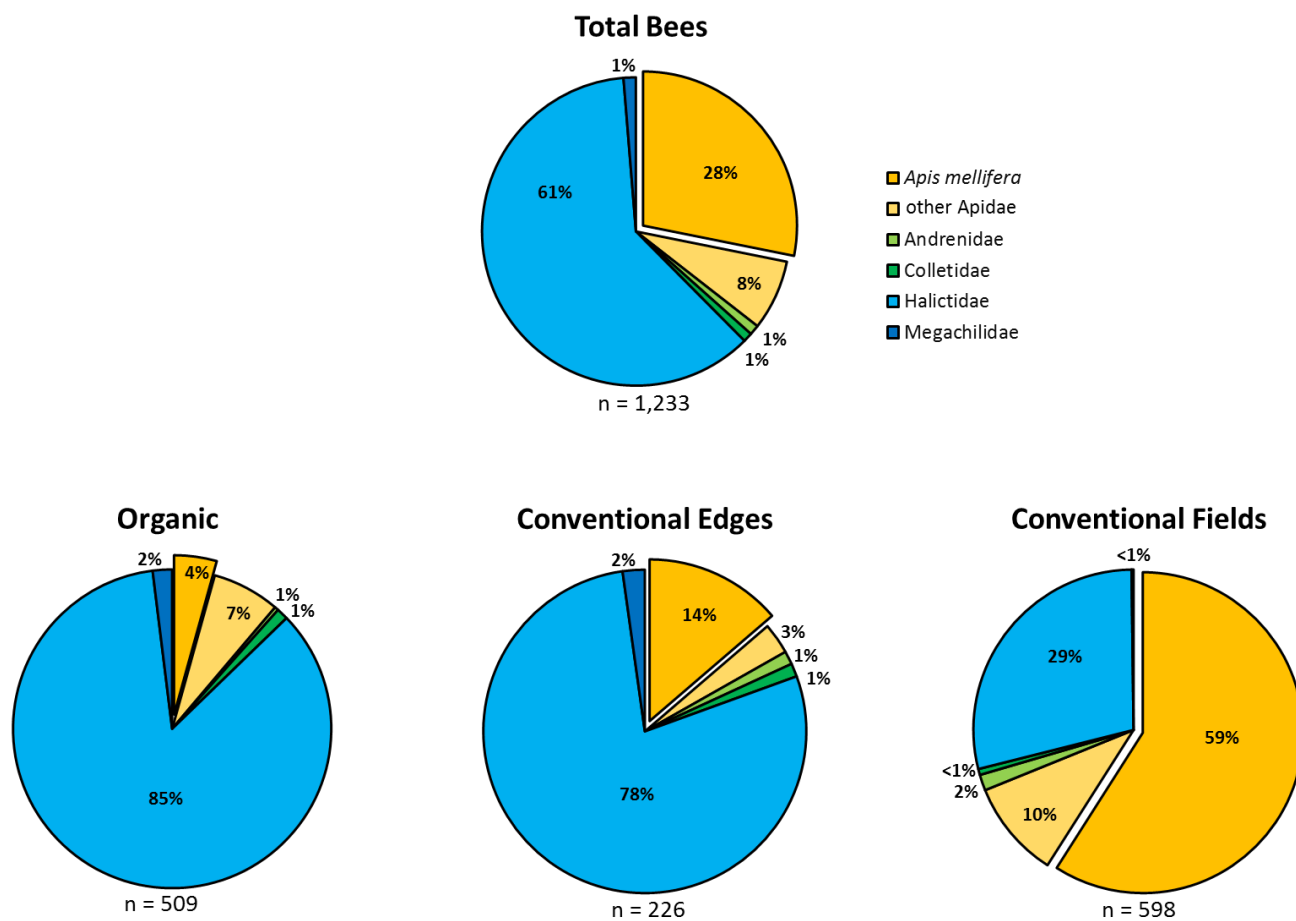
Figure 4. Mean size of conventional processing vegetable fields with bee diversity H-values (indicating the presence of more than one species of bee captured) and without bee diversity (one or zero bee species captured).
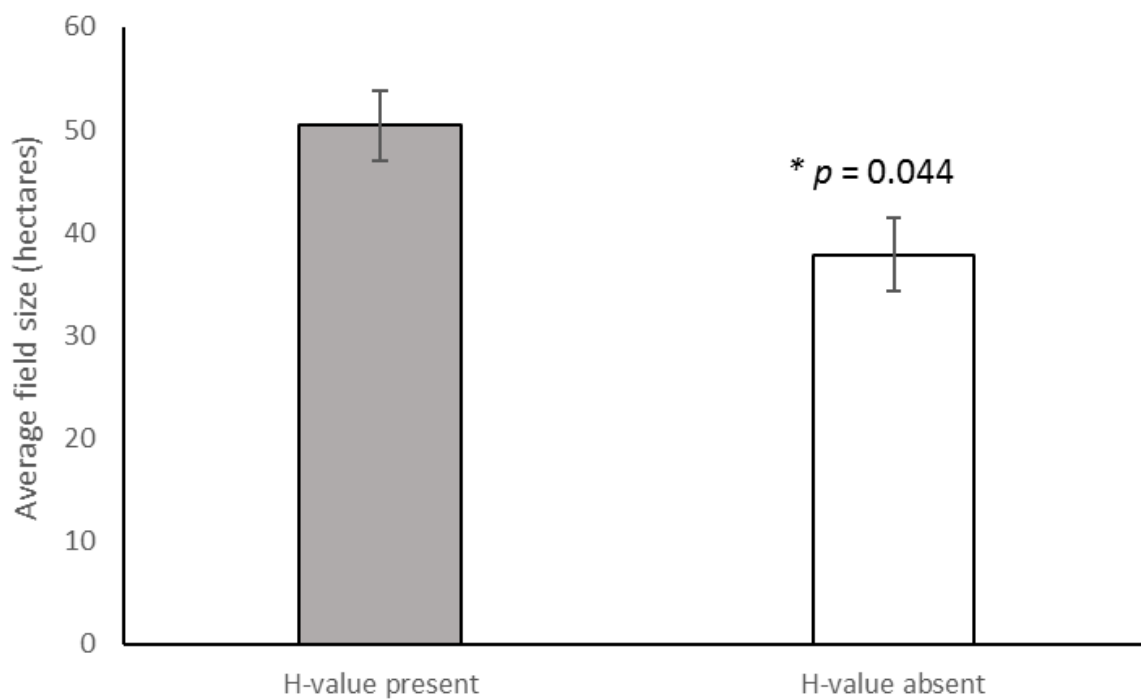
Figure 5. Log-transformed abundance of all bees within snap bean fields (Y = 115.855 + 6.523X), pea fields (Y = 73.564 – 1.384X), and sweet corn fields (Y = 108.74 – 5.77X) as a function of time from April first. Fields were located within Wisconsin's Central Sands growing region in 2014 and 2015.
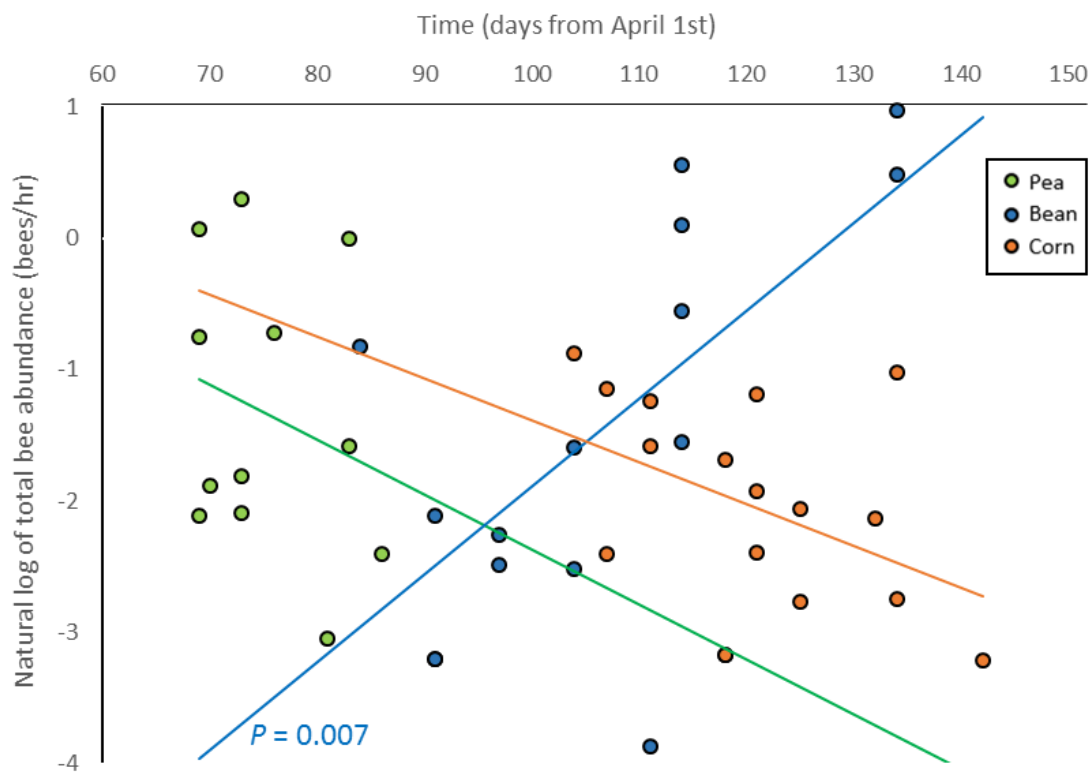
Figure 6. Log-transformed abundance of wild bee species (including *Apis mellifera*) in processing vegetable fields as a function of A) the percentage of natural land within 2,000 meters ($Y = 0.533 + 0.092X$), and B) the proportion of agricultural land within 2,000 meters ($Y = -0.867 - 2.139X$). Crops were grown within Wisconsin's Central Sands growing region in 2014 and 2015.
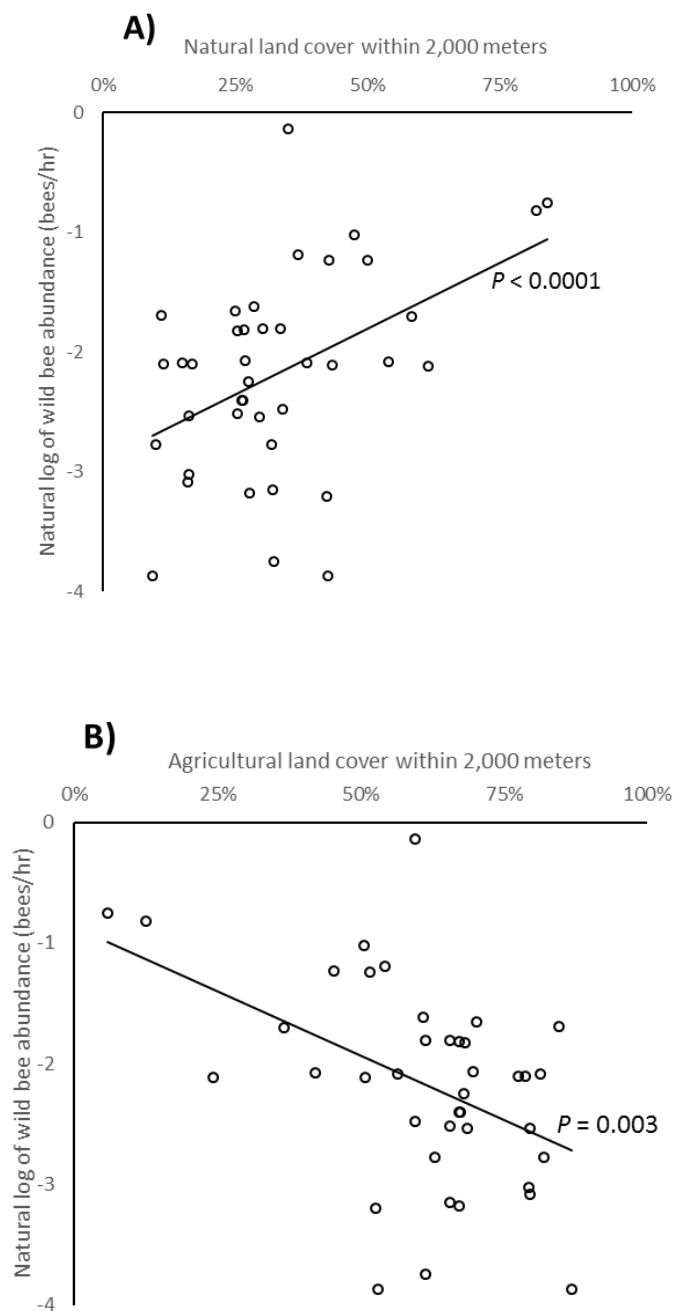
Figure 7. The bee diversity of conventionally grown fields of sweet corn, snap beans, and peas as a function of the proportion of natural land cover at A) 500 m, B) 1,000 m, and C) 2,000 m, and as a function of the percentage of surrounding agricultural land cover at D) 1,000 m and E) 2,000 m. All crops were grown within Wisconsin's Central Sands region in 2014 and 2015.
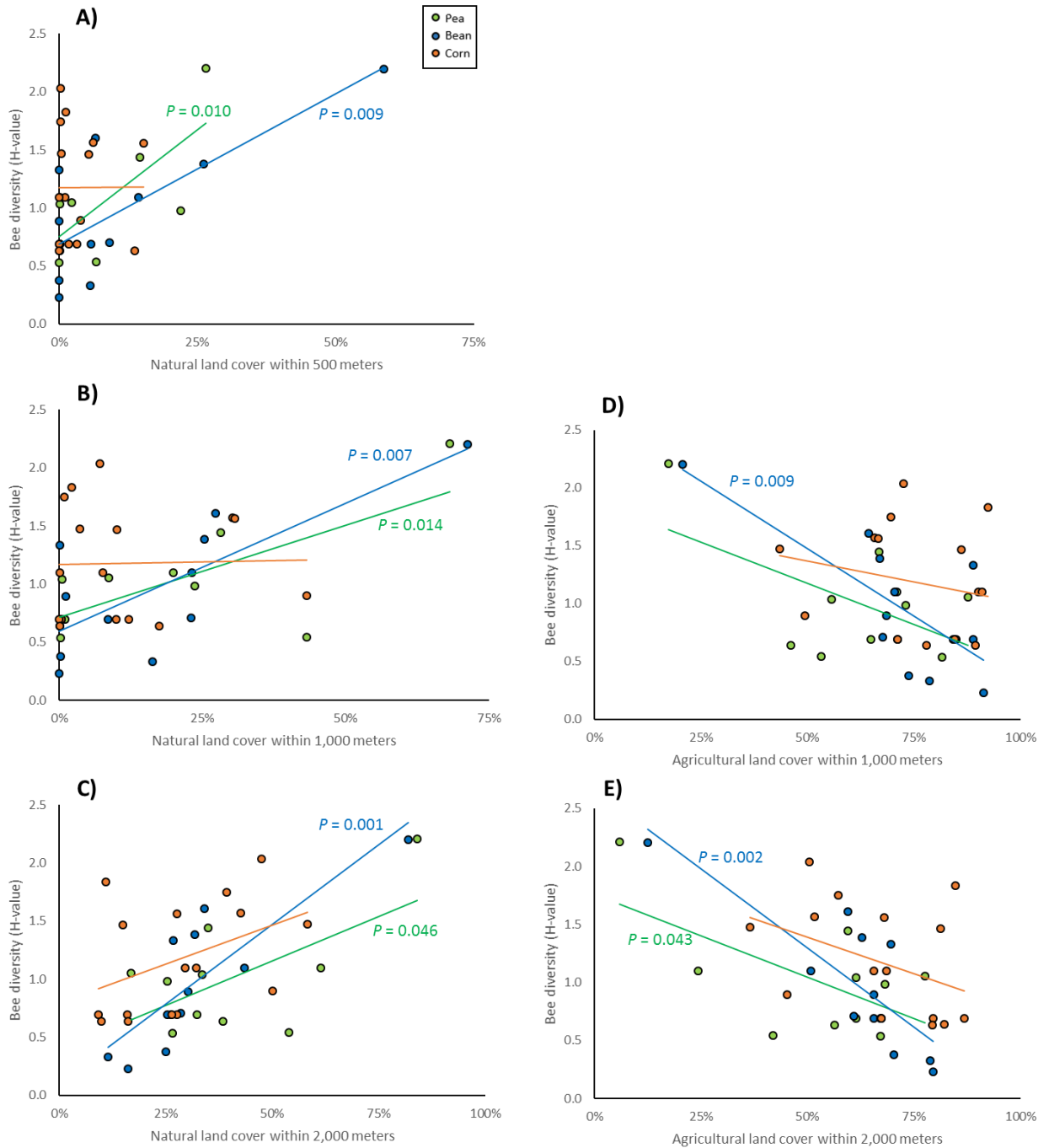
Figure 8. Comparison of the mean total abundance (A), species richness (B), wild abundance (C), and diversity (D) ± SE of bees in paired conventionally grown vegetable fields and vegetable field margins (n = 13) located in Wisconsin's Central Sands region. Untransformed abundance and species richness data are represented here, but *p*-values are taken from comparisons of natural-log transformed values.
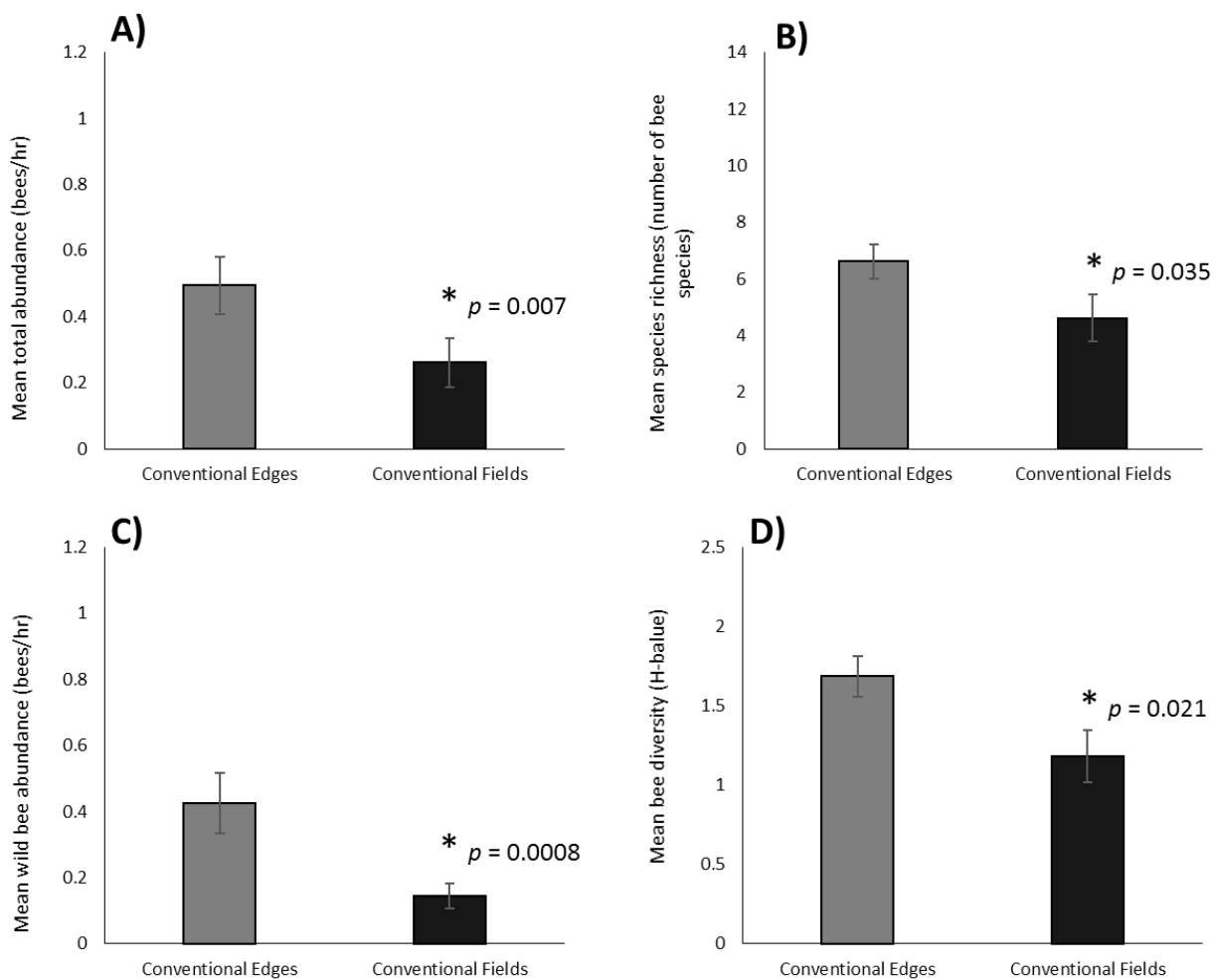
Figure 9. Comparisons of the mean diversity, species richness, and abundance of bees ± SE in A) small- versus large-scale organic field margins and B) combined small- and large-scale organic field margins versus conventional field margins located in Wisconsin's Central Sands region. Untransformed abundance data are represented here, but *p*-values are taken from comparisons of natural-log transformed values.
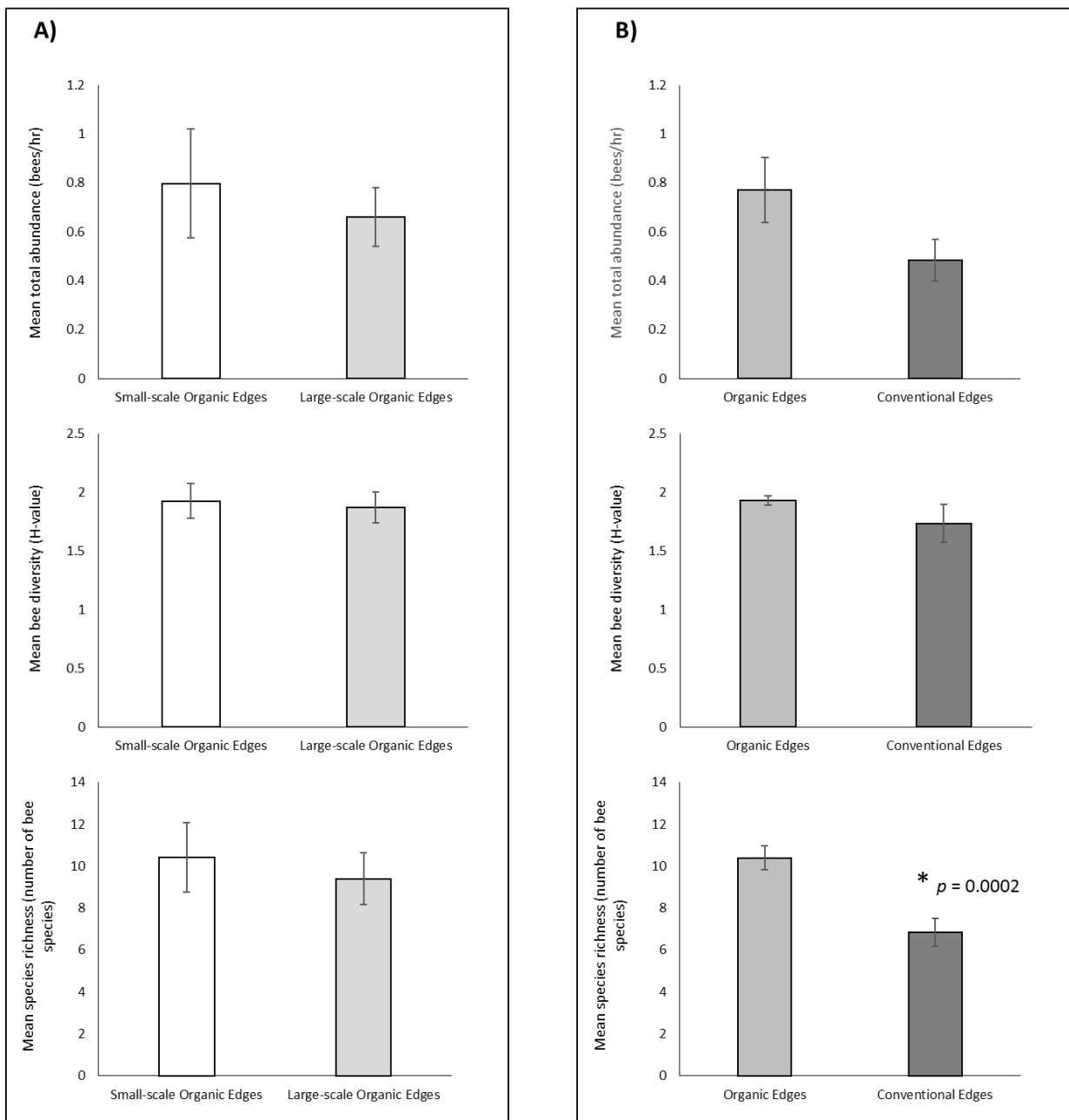
Table 1. The total number of honey bees (*Apis mellifera*) and other bees observed visiting flowers in different conventional vegetable fields (bean n = 9, corn n = 9) and conventional vegetable field edges (bean n = 6, corn n = 5), as well as within a small-scale (n = 10) and a large-scale (n = 10) organic farm's field margins over time in 2015. Conventional pea data are absent. All sites were located within the Central Sands vegetable growing region of Wisconsin.

	2015 Conventional Fields (n = 18)			2015 Conventional Edges (n = 11)			2015 Organic Edges (n = 20)	
	Pea	Bean	Corn	Pea	Bean	Corn	Small-scale	Large-scale
Apis mellifera			1		3	29	6	4
Other bee species			1		12	17	25	13
TOTAL	N/A	0	2	N/A	15	46	31	17

Table 2. Model statistics from a fitted multiple linear regression between variables describing A) diversity, B) species richness, and C) wild bee abundance at different buffer distances from bee sampling sites in central Wisconsin vegetable fields. Values in bold indicate significant models ($R^2_{adj} > 0.15$ and $p \leq 0.05$).

A)

Land use type	Buffer size	$F_{1,36}$	P	R^2_{adj}
Diversity				
Natural	250 m	3.22	0.081	0.057
	500 m	**10.63**	**0.002**	**0.207**
	1,000 m	**10.22**	**0.003**	**0.200**
	2,000 m	**15.93**	**0.0003**	**0.288**
Agricultural	250 m	3.98	0.054	0.075
	500 m	0.41	0.525	-0.016
	1,000 m	**8.04**	**0.008**	**0.160**
	2,000 m	**13.12**	**0.0009**	**0.247**
Developed	250 m	3.00	0.092	0.051
	500 m	0.01	0.906	-0.027
	1,000 m	1.75	0.195	0.020
	2,000 m	0.54	0.468	-0.013

B)

Land use type	Buffer size	$F_{1,45}$	P	R^2_{adj}
Species Richness*				
Natural	250 m	< 0.01	0.975	-0.022
	500 m	2.99	0.091	0.041
	1,000 m	5.08	0.029	0.081
	2,000 m	3.02	0.089	0.042
Agricultural	250 m	0.12	0.729	-0.019
	500 m	0.78	0.383	-0.005
	1,000 m	1.48	0.231	0.010
	2,000 m	2.59	0.12	0.033
Developed	250 m	3.26	0.078	0.047
	500 m	0.14	0.715	-0.019
	1,000 m	1.32	0.257	0.007
	2,000 m	0.05	0.818	-0.021

C)

Land use type	Buffer size	$F_{1,38}$	P	R^2_{adj}
Wild Bee Abundance*				
Natural	250 m	0.07	0.790	-0.024
	500 m	1.07	0.307	0.002
	1,000 m	3.37	0.074	0.057
	2,000 m	**9.59**	**0.004**	**0.180**
Agricultural	250 m	0.01	0.910	-0.026
	500 m	1.77	0.191	0.019
	1,000 m	7.52	0.009	0.143
	2,000 m	**10.02**	**0.003**	**0.188**
Developed	250 m	0.54	0.469	-0.012
	500 m	0.88	0.355	-0.003
	1,000 m	1.11	0.298	0.003
	2,000 m	1.39	0.246	0.010

* Transformed to natural log values for analysis

Table 3. Model statistics from a fitted multiple linear regression between variables describing the bee diversity in pea (n = 11), bean (n = 11), and sweet corn (n = 16) fields grown in central Wisconsin. Rows in bold indicate significant models ($R^2_{adj} > 0.15$ and $p \leq 0.05$).

Diversity in Pea Fields

Land use type	Buffer size	Mean	SE	$F_{1,9}$	P	R^2_{adj}
Natural	250 m	0.198	0.08	1.74	0.22	0.069
	500 m*	**0.066**	**0.03**	**10.61**	**0.010**	**0.490**
	1,000 m*	**0.176**	**0.07**	**9.39**	**0.014**	**0.456**
	2,000 m*	**0.394**	**0.06**	**5.34**	**0.046**	**0.303**
Agricultural	250 m	0.743	0.08	1.79	0.21	0.073
	500 m	0.743	0.05	< 0.01	0.98	-0.111
	1,000 m	0.625	0.06	4.07	0.074	0.235
	2,000 m*	**0.537**	**0.07**	**5.57**	**0.043**	**0.314**

Diversity in Bean Fields

Land use type	Buffer size	Mean	SE	$F_{1,9}$	P	R^2_{adj}
Natural	250 m	0.124	0.08	0.19	0.67	-0.088
	500 m*	**0.115**	**0.06**	**11.15**	**0.009**	**0.504**
	1,000 m*	**0.179**	**0.07**	**12.01**	**0.007**	**0.524**
	2,000 m*	**0.323**	**0.06**	**20.57**	**0.001**	**0.662**
Agricultural	250 m	0.853	0.08	0.29	0.61	-0.077
	500 m	0.775	0.07	2.43	0.15	0.126
	1,000 m*	**0.709**	**0.06**	**10.82**	**0.009**	**0.495**
	2,000 m*	**0.614**	**0.06**	**17.80**	**0.002**	**0.627**

Diversity in Corn Fields

Land use type	Buffer size	Mean	SE	$F_{1,14}$	P	R2adj
Natural	250 m	0.036	0.02	1.91	0.19	0.057
	500 m	0.033	0.02	< 0.01	0.992	-0.071
	1,000 m	0.110	0.04	0.01	0.921	-0.071
	2,000 m	0.394	0.06	3.09	0.100	0.123
Agricultural	250 m	0.950	0.030	2.49	0.14	0.090
	500 m	0.882	0.03	0.11	0.75	-0.063
	1,000 m	0.761	0.05	0.71	0.413	-0.020
	2,000 m	0.669	0.05	2.52	0.135	0.092

Table 4. Model statistics from a paired analysis of A) conventional vegetable field edges vs. conventional fields, B) cumulative organic field edges vs. conventional field edges, and C) large-scale organic field edges vs. small-scale organic field edges. All sites were located in central Wisconsin. Columns in bold indicate significant models.

A)	Conventional fields vs. Conventional Edges			
	Diversity	**Richness***	**Wild Abundance***	**Total Abundance***
Conventional Edge Mean ± SE	**1.69 ± 0.47**	**6.62 ± 0.62**	**0.43 ± 0.08**	**0.49 ± 0.09**
Conventional Field Mean ± SE	**1.18 ± 0.162**	**4.62 ± 0.82**	**0.14 ± 0.03**	**0.26 ± 0.07**
t_{12}	**2.67**	**2.37**	**4.41**	**3.23**
p	**0.021**	**0.035**	**0.0008**	**0.007**

B)	Small-scale vs. Large-scale Organic Edges		
	Diversity	Richness	Total Abundance*
Small-scale Edge Mean ± SE	1.93 ± 0.15	10.40 ± 1.65	0.80 ± 0.22
Large-scale Edge Mean ± SE	1.87 ± 0.13	9.40 ± 1.25	0.66 ± 0.12
t_9	0.214	0.389	0.109
p	0.835	0.835	0.916

C)	Organic Edges vs. Conventional Edges		
	Diversity	**Richness**	Total Abundance*
Conventional Edge Mean ± SE	1.74 ± 0.16	**6.83 ± 0.67**	0.48 ± 0.09
Organic Edge Mean ± SE	1.93 ± 0.04	**10.39 ± 0.56**	0.77 ± 0.13
t_8	1.24	**4.44**	1.66
p	0.252	**0.0002**	0.135

*transformed to natural log values for analysis

Table 5. Cumulative number of bees collected from conventional vegetable fields, conventional field edges, and small- and large-scale organic farms within the Central Sands vegetable growing region of Wisconsin. Sampling in organic field margins was replicated across time.

Species	Conventional Pea (n = 16)	Conventional Bean (n = 18)	Conventional Corn (n = 19)	Conventional Edges (n = 13)	Small Organic (n = 10)	Large Organic (n = 10)	Total (n = 86)
ANDRENIDAE							
Andrena alleghaniensis						1	1
Andrena ceanothi		1		2			3
Andrena crataegi						1	1
Andrena cressonii	1						1
Andrena miranda	1						1
Andrena perplexa	1						1
Andrena vicina	2						2
Andrena wilkella	2			1			3
APIDAE							
Apis mellifera	90	186	18	31	10	12	347
Bombus bimaculatus						2	2
Bombus borealis	1			1	1	1	4
Bombus fervidus		2	2		3	9	16
Bombus griseocollis	1						1
Bombus impatiens	1	3			6	1	11
Bombus perplexus		1				2	3
Bombus rufocinctus					1		1
Bombus ternarius		2		1	1		4
Bombus vagans			2	2	1		5
Eucera hamata	11			1			12
Melissodes agilis		1		1		1	3
Melissodes bimaculata		1	7		1	1	10
Melissodes dentiventrus			1				1
Melissodes druriella			1		1	3	5
Melissodes illata		1					1
Melissodes nivea		1	6	1			8
Melissodes trinodis		1	1				2
Nomada (spp.)	1						1
Xenoglossa kansensis		1					1
COLLETIDAE							
Hylaeus affinis				2			2
Hylaeus annulatus			2	1	2	2	7

Species	Conventional Pea (n = 16)	Conventional Bean (n = 18)	Conventional Corn (n = 19)	Conventional Edges (n = 13)	Small Organic (n = 10)	Large Organic (n = 10)	Total (n = 86)
Hylaeus rudbeckiae			1			1	2
Perdita halictoides						1	1
HALICTIDAE							
Agapostemon sericeus						1	1
Agapostemon splendens		3		1			4
Agapostemon texanus	6		1	1	5	3	16
Agapostemon virescens	7	2			18	6	33
Augochlora pura			1				1
Augochlorella aurata	1		1	11	6	20	39
Augochloropsis metallica		1					1
Halictus confusus	1		3	13	19	7	43
Halictus ligatus				1		2	3
Halictus rubicundus				1			1
Lasioglossum acuminatum	2	6			1		9
Lasioglossum admirandum			7	16	7	15	45
Lasioglossum albipenne		1	1	1	5	2	10
Lasioglossum cinctipes		2			2		4
Lasioglossum coriaceum	12	2		7	4	2	27
Lasioglossum cressonii		1		13	3		17
Lasioglossum floridanum						1	1
Lasioglossum hitchensi			1		1		2
Lasioglossum laevissimum		1					1
Lasioglossum leucocomum	3	3	5	37	14	35	97
Lasioglossum leucozonium	4	3	14	6	16	9	52
Lasioglossum lineatulum		11	1	3	7	2	24
Lasioglossum lustrans				1	1		2
Lasioglossum oceanicum			7	3	9	4	23
Lasioglossum paradmirandum					1	1	2
Lasioglossm paraforbesii		2	1	5		4	12
Lasioglossum pectorale				4	27	1	32
Lasioglossum perpunctatum	1	3	1	6		8	19
Lasioglossum pictum				1	7	5	13
Lasioglossum pilosum	1	2	13	30	34	33	113
Lasioglossum planatum						1	1
Lasioglossum pruinosum			1			1	2
*Lasioglossum semicaeruleum**						1	1
Lasioglossum smilacinae					1		1

Species	Conventional Pea (n = 16)	Conventional Bean (n = 18)	Conventional Corn (n = 19)	Conventional Edges (n = 13)	Small Organic (n = 10)	Large Organic (n = 10)	Total (n = 86)
Lasioglossum subviridatum						2	2
Lasioglossum swenki				1			1
Lasioglossum tegulare					1		1
Lasioglossum timothyi				3			3
Lasioglossum versatum		2			64	4	70
Lasioglossum vierecki				10	3	3	16
Lasioglossum viridatum			1				1
Lasioglossum zephyrum			1				1
Sphecodes davisii				1	3		4
Sphecodes dichrous					1		1
Sphecodes mandibularis				1	1		2
MEGACHILIDAE							
Hoplitis producta	1						1
Megachile addenda						1	1
Megachile campanulae						1	1
Megachile latimanus						5	5
Megachile pugnata						2	2
Megachile relativa				1			1
Megachile rotundata				1			1
Osmia distincta					1		1
Osmia pumila				1			1
Osmia simillima				2			2
Total Individuals Captured	151	246	101	226	289	220	1233

*Indicates new regional record

Chapter 3: Seasonal Variation of Thiamethoxam Concentrations

in Central Wisconsin Processing Vegetables

Abstract

Previous research suggests that pollinator decline is being caused by interacting factors, including agrochemical exposure. Although bees forage in processing vegetable fields where they may be exposed to insecticides such as neonicotinoids, many of these crops are not pollinator-dependent and have thus been overlooked by previous pollinator research. We used ultra-high pressure liquid chromatography (UHPLC) to assess seasonal changes in concentration of the neonicotinoid thiamethoxam within flower and leaf tissues of three processing vegetable crops grown with a thiamethoxam seed coating. Floral concentrations of thiamethoxam, which were collected 5-7 weeks post-emergence, were negligible across all crops in all years. Thiamethoxam was present in the leaf tissues of all crops. Concentrations were highest in the first week following emergence (as high as 18.4 ppm) before dropping to near zero by the third week. These concentrations do not convey an idea of doses that bees might be exposed to, but they do indicate that the period of time one to two weeks post-planting is when these crops are most likely to pose a risk to bees coming into direct contact with plant structures. Finally, significant thiamethoxam concentrations were observed in untreated bean leaf tissue collected in the first week following emergence, which suggests that thiamethoxam contamination could occur in untreated plants that share proximity or planting equipment with treated crop seeds.

Introduction

Neonicotinoid use and insect risk.

Conventionally grown food crops regularly employ insecticide to control pest insects. In the 1990's, agrochemicals that can have high human toxicity and/or environmental persistency –such as certain carbamates, organophosphates, and chlorinated hydrocarbons - began to be replaced with newer, safer insecticides (Jeschke & Nauen 2008, Grafton-Cardwell et al 2005). Recognition of both the importance of pesticides to crop production and the need for new chemistry that reduces risks to humans and the environment led to the Conventional Reduced-Risk (RR) Pesticide Program, which was created within the Environmental Protection Agency to expedite the review and registration of pesticides classified as RR or organophosphate-alternative; i.e., chemicals that pose a lower risk to humans and the environment compared to older, more hazardous pesticides like carbamate, organophosphate, and cyclodiene compounds. The recent registration of several new RR insecticides on processing vegetables and potatoes, many of which move systemically within the plant when taken up by roots, had the potential to mitigate adverse effects to human health, the environment, and non-target organisms that were associated with current insect management practices (EPA 2016). One of these new insecticide classes, the neonicotinoids, has since become a popular choice for early season pest control in many crops (Elbert et al 2008) in part because these compounds are much less toxic to mammals like humans (Tomizawa & Casida 2005, Jeschke & Nauen 2008). Since their introduction the neonicotinoids have grown in popularity to comprise a third of the global insecticide market (Simon-Delso et al 2015), making them the most widely used insecticide class in the world (van Lexmond et al 2015).

Each year millions of pounds of neonicotinoids are applied to the United States (USGS 2016). Three compounds comprise the majority of agricultural neonicotinoid use: thiamethoxam, clothianidin, and imidacloprid (Elbert et al 2008). These can be sprayed directly onto leaves, placed into seed furrows in soil, or applied as a seed coating prior to planting, which is the most popular method for agricultural production (Hopwood et al 2013). Regardless of application method, once a plant is treated the neonicotinoid becomes systemic, moving acropetally through the xylem or phloem tissues of roots, shoots, and flower structures to provide protection against tissue- and sap-feeding insects like aphids, thrips, and leaf hoppers (Bonmatin et al 2015, Goulson 2013) while simultaneously providing protection against the viruses these species can transmit (Simon-Delso et al 2015). Neonicotinoids are not designed to control a specific insect target, which has made them effective against many different crop pests. Unfortunately, this lack of target specificity has also made neonicotinoids a danger to non-pest insects in proximity to their use (Klein et al 2007, Larson et al 2014, Hopwood et al 2013).

Globally, many insect species are in the midst of a decline (van Lexmond et al 2015, Potts et al 2010). Declining bee populations are of concern because these animals provide vital pollination that makes possible 35% of the world's food crops (Klein et al 2007), and because they contribute to the continued function of many and ecosystems across the globe (National Academic Press 2007, Moisset & Buchmann 2011). The decline of wild and domestic bees has been attributed to many interacting factors, including habitat loss (Klein et al 2007, Kennedy et al 2013, Kremen et al 2007, Ricketts et al 2008, Winfree et al 2009) and pathogen spread (James 2008, Gensersch 2010, Graystock et al 2014, Otterstatter & Thomson 2008). Evidence also points to the increasingly widespread use of neonicotinoid pesticides as

a contributor to bee decline (Larson et al 2014, Brittain et al 2010, Hopwood et al 2013, Krupke et al 2012, Fischer et al 2014, Samson-Robert et al 2014).

Bees' susceptibility to neonicotinoids depends on the bee species, the compound, the dose, and the route of exposure. For honey bees a median lethal dose (LD_{50}) ranges between 3.7 ng/bee and 24-81 ng/bee for oral and contact exposure, respectively (Godfray et al 2014), though Iwasa et al (2004) reported that contact doses of clothianidin, thiamethoxam, and clothianidin at much lower levels of 0.02-0.03 µg/bee were enough to cause mortality. Bees that are not killed from neonicotinoid exposure can still exhibit sub-lethal effects including inhibited larval growth, disrupted foraging and navigation, and reduced queen production (Hopwood et al 2013).

While it's true that the widespread use of neonicotinoids has the potential to harm bees, the extent of this risk –especially towards less studied bee species- can be difficult to pinpoint. Thus, research dealing with pollinator/neonicotinoid interactions has turned its focus from whether high doses of neonicotinoids can kill or harm bees, to whether these harmful levels exist in the natural landscape where bees are found and how bees might be exposed.

Bees living in proximity to agricultural areas where neonicotinoids are used could be exposed in a number of ways. Dust clouds containing concentrated amounts of active ingredient form when coated seeds are planted and may drift from the planted field into surrounding uncultivated areas (Krupke et al 2012). Bees may also come into contact with neonicotinoids by drinking contaminated surface water found in farm fields such as irrigation puddles, where concentrations have been found at sublethal levels (Samson-Robert et al 2014), or by drinking a treated crop plant's guttation fluid (Girolami et al 2009). Guttation is

an excretion of xylem from a plant's leaf tips that often appears in the early morning during humid conditions. If a foraging bee were to consume a treated crop plant's guttation fluid, especially a young plant, the dose would be as much as 100,000 times higher than that found in nectar (Godfray et al 2014), which is a concentration well within the range that can harm bees (Hopwood et al 2013, Godfray et al 2014, Iwasa et al 2004).

Perhaps the most obvious route of potential exposure for bees is via contact with neonicotinoid contaminated flowers, pollen and nectar –those plant structures with which bees most often interact. Although product labelling attempts to limit the application of neonicotinoids during a crop's flowering period, detectable neonicotinoid concentrations may remain in the nectar and pollen of flowering crops planted with a neonicotinoid in-furrow or seed coating application (Hopwood et al 2013, Mogren & Lundgren 2016, Sanchez-Hernandez et al 2015, Pohorecka et al 2013).

Unique risks of central Wisconsin

Commodity crop rotations of soy and maize, which do not require insect pollination, are predominant across Wisconsin (Haines et al 2010), as is the case in much of the Midwest. Yet Wisconsin is also a state with a high reliance upon bees for agricultural pollination. The state ranks second in the nation in major processing vegetable production (USDA NASS 2015) and sells about $400 million worth of vegetables each year (USDA NASS 2015). Many of Wisconsin's specialty crops -such as squash, cucumbers, melons, apples, and cranberries- require insect pollination to produce viable yields (USDA 2015). Even some specialty crops that are self-fertile or wind pollinated can experience a yield boost when visited by bees (Bartomeus et al 2014, Woodcock 2012).

Many of Wisconsin's specialty crops are grown in central Wisconsin (Haines et al 2010), within an ecological region known as the Central Sands. The Central Sands has been a historically difficult place for agriculture due to its sandy soil and undesirable drainage (WI DNR 2014). But with the onset of center-pivot irrigation systems that draw from the region's easily accessible ground water, valuable vegetable crops have prospered in the area during the last fifty years. Each of the Sands' seven counties now has a quarter or more of its land in agricultural production (WI DNR 2014). In the three most intensively irrigated counties (Portage, Adams, and Waushara Counties) production is primarily dedicated to the production of specialty crops, and more specifically processing vegetables (Haines et al 2010).

Due to the concentration of irrigated specialty agriculture in central Wisconsin, there exist specific risks to pollinator communities here that are different from elsewhere in the state. Vegetable crops that do not require insect pollination, such as sweet corn (*Zea mays*), snap bean (*Phaseolus vulgaris*), and field pea (*Pisum sativum*) share space (and rotation) with heavily pollinator-dependent crops each year, resulting in a mosaic of diverse crops and management regimes across the landscape. Many of these vegetables rely on seed coatings of neonicotinoids, including thiamethoxam, imidacloprid, and clothianidin (Colquhoun et al 2016). The use of neonicotinoids is prevalent such that trace residues of thiamethoxam have consistently been found in central Wisconsin's ground water since 2008 (Huseth & Groves 2014). In-field concentrations of neonicotinoids in non-pollinator dependent processing crops have not been evaluated, though the EPA has set tolerance levels for vegetable tissues of field peas and sweet corn at 0.02 ppm (USDA 2016). Furthermore, the bee community of the Central Sands region is the least-studied in the state (Wolf & Ascher 2008). Previous

pollinator research in the area has largely focused on pollinator dependent crops like

cranberries (Gaines Day 2013) and cucurbits (Lowenstein et al 2012). A need exists for

research to examine a broader diversity of crops that receive visits from bees, and to combine

these investigations with field studies that inform more realistic neonicotinoid concentrations

present during the growing season (Lundin et al 2015).

Research rationale

This study examined three thiamethoxam-treated specialty crops grown within the

Central Sands that do not exhibit dependence on insect pollination. The study's goal was to

examine the concentration of thiamethoxam within leaf and floral tissues from plants

receiving seed treatments during the course of a given growing season to determine whether

detectable thiamethoxam concentrations were present in these tissues and how long they

would remain present. Sweet corn, snap beans, and field peas were chosen for analysis due to

their prevalence within the region. These vegetables all commonly receive a seed coating of

thiamethoxam (Cruiser® 5FS) prior to planting, a pesticide in which the active ingredient is

formulated as a 47.6% concentrate per unit (Syngenta 2015).

Materials and Methods

Tissue collection

All the crop plants in this study were grown at the Del Monte Foods, Incorporated

experimental agricultural fields in Plover, Wisconsin (44.453567°N, 89.489896°W) during

the 2014 and 2015 growing seasons. Sweet corn, field peas, and snap beans were planted in a

randomized complete block design, with the insecticide main effect including thiamethoxam-

treated and untreated plants. Four replicate plots each of treated and untreated seeds from the three crops were planted, for a total of 24 plots. In 2014 the seeding rate was 100 peas per plot, 60 beans per plot, and 25 corn kernels per plot. In 2015 the rate was the same but the plots were bigger, resulting in 165 peas per plot, 100 beans per plot, and 40 corn kernels per plot.

In 2014, each plot measured 4.57 m by 0.91 m with 0.91 m row spacing for a total field size of 60.96 m by 2.74 m. In 2015 the plots were larger, each measuring 7.62 m long with 0.91 m row spacing for a total field size of 60.96 m by 6.40 m. In both 2014 and 2015, the seeds were planted in late May/early June and left to grow until either mid-August or natural senescence. According to the crops' natural phenology, peas were the first to fully flower (36-39 days after planting), then snap beans (42-45 days after planting), and finally sweet corn (59-64 days after planting).

All treated seed was planted with a coating of thiamethoxam, Cruiser® 5FS, at a labeled rate consistent with industry standard application. For both legumes (peas and snap beans), the thiamethoxam application was applied at 16.84 g AI/acre. For sweet corn, the application rate was 30.24 g AI/acre. In addition to thiamethoxam, both treated and untreated legumes received co-application of seed coatings of fungicide as per industry standard. Snap beans received Apron XL® (mefenoxam 33.3% AI) and Maxim® (fludioxonil 40% AI), while peas received only Apron XL® at an equivalent concentration.

To obtain tissue samples the plants were visited weekly once the first set of true leaves were large enough to be sampled without complete defoliation. In 2014, all crops were sampled for the first time eleven days after planting. In 2015, corn and peas were first sampled fourteen days after planting, while beans, which tend to emerge more quickly, were

first sampled seven days after planting. Each leaf sample was an amalgam of leaf tissue

collected from five randomly selected plants within a plot. In addition, when each crop was

at or near full flower, floral structures were collected following the same procedure used for

leaf tissue. Immediately following each weekly tissue collection, samples were weighed and

frozen at -80°C until analysis could begin.

Thiamethoxam quantification

      Thiamethoxam concentrations present within tissue samples were analyzed from

March through September of 2016 using ultra high pressure liquid chromatography

(UHPLC). We used a Waters I-Class UPLC® system with detection by positive ionization

single quadrupole mass spectrometry (ESI (+)MS; Waters Corporation, Milford, MA, USA).

To prepare plant tissue for UHPLC analysis, a preparation procedure was adapted from an

existing procedure for quantifying thiamethoxam in cereals (Perez et al 2009).

      For each leaf sample, approximately 200 mg of wet-weight leaf tissue was initially

combined with 700 µL of HPLC-grade acetonitrile extraction reagent. Floral samples were

also analyzed using this tissue: solvent ratio. The legumes' floral tissues included the petals,

keel, ovary, stigma, and stamens, but care was taken not to include vascular tissues below the

floral pedicel. The corn floral tissue consisted of pollen-laden anthers and spikelets and again

did not include vascular tissue from the mother plant.

      Samples were macerated until finely ground in a FastPrep® 120 machine using 2 mL

lysing matrix tubes, which were filled with a matrix of garnet granules and a porcelain bead

(MP Biomedicals, Santa Ana CA). After maceration, the tubes were centrifuged in an

Eppendorf Centrifuge 5417R at 10,000 rpm for five minutes. The supernatant of each

centrifuged sample was syringe-filtered through a PTFE membrane of pore size 0.22 µm, then the remaining pellet was re-macerated and re-centrifuged using an additional 700 µL of acetonitrile for a resultant final ratio of 200 mg wet weight tissue: 1,400 µL acetonitrile solvent. This solution was then evaporated until the total volume of each sample was at or below 100 mL. Final volume measurements were taken with a serological pipette, and samples were stored at -20°C until same-day UHPLC analysis. During analysis, the autosampler was run at a temperature of 10°C, and the injection volume was 4.0 µL. The column temperature was 30°C, with a flow rate of 0.5 mL/min.

Plant extracts were diluted by 50% with water, mixed with internal standard (5 mg/L 6-benzylaminopurine or 1 mg/L caffeine), held at 10°C, injected (4.0 µL) onto a Waters C_{18} column (CSH 1.7µm, 2.1 x 100-mm) equipped with a 5-mm guard column, and separated with a gradient of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The absence of thiamethoxam carryover between injections was assured by including a needle wash with 9:1 water:acetonitrile between injections, and by analyzing solvent "blanks" after running samples with high analyte concentrations.

A series of standard stock solutions were prepared using technical-grade thiamethoxam. These were run alongside each set of samples to serve as a calibration curve. Additionally, three samples of untreated leaf tissue from plants grown under greenhouse conditions were spiked with different known amounts of thiamethoxam and used to calculate a percent recovery estimate. Snap bean tissue spikes were run alongside the experimental legume tissue samples, while sweet corn tissue spikes were run alongside the experimental corn tissue samples. To obtain a final percent recovery for the legume and corn crops, the percent recoveries of all tissue spikes reported within the experimental quantification range

were averaged and used to estimate the percent recovery of like crop tissues. These final thiamethoxam values were converted from units of ng/μg to parts per billion (ppb). The instrumental detection limit of thiamethoxam was 0.008 mg/L, based on a signal to noise ratio of 1:3. The lower quantification limit was 0.01 mg/L, based on a signal to noise ratio of 1:10. The corresponding method detection and lower quantification limits were respectively 30 ppb and 50 ppb for wet plant material.

Statistical analysis

Comparisons of crop and phenology were analyzed as a repeated measures analysis of variance (ANOVA) with a linear model using R software, version 3.3.0 (R Core Team 2016). Due to an interactive effect of year, data from 2014 and 2015 were analyzed separately. Crop type, treatment, and time were analyzed as fixed variables in the models. For each sample date, the mean concentration plus or minus standard error in parts per billion was plotted until thiamethoxam concentrations diminished past the detection limits of our procedure and registered at or near zero. Pairwise comparisons of treatment least squares means were performed using SAS 9.4 (SAS Institute Inc., Cary NC). Resultant probability values were adjusted using the Tukey-Kramer post-hoc test.

Results

Thiamethoxam in floral tissue

None of the floral tissues analyzed registered concentrations of thiamethoxam above this study's detection limit of 30 ppb (**Table 1**). In both years, the concentrations that were

recorded below the detection limit ranged from seven to 27 ppb, and did not differ

significantly among treatments or crop types.

Thiamethoxam in leaf tissue

In all crops measured from 2014, leaf tissue samples were analyzed across four time

points spanning the second through fifth weeks following emergence. Thiamethoxam

concentrations decreased sharply over the course of four weeks of growth (**Fig. 1**). There

were significant effects of crop ($F = 5.17$, $p = 0.008$), time ($F = 55.43$, $p < 0.0001$), and

treatment ($F = 119.52$, $p < 0.0001$) on thiamethoxam concentrations in 2014 leaf tissue.

Taken together, untreated leaf tissue in 2014 bore significantly lower mean

thiamethoxam concentrations (54 ppb ± 12) than treated (1,838 ppb ± 351; $t = 10.86$, $p <$

0.0001), and untreated concentrations did not statistically differ from zero. Treated leaves in

2014 had significantly higher mean thiamethoxam levels than untreated leaves for the first

two collection weeks ($t = 16.06$, $p = <0.0001$ in week 1; $t = 3.91$, $p = 0.0002$ in week 2). By

the third collection week treated and untreated tissues no longer differed significantly from

each other, though treated concentrations were still greater than zero in the third week ($t =$

2.78, $p = 0.008$).

There was an interactive effect of time x treatment ($F = 52.06$, $p < 0.0001$) and crop x

treatment ($F = 5.03$, $p = 0.009$) in 2014. Thiamethoxam in treated corn leaves was significant

only in the first collection week ($t = 11.05$, $p < 0.0001$), while thiamethoxam in treated bean

leaves was significant in the first ($t = 17.55$, $p < 0.0001$) and second ($t = 4.94$, $p < 0.0001$)

weeks. Thiamethoxam in treated pea leaves was significant for the first ($t = 11.52$, $p <$

0.0001), second ($t = 11.72$, $p < 0.0001$), and third ($t = 2.87$, $p = 0.005$) weeks. In the first

collection week, treated beans bore significantly higher mean thiamethoxam concentrations than corn ($t = 4.46$, $p = 0.0004$), but were no different from peas at the same time point ($t = 1.60$, $p = 0.60$).

In 2015, leaf tissue thiamethoxam concentrations followed a similar pattern, decreasing sharply in the month following emergence (**Fig. 1**). Bean leaf sampling spanned the first through fourth weeks following planting, while peas and corn experienced a delayed sampling start time resulting from variation in emergence. The sampling interval for pea and corn spanned the second through fifth weeks following planting. There were significant effects of crop ($F = 149.41$, $p < 0.0001$), time ($F = 129.19$, $p < 0.0001$) and treatment ($F = 126.79$, $p < 0.0001$) on thiamethoxam concentration in 2015 leaf tissue.

The mean thiamethoxam concentration of untreated bean tissue was significantly greater than zero at the first time point ($t = 4.41$, $p < 0.0001$), though not greater than that of treated corn ($t = 1.84$, $p = 0.97$) nor treated pea ($t = 2.94$, $p = 0.36$) at this time point. Across all other weeks and among all other crops, untreated leaf tissue concentrations did not differ significantly from zero.

As a whole, treated leaf tissue from 2015 retained significantly higher thiamethoxam levels (1,612 ppb ± 672) than untreated leaves (144 ppb ± 63) only in the first collection week ($t = 19.66$, $p < 0.0001$, but remained significantly greater than zero during the first ($t = 30.45$, $p < 0.0001$) and second ($t = 3.37$, $p = 0.001$) weeks.

There was an interactive effect of crop x time ($F = 115.34$, $p < 0.0001$), crop x treatment ($F = 109.5$, $p < 0.0001$), time x treatment ($F = 91.36$, $p < 0.0001$), and crop x time x treatment ($F = 79.25$, $p < 0.0001$). The difference between treated beans and treated corn was significant during the first sampling week ($t = 34.57$, $p < 0.0001$), while the difference

between treated beans and treated peas remained significant through the second week of sampling ($t = 35.66$, $p < 0.0001$ week 1; $t = 3.86$, $p = 0.041$ week 2). Treated pea leaves from 2015 did not differ significantly from zero at any sampling point.

Discussion

Although thiamethoxam concentrations in leaf tissues from both years were present throughout all four weeks of leaf tissue analysis, these concentrations were highest in the first week following emergence and close to zero by the third week (**Fig. 1**). The overall decay pattern of thiamethoxam in the leaf tissues of these crops is consistent with previous findings examining in-tissue concentrations of thiamethoxam from seed-treated potato (Huseth et al 2014), though thiamethoxam in this study's crop plants appeared to peak and degrade on a much shorter time scale than potato, which peaked as high as 21,300 ppb before degrading to near-zero 100 days post planting.

It is possible for neonicotinoids from a seed coating to be transported into a plant's floral structures (Bredeson & Lundgren 2015), but this study's floral thiamethoxam concentrations were negligible across all crops in all years. These results are consistent with thiamethoxam non-detections reported from soybean flowers (Camargo Gil 2016), and in maize pollen (Sánchez-Hernández et al 2016) grown from seed coats. Because the floral structures analyzed in this study included more than pollen and (in the case of legumes) nectar, the true thiamethoxam levels of isolated pollen and nectar are likely higher than reported by this study.

Although bees do collect pollen from corn, snap beans, and peas (USDA 2015), there appears to be no research indicating that these plants are visited preferentially by any bee

species. Cutler & Scott-Dupree (2014) observed bumble bees foraging for pollen from conventional corn, but the bumble bees did not favor corn as a pollen source and tended to have fewer workers when in proximity to corn fields. Beans of the species *Phaseolus vulgaris* bear flowers that may attract bees seeking nectar rewards, but not foraging for pollen, while the structure of a pea flower is not designed for visitation by insect pollinators (Woodcock 2012).

In all three of this study's crops, untreated control plants yielded detectable levels of thiamethoxam in their leaf tissue (**Table 1**), and untreated bean leaves from 2015 possessed thiamethoxam concentrations significantly greater than zero during the first week of sampling (**Fig. 1**). It is unlikely that the source of this thiamethoxam contamination came from overhead irrigation, since irrigation water has been found to have a much lower neonicotinoid concentration in central Wisconsin ($0.31 - 0.58$ ppb for thiamethoxam, Huseth & Groves 2014). More likely, the shared seed planting equipment used for all the trial plots may have introduced contamination to untreated seeds via talc dust from the coatings of treated seeds, as can happen during planting (Krupke et al 2012, Hopwood et al 2013). Thiamethoxam can remain detectable in soil for 25 to 100 days (Syngenta Group 2005), while clothianidin, which is the primary metabolite of thiamethoxam, lingers in soil as long as 148-1,555 days (EPA 2003) after delivery or application. Given the long-standing agricultural history of the test plot area, it is possible that thiamethoxam residues from a previous planting remained in the soil. Regardless of the origin of this study's observed neonicotinoid contamination in untreated crop plants, there are implications for the mobility of thiamethoxam from seed-coated crops into the ambient environment as well as into the tissues of plants growing within the vicinity of these crops.

In 2015, corn and pea plants treated with thiamethoxam seed coats yielded unexpectedly low concentrations of the pesticide in their leaf tissue. Corn and peas emerged more slowly than beans in 2015, and it was not possible to sample them until a week later than the first bean sampling point. It is possible that because the crops from this year were planted –and subsequently sampled- later than those of 2014, the results in peas and corn may be due to weather-related differences. Thiamethoxam is transported through a plant primarily via xylem (Jeschke et al 2011), and plants experience greater rates of xylem evapotranspiration from pores during warmer weather, which could speed plant metabolism and thus allocate less of this systemic insecticide to subsequent leaf growth.

An important distinction must be made regarding pesticide concentration versus pesticide dose. The median lethal dose of thiamethoxam taken orally is approximately 3.7 ng/honey bee (Godfray et al 2014). This study found concentrations of thiamethoxam as high as 18,375 ppb in crop tissues, but it is unlikely that a bee would be exposed to a dose of thiamethoxam at these concentrations because bees do not ingest leaves. These concentrations are relevant because bees have been found flying within these crop fields and in the semi-natural edges that border them, where they could come into contact with thiamethoxam residues in the soil, in water, or possibly from interacting with plant structures.

Of the bee species that have been documented in Wisconsin's Central Sands region (**Appendix I**), the majority have activity periods between the months of May and July (Wolf & Ascher 2008). The bee species active in May and early June, when processing vegetable leaves bore their respective peak concentrations of thiamethoxam, include solitary soil-nesting andrenid bees, eusocial soil-nesting halictid bees, cavity-nesting megachilid bees, as well as bumble bee queens and honey bees (Wolf & Ascher 2008, Ascher & Pickering 2016).

During a given growing season in central Wisconsin, this study's processing vegetables are planted and harvested at a range of different times. Field peas are planted first, usually by mid-May, and have a very short flowering window before being harvested around mid-June. In this study, field peas were planted unusually late for the region (May 19 in 2014, June 3 in 2015). Sweet corn is typically planted later than peas, between late April and June, and different sweet corn fields within the region bear pollen at times that can vary by as much as three weeks. Snap beans display the greatest phenological variety, with staggered plantings occurring between mid-May and late July. Subsequently, there are snap beans in flower across the Central Sands from late June through early September. Since snap bean crops are planted at such frequency, the window of elevated thiamethoxam concentration 1-2 weeks post-emergence may be recurrent in the area.

Summary

Thiamethoxam concentrations in leaf tissue from seed-treated processing vegetable crops were highest one to two weeks post emergence, while thiamethoxam concentrations in floral tissue was below this study's detection limit. Based on existing foraging records of wild Wisconsin bees and the low or non-existent thiamethoxam concentrations in flowers found by this study, it is unlikely that the flowers of neonicotinoid-treated processing vegetable crops commonly grown in Wisconsin's Central Sands region pose an elevated risk to foraging pollinators. However, further research is needed to compare field-level tissue concentrations such as these to possible doses bees may receive, especially in regards to less-studied wild bee species.

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Figure 1. Mean concentration ± standard error of thiamethoxam in the leaf tissue of field pea, snap bean, and sweet corn grown from thiamethoxam seed-coated (black) and untreated (white) plants within Wisconsin's Central Sands region in 2014 and 2015.
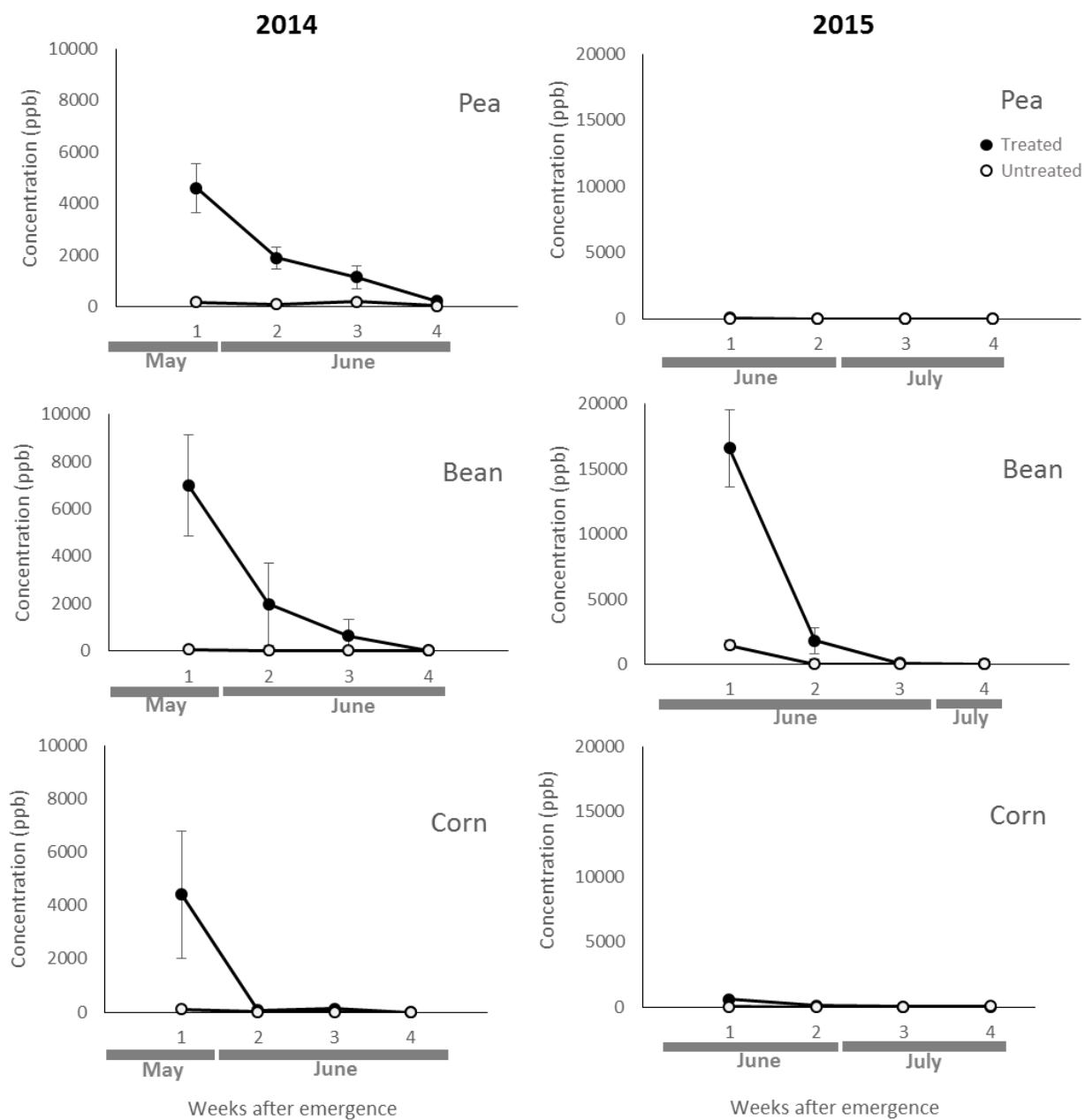
Table 1. Total number of snap bean, field pea, and sweet corn A) floral tissue and B) leaf tissue yielding levels of thiamethoxam above this study's detection limit of 30 ppb. Plants were grown with and without seed treatments in central Wisconsin during the years 2014 and 2015.

A)

Floral Tissue	Treatment	Number of samples	Number of detects	Percent of samples with detects
Pea	16.84 g AI/acre	8	0	0%
	Untreated	8	0	0%
Bean	16.84 g AI/acre	8	0	0%
	Untreated	8	0	0%
Corn	30.24 g AI/acre	8	0	0%
	Untreated	8	0	0%

B)

Leaf Tissue	Treatment	Number of samples	Number of detects	Percent of samples with detects
Pea	16.84 g AI/acre	32	18	56%
	Untreated	31	12	39%
Bean	16.84 g AI/acre	32	24	75%
	Untreated	32	9	28%
Corn	30.24 g AI/acre	32	23	72%
	Untreated	29	7	24%

Appendix I. Combined list of the bee species that have been recorded within Wisconsin's Central Sands region (Wolf & Ascher 2008), as well as within the region's agricultural fields of pickling cucumbers (Lowenstein 2011), cranberry (Gaines Day 2013) and organic and conventional vegetable crops (Prince chp 2, 2016).

Species		Wolf & Ascher 2008	Lowenstein 2011	Gaines Day 2013	Prince 2016
ANDRENIDAE					
Andrena alleghaniensis	Viereck 1907			x	x
Andrena arabis	Robertson 1897			x	
Andrena asteris	Robertson 1891	x			
Andrena barbilabris	(Kirby 1802)			x	
Andrena canadensis	Dalla Torre 1896		X		
Andrena carlini	Cockerell 1901			x	
Andrena carolina	Viereck 1909			x	
Andrena ceanothi	Viereck 1917			x	x
Andrena chromotricha	Cockerell 1899	x		x	
Andrena commoda	Smith 1879			x	
Andrena crataegi	Robertson 1893	x		x	x
Andrena cressonii	Robertson 1891			x	x
Andrena distans	Provancher 1888			x	
Andrena dunningi	Cockerell 1898	x			
Andrena erigeniae	Robertson 1891			x	
Andrena erythrogaster	(Ashmead 1890)			x	
Andrena forbesii	Robertson 1891			x	
Andrena fragilis	Smith 1853 (Svida)	x		x	
Andrena geranii	Robertson 1891	x			
Andrena helianthi	Robertson 1891	x			
Andrena hippotes	Robertson 1895			x	
Andrena hirticincta	Provancher 1888	x			
Andrena imitatrix	Cresson 1872		x	x	
Andrena krigiana	Robertson 1901			x	
Andrena milwaukeensis	Graenicher 1903			x	
Andrena miranda	Smith 1879			x	x
Andrena miserabilis	Cresson 1872	x		x	
Andrena nasonii	Robertson 1895			x	
Andrena nivalis	Smith1853		x	x	
Andrena perplexa	Smith 1853		x	x	x
Andrena placata	Mitchell 1960	x		x	
Andrena platyparia	Robertson 1895		x		
Andrena rufosignata	Cockerell 1902			x	
Andrena rugosa	Robertson 1891			x	

Species		Wolf & Ascher 2008	Lowenstein 2011	Gaines Day 2013	Prince 2016
Andrena sigmundi	Cockerell 1902			x	
Andrena simplex	Smith 1853	x			
Andrena vicina	Smith 1853			x	x
Andrena violae	Robertson 1891			x	
Andrena wilkella	(Kirby 1802)			x	x
Andrena wilmattae	Cockerell 1906			x	
Andrena w-scripta	Viereck 1904			x	
Calliopsis andreniformis	Smith 1853			x	
Perdita halictoides	Smith 1853				x
Perdita maculigera	Cockerell 1896			x	
Pseudopanurgus helianthi	Mitchell 1960			x	
APIDAE					
Anthophora terminalis	Cresson 1869	x	x	x	
Apis mellifera	Linnaeus 1758	x	x	x	x
Bombus ashtoni	(Cresson 1864)	x			
Bombus auricomus	(Robertson 1903)	x	x	x	
Bombus bimaculatus	Cresson 1863	x		x	x
Bombus borealis	Kirby 1837	x	x	x	x
Bombus citrinus	(Smith 1854)	x	x		
Bombus fernaldae	(Franklin 1911)			x	
Bombus fervidus	(Fabricius 1798)	x		x	x
Bombus griseocollis	(DeGeer 1773)	x	x	x	x
Bombus impatiens	Cresson 1863	x	x	x	x
Bombus pensylvanicus	(DeGeer 1773)	x		x	
Bombus perplexus	Cresson 1863	x			x
Bombus rufocinctus	Cresson 1863	x	x	x	x
Bombus sandersoni	Franklin 1913			x	
Bombus ternarius	Say 1873	x		x	x
Bombus terricola	Kirby 1837	x		x	
Bombus vagans	Smith 1854	x	x	x	x
Bombus variabilis	(Cresson 1872)	x			
Ceratina calcarata	Robertson 1900	x		x	
Ceratina dupla	Say 1837	x	x	x	
Eucera atriventris	(Smith 1854)			x	
Eucera hamata	(Bradley 1942)		x	x	x
Melissodes agilis	Cresson 1878		x	x	x
Melissodes bimaculata	(Lepeletier 1825)			x	x
Melissodes communis	Cresson 1878		x		
Melissodes coreopsis	Robertson 1905		x		
Melissodes denticulata	Smith 1854	x			

Species		Wolf & Ascher 2008	Lowenstein 2011	Gaines Day 2013	Prince 2016
Melissodes dentiventrus	Smith 1854	x			x
Melissodes desponsa	Smith 1854	x		x	
Melissodes druriella	(Kirby 1802)	x		x	x
Melissodes illata	Lovell & Cockerell 1906				x
Melissodes nivea	Robertson 1895		x		x
Melissodes subillata	LaBerge 1961	x	x	x	
Melissodes wheeleri	Cockerell 1906		x		
Melissodes trinodis	Robertson 1901			x	x
Nomada armatella	Cockerell 1903	x			
Nomada articulata	Smith 1854	x			
Nomada (spp)					x
Nomada bella	Cresson 1863	x			
Nomada cressonii	Robertson 1893	x			
Nomada cuneata	(Robertson 1903)	x			
Nomada denticulata	Robertson 1902	x			
Nomada luteoloides	Robertson 1895	x			
Nomada maculata	Cresson 1863	x			
Nomada pygmaea	Cresson 1863	x			
Nomada superba	Cresson 1863	x			
Nomada texana	Cresson 1872	x			
Nomada vincta	Say 1837	x			
Peponapis pruinosa	(Say 1837)		x	x	
Svastra obliqua	(Say 1837)	x			
Xenoglossa kansensis	Cockerell 1905		x	x	x
COLLETIDAE					
Colletes compactus	Cresson 1868	x			x
Colletes inaequalis	Say 1837			x	
Hylaeus affinis	(Smith 1853)			x	x
Hylaeus annulatus	(Linneaeus 1758)				x
Hylaeus basalis	(Smith 1853)			x	
Hylaeus mesillae	(Cockerell 1896)			x	x
Hylaeus modestus	Say 1837		x	x	
Hylaeus rudbeckiae	(Cockerell & Casad 1895)				x
HALICTIDAE					
Agapostemon sericeus	(Förster 1771)		x	x	x
Agapostemon splendens	(Lepeletier 1841)		x	x	x
Agapostemon texanus	Cresson 1872	x	x	x	x
Agapostemon virescens	(Fabricius 1775)		x	x	x
Augochlora pura	(Say 1837)		x	x	x

Species		Wolf & Ascher 2008	Lowenstein 2011	Gaines Day 2013	Prince 2016
Augochlorella aurata	(Smith 1853)		x	x	x
Augochloropsis metallica	(Fabricius 1793)		x	x	x
Augochloropsis sumptuosa	(Smith 1853)		x	x	
Dufourea monardae	(Viereck 1924)			x	
Halictus confusus	Smith 1853	x	x	x	x
Halictus ligatus	Say 1837	x	x	x	x
Halictus parallelus	Say 1837		x	x	
Halictus rubicundus	(Christ 1791)			x	x
Lasioglossum acuminatum	McGinley 1986	x	x	x	x
Lasioglossum admirandum	(Sandhouse 1924)			x	x
Lasioglossum albipenne	(Robertson 1890)			x	x
Lasioglossum anomalum	(Robertson 1892)		x	x	
Lasioglossum athabascense	(Sandhouse 1933)			x	
Lasioglossum bruneri	(Crawford 1902)	x		x	
Lasioglossum cinctipes	(Provancher 1888)		x	x	x
Lasioglossum coreopsis	(Robertson 1902)			x	
Lasioglossum coriaceum	(Smith 1853)	x	x	x	x
Lasioglossum cressonii	(Robertson 1890)	x	x	x	x
Lasioglossum ellisiae	(Sandhouse 1924)		x	x	
Lasioglossum ephialtum	Gibbs 2010		x	x	
Lasioglossum fedorense	(Crawford 1906)			x	
Lasioglossum floridanum	(Robertson 1892)			x	x
Lasioglossum forbesii	(Robertson 1890)	x		x	
Lasioglossum foxii	(Robertson 1895)			x	
Lasioglosum heterognathum	(Mitchell 1960)		x	x	
Lasioglossum hitchensi	Gibbs 2012				x
Lasioglossum laevissimum	(Smith 1853)			x	x
Lasioglossum leucocomum	(Lovell 1908)			x	x
Lasioglossum leucozonium	(Schrank 1781)	x	x	x	x
Lasioglossum lineatulum	(Crawford 1906)		x	x	x
Lasioglossum lusorium	(Cresson 1872)			x	
Lasioglossum lustrans	(Cockerell 1897)		x	x	x
Lasioglossum michiganense	(Mitchell 1960)			x	
Lasioglossum mitchelli	Gibbs, manuscript			x	
Lasioglossum nelumbonis	(Robertson 1890)	x	x	x	
Lasioglossum nigroviride	(Graenicher 1911)		x	x	
Lasioglossum novascotiae	(Mitchell 1960)			x	
Lasioglossum nymphaearum	(Robertson 1895)	x		x	
Lasioglossum oblongum	(Lovell 1905)			x	
Lasioglossum oceanicum	(Cockerell 1916)		x	x	x

Species		Wolf & Ascher 2008	Lowenstein 2011	Gaines Day 2013	Prince 2016
Lasioglossum paradmirandum	(Knerer & Atwood 1966)				x
Lasioglossm paraforbesii	McGinley 1986	x	x	x	x
Lasioglossum pectorale	(Smith 1853)	x	x	x	x
Lasioglossum perpunctatum	(Ellis 1913)	x	x	x	x
Lasioglossum pictum	(Crawford 1902)		x	x	x
Lasioglossum pilosum	(Smith 1853)		x	x	x
Lasioglossum planatum	(Lovell 1905)				x
Lasioglossum pruinosum	(Robertson 1892)		x	x	x
Lasioglossum rohweri	(Ellis 1915)	x		x	
Lasioglossum semicaeruleum	(Cockerell 1895)				x
Lasioglossum smilacinae	(Robertson 1897)			x	x
Lasioglossum subviridatum	(Cockerell 1938)			x	x
Lasioglossum succinipenne	(Ellis 1913)		x		
Lasioglossum swenki	(Crawford 1906)		x	x	x
Lasioglossum taylorae	Gibbs 2010			x	
Lasioglossum tegulare	(Robertson 1890)		x		x
Lasioglossum timothyi	Gibbs 2010			x	x
Lasioglossum versans	(Lovell 1905)		x		
Lasioglossum versatum	(Robertson 1902)		x	x	x
Lasioglossum vierecki	(Crawford 1904)		x	x	x
Lasioglossum viridatum	(Lovell 1905)			x	x
Lasioglossum weemsi	(Mitchell 1960)			x	
Lasioglossum zonulum	(Smith 1848)	x		x	
Lasioglossum zophops	(Ellis 1914)		x		
Lasioglossum zephyrum	(Smith 1853)	x		x	x
Sphecodes atlantis	Mitchell 1956			x	
Sphecodes confertus	Say 1837			x	
Sphecodes coronus	Mitchell 1956			x	
Sphecodes davisii	Robertson 1897	x		x	x
Sphecodes dichrous	Smith 1853	x		x	x
Sphecodes levis	Lovell & Cockerell 1907			x	
Sphecodes mandibularis	Cresson 1872	x		x	x
Sphecodes ranunculi	Robertson 1897	x		x	
Sphecodes solonis	Graenicher 1911			x	
Coelioxys funeraria	Smith 1854	x			
Coelioxys immaculata	Cockerell 1912	x		x	
Coelioxys rufitarsis	Smith 1854	x			
Heriades carinatus	Cresson 1864	x			
Hoplitis pilosifrons	(Cresson 1864)	x		x	

Species		Wolf & Ascher 2008	Lowenstein 2011	Gaines Day 2013	Prince 2016
Hoplitis producta	(Cresson 1864)	x		x	x
Hoplitis rubicundus				x	
Hoplitis spoliata	(Provancher 1888)	x		x	
Hoplitis truncata	(Cresson 1878)	x		x	
MEGACHILIDAE					
Megachile addenda	Cresson 1878	x		x	x
Megachile brevis	Say 1837			x	
Megachile gemula	Cresson 1878			x	
Megachile campanulae	(Robertson 1903)	x			x
Megachile latimanus	Say 1823	x	x	x	x
Megachile melanophaea	Smith 1853	x			
Megachile montivaga	Cresson 1878	x			
Megachile pugnata	Say 1837	x	x		x
Megachile relativa	Cresson 1878	x		x	x
Megachile rotundata	(Fabricius 1793)	x			x
Osmia albiventris	Cresson 1864	x		x	
Osmia atriventris	Cresson 1864	x	x	x	
Osmia distincta	Cresson 1864	x		x	x
Osmia georgica	Cresson 1878	x	x	x	
Osmia inspergens	Lovell & Cockerell 1907			x	
Osmia lignaria	Say 1837			x	
Osmia proxima	Cresson 1864	x			
Osmia pumila	Cresson 1864			x	x
Osmia simillima	Smith 1853			x	x
Osmia tersula	Cockerell 1912	x		x	
Osmia virga	Sandhouse 1939			x	
Stelis labiata	(Provancher 1888)			x	
MELITTIDAE					
Macropis nuda	(Provancher 1882)			x	