

Insecticide drift and impacts on arthropod prey resources of birds in public grasslands in
Minnesota

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Dedication

This work is dedicated to my family. Mom and Dad – for your endless support and encouragement of my love of nature, thank you. Caro – I’m so thankful to have grown up and spent so many hours playing outside with you. I love you.

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Chapter 1

Grassland wildlife exposure to insecticide spray drift on public lands in Minnesota's farmland landscape

OVERVIEW

Soybean aphid (*Aphis glycines*) insecticides are widely used in the farmland region of Minnesota to combat insect pests. In Minnesota, the most commonly used broad spectrum foliar insecticides have been shown to be toxic to wildlife in laboratory settings. This is of concern to wildlife conservation because increasing evidence suggests that insecticide exposure is a significant threat to grassland birds and pollinators. However, little information exists regarding drift and deposition of insecticides in grasslands in the farmland region of Minnesota. To address this information gap, I measured insecticide drift and deposition onto passive samplers and arthropods in grasslands adjacent to soybean fields. I collected samples immediately following insecticide application at treatment sites and at control sites without insecticide application. I detected insecticides in grasslands up to 400 m from field edges regardless of whether adjacent fields were sprayed with insecticides, and deposition was greatest within 25 m of field edges. The insecticide chlorpyrifos is especially toxic to wildlife, and I measured residues that were above the contact LD₅₀ for honey bees (*Apis mellifera*) up to 25 m from field edges in grasslands. The masses of chlorpyrifos that birds could consume in a day (if food items contained chlorpyrifos residues equivalent to those in my arthropod samples) were below the acute oral lethal doses (LD₅₀ values) for common grackles (*Quiscalus quiscula*), house sparrows (*Passer domesticus*), northern bobwhites (*Colinus virginianus*), red-winged blackbirds (*Agelaius phoeniceus*), and ring-necked

pheasants (*Phasianus colchicus*). I used linear mixed models in a hierarchical selection approach to assess the importance of distance from field edge, spray method (plane or ground sprayer), and sampler height (mid-canopy or ground) in explaining insecticide deposition in grasslands. The best-supported model of deposition on passive sampling devices included an inverse association of distance from the field edge with deposition ($\beta = -0.62$, 95% CI = -1.30 – 0.06) and positive association of samplers being placed at the mid-canopy level ($\beta = 146.81$, 95% CI = -28.99 – 322.60) compared to ground level. Canopy cover of live vegetation had an inverse association with deposition ($\beta = -6.02$, 95% CI = -12.06 – 0.12). The best-supported model of insecticide deposition on arthropods included effects of air temperature ($\beta = -544.19$, 95% CI = -937.41 – -150.98) and maximum height of vegetation ($\beta = 272.97$, 95% CI = 2.10 – 543.84). Grasslands with cover ≥ 25 m from row crop edges may provide wildlife habitat with lower exposure to insecticides. Management regimes that increase the percent canopy cover in grasslands also have the potential to reduce exposure of grassland wildlife to foliar insecticides.

Key Words: insecticides, chlorpyrifos, chemical drift, farmland landscape, row crops, grasslands, grassland birds, non-target arthropods, direct exposure, indirect exposure

INTRODUCTION

Insecticides are widely used on soybeans throughout the farmland region of Minnesota to control insect pests, but little is known about the environmentally relevant impact of these chemicals on non-target grassland wildlife. Soybean aphids (*Aphis glycines*) are common agricultural pests that were first discovered in Minnesota in 2000 and quickly spread throughout the state's farmland region by 2001 (Venette and Ragsdale 2004). Given that large, untreated populations of this pest can decrease crop yields by 40% (Ragsdale et al. 2011), many producers apply broad spectrum foliar insecticides on their soybeans to control aphid outbreaks. Between 2015-2018, approximately 41% of the area planted to soybeans in Minnesota was treated with insecticides (U.S. Department of Agriculture [USDA] National Agricultural Statistics Service [NASS] 2016, 2018, 2019). With over 3 million ha of soybeans planted in Minnesota annually (USDA 2019a) and much of the state's grassland cover located in the farmland region (Minnesota Prairie Plan Working Group 2018), grassland wildlife have the potential to be exposed to soybean aphid insecticides in Minnesota.

Chlorpyrifos, lambda-cyhalothrin, and bifenthrin are the 3 most common active ingredients applied on soybeans to control soybean aphids in Minnesota (USDA NASS 2016). Foliar applications of these chemicals using airplanes or ground sprayers are common when aphids reach threshold levels. Because these ingredients have varying withholding times and modes of action, landowners may use a single active ingredient during a growing season, apply a product that combines insecticides with different modes of action, or apply rotations of insecticides (Koch et al. 2016). Although these chemicals can be very effective against soybean aphids when used individually, using multiple

active ingredients on a field during a single growing season is becoming increasingly common to combat aphids' resistance to lambda-cyhalothrin and bifenthrin (Koch and Potter 2019).

Growing evidence suggests that insecticide exposure is a threat to grassland wildlife, especially birds (Avery et al. 2004, Mineau and Whiteside 2006, 2013). Chlorpyrifos, lambda-cyhalothrin, and bifenthrin have all been shown to be toxic to non-target organisms in laboratory settings (National Pesticide Information Center 2001, Christensen et al. 2009, Johnson et al. 2010). These chemicals disrupt nervous systems of organisms and can cause mortality at high doses. At sublethal dosages, insecticides have been shown to have negative effects on the behavior and physiology of animals. Symptoms of sublethal exposure include increased susceptibility to predation, lost breeding opportunities, impaired development of offspring, loss of mobility and orientation, and impaired feeding and breeding behavior (Mitra et al. 2011, Moore et al. 2014, Eng et al. 2017).

Chlorpyrifos is a broad-spectrum organophosphate insecticide that disrupts nervous system functioning of target and non-target organisms through direct contact, ingestion, and inhalation (Christensen et al. 2009). This chemical is very highly toxic to bird species including common grackles (*Quiscalus quiscula*), house sparrows (*Passer domesticus*), northern bobwhites (*Colinus virginianus*), red-winged blackbirds (*Agelaius phoeniceus*), and ring-necked pheasants (*Phasianus colchicus*) with acute oral lethal doses (LD₅₀ values) of 8.5, 29.5, 32, 13.2, and 12.2 mg/kg, respectively (Solomon et al. 2001, Table 1). Chlorpyrifos is also highly toxic to honey bees (*Apis mellifera*) with a contact LD₅₀ of 100 ppb or 66.67 ng/cm² (Tomlin 2000, Ostiguy et al. 2019, see Table 1

for further explanation). LD₅₀ values of chlorpyrifos in contaminated pollen and nectar of adult honey bees are also representative of non-*Apis* bee species (e.g., bumblebees; Cutler et al. 2014, U.S. Environmental Protection Agency [EPA] et al. 2014). At sublethal levels, chlorpyrifos has been shown to impair orientation in white-crowned sparrows (*Zonotrichia leucophrys*) at doses equivalent to 10% of the oral LD₅₀ for house sparrows (2.95 mg/kg; Solomon et al. 2001, Eng et al. 2017).

Lambda-cyhalothrin is a broad-spectrum pyrethroid insecticide that also disrupts the nervous systems of target and non-target organisms (National Pesticide Information Center [NPIC] 2001). This chemical is low in toxicity to birds but highly toxic to pollinators including bees (World Health Organization [WHO] 1990, NPIC 2001).

Lambda-cyhalothrin is reported to have an oral LD₅₀ of >3,950 mg/kg for mallards (*Anas platyrhynchos*), and cyhalothrin's oral LD₅₀ for domestic hens (*Gallus domesticus*) is >10,000 mg/kg (WHO 1990). This chemical is very highly toxic to honey bees with an oral LD₅₀ of 0.97 µg/bee and contact LD₅₀ of 0.051 µg/bee (WHO 1990, NPIC 2001).

Bifenthrin is a broad-spectrum pyrethroid insecticide that affects the central and peripheral nervous systems of organisms by direct contact or ingestion (Johnson et al. 2010). This chemical is low in toxicity to birds including northern bobwhites and mallards with oral LD₅₀ values of 1,800 mg/kg and 2,150 mg/kg, respectively (Tomlin 2000). However, terrestrial insects are especially susceptible to this chemical, with bifenthrin being highly toxic to bumblebees. One study showed that direct exposure to bifenthrin killed 100% of worker bumblebees (Besard et al. 2010). Reported toxicity values for bumblebees include an oral LD₅₀ value of 0.1 µg/bee and contact LD₅₀ of 0.01462 µg/bee (Tomlin 2000).

One important avenue for grassland birds and other wildlife to be exposed to insecticides is through drift associated with routine airplane or ground-based applications to prevent and control soybean aphid outbreaks. Drift occurs when insecticides are sprayed on crops but environmental factors (e.g., temperature, wind speed, or wind direction) result in their transport to areas beyond the targeted application area. The distances over which drift occurs vary widely, with reported distances ranging from 1 m to 2,000 m (Davis and Williams 1990, Langhof et al. 2005, Carlsen et al. 2006, Antuniassi et al. 2014, Holterman et al. 2017, Runquist et al. 2018, Baio et al. 2019). There is little information about drift and environmentally relevant exposure of wildlife in grasslands for standard foliar insecticide application regimes in Midwestern farmland landscapes. This information is necessary to effectively design grasslands that are protected and managed for wildlife.

Knowledge of wildlife's exposure to insecticides will help managers with grassland conservation efforts in Minnesota. Several conservation plans aim to add grassland cover to Minnesota's landscape in the coming years. For example, Minnesota's Pheasant Action Plan (Minnesota Department of Natural Resources [MNDNR] 2020) and Prairie Conservation Plan (Minnesota Prairie Plan Working Group 2018) both aim to offset grassland cover losses due to declining Conservation Reserve Program (CRP) enrollments by establishing grassland/wetland habitat complexes within the farmland region of the state. Additionally, a 2016 Minnesota law that requires perennial vegetation buffers averaging 15-m wide along public lakes, rivers, and streams and 5-m wide along public ditches has resulted in the addition of narrow strips of grassland cover to farmland landscapes in recent years. Understanding the potential for grassland wildlife to be

exposed to insecticide drift will provide managers and landowners with better information for managing grasslands and buffers in the farmland region of the state.

My objective was to quantify drift of soybean aphid insecticides into grasslands in the farmland region of Minnesota. To address this objective, I measured the deposition of insecticides onto filter paper to assess the potential for grassland wildlife to be directly exposed to these chemicals through drift. I also quantified insecticide deposition on arthropods to assess the potential for grassland birds and other insectivores to be exposed to these chemicals indirectly through consumption of contaminated arthropod prey. I hypothesized that the distance from the grassland/soybean field edge, spraying method used, and sample height would influence the amount of insecticide residues measured in grasslands. In particular, I predicted that samples collected closer to the field edge would collect more residues, airplane spraying would result in greater measures of drift in grasslands than ground spraying, and filter paper samples placed above the ground would contain more residues than samples collected at ground level. I also hypothesized that chlorpyrifos residues would exceed the contact LD₅₀ for honey bees and acute oral LD₅₀ values for birds in grasslands near the edges of sprayed fields.

METHODS

Study Area

I conducted this study in the southwest (SW), west-central (WC), and central (C) regions of Minnesota (Fig. 1). Corn and soybeans accounted for approximately 90%, 67%, and 71% of the landscape in these 3 regions, respectively (USDA 2019*a, b*). Grassland cover on public and private land accounted for 6.9%, 10.0%, and 5.6% of the landscape in these regions (Messinger and Davros 2018). Since 2003, these areas have

also experienced some of the greatest estimated uses of chlorpyrifos and lambda-cyhalothrin in Minnesota (Minnesota Department of Agriculture [MDA] 2005, 2012, 2014, 2016).

My study sites consisted of public Wildlife Management Areas (WMAs) comprised of reconstructed grasslands or grassland/wetland complexes. These sites were managed by the Minnesota Department of Natural Resources (MNDNR) with the intent of providing high quality habitat for wildlife. I selected study sites in ArcGIS (version 10.6.1, ESRI 2021) by first choosing WMAs that were bordered by row crop fields and were of sufficient size. I focused on potential treatment sites that were predicted to be downwind (i.e., east or north) from cooperators' soybean fields based on archived National Weather Service data (TWC Product and Technology LLC 2015).

I visited potential study site WMAs to examine the vegetation diversity and to identify the crops planted in adjacent fields. I chose sites dominated by a diverse mesic tallgrass prairie mix containing warm-season grasses and forbs because this assemblage is commonly used by MNDNR managers and agency partners to restore habitat for grassland birds and pollinators. In my study sites, predominant grass species included big bluestem (*Andropogon gerardii*), smooth brome (*Bromus inermis*), Canada wild rye (*Elymus Canadensis*), and Kentucky bluegrass (*Poa pratensis*). Dominant forb species were wild bergamot (*Monarda fistulosa*), smooth oxeye (*Heliopsis helianthoides*), and Canada goldenrod (*Solidago canadensis*). Canada thistle (*Cirsium arvense*) was also commonly present but was not planted by the MNDNR.

Landowner Contact

Landowner cooperation was vital to timing my field sampling efforts. To request the cooperation of landowners and learn about their insecticide spraying practices, I mailed surveys to 206 landowners who owned land bordering 29 potential study sites in March and April 2017 (Appendix A). Although the mailed surveys helped me gather useful information about common spraying habits, I ultimately found that soliciting landowner cooperation through in-person visits and phone calls was more effective. Therefore, I did not mail surveys to landowners in 2018. Once I secured landowner cooperation, I kept in contact with them throughout the growing season to determine if and when they would be applying insecticides on their soybean fields. Several landowners rented their land and/or hired farming cooperatives to spray their fields, so I contacted combinations of landowners, renters, agronomists, cooperative representatives, and pilots to determine the exact time of spraying and to obtain additional relevant data after spraying (e.g., insecticide product used, application rate, and tank pressure).

Experimental Design

I conducted sampling in July-August 2017 and 2018, the peak period in which insecticides were used to control soybean aphids. Each of my treatment study sites consisted of upland grassland cover directly adjacent to a soybean field. The soybean field adjacent to each treatment study site was treated with foliar insecticides used to control soybean aphids. Insecticide applicators treated fields using ground sprayers or airplanes. I worked closely with cooperating landowners to learn the exact dates of spraying and to verify the chemical formulations applied to soybean fields bordering my sites. My control study sites had similar site characteristics except that they were adjacent

to corn fields. I was not in contact with the landowners of these corn fields and did not observe foliar pesticide spraying on these fields during sampling. Standard management practices for corn in this region do not include foliar insecticide applications in late summer.

Within each study site, I established 3 primary transects 90-100 m apart that extended perpendicular from the soybean field edge to the grassland interior (Fig. 2). I conducted sampling at stations placed at 6 distances (0, 5, 25, 50, 100, and 200 m) along each of these 3 primary transects. If the site was large enough, I also established a station at 400 m along each transect. I created transects with the same orientation at control sites. Therefore, I established 18-21 drift sampling stations at each study site. At treatment sites, I aligned primary transects perpendicular to the cooperators' soybean field edge. At control sites, I established primary transects perpendicular to a grassland edge that was east or north of a corn field.

Measuring Insecticide Deposition with Passive Sampling Devices

To assess the potential for birds and other wildlife to be directly exposed to soybean aphid insecticide drift, I deployed passive sampling devices (PSDs) to collect drift residues. PSDs consisted of Whatman™ Qualitative Filter Paper (grade 2; GE Healthcare U.K. Ltd., Little Chalfont, U.K.) attached to 1.27-cm hardware cloth formed to a cylinder shape. This structure approximated the size and shape of a large songbird or a gamebird chick. The surface area of the filter paper on each PSD was 354.75 cm²: the top contained a 7-cm-diameter circle and the vertical plane was covered by a 11.5 by 28.5 cm sheet of filter paper with 1 cm of overlap for attachment.

I deployed PSDs in treatment study sites ≤ 4.5 hours before insecticide application. I placed PSDs at ground level (0 m) and mid-canopy height (0.5 m) at each sampling station for a total of 36–42 PSDs per site. Ground-level sampling measured potential insecticide drift exposure for ground-nesting birds and other ground-dwelling wildlife (e.g., gamebirds, spiders, beetles, ants, and small mammals). Mid-canopy sampling measured potential exposure of above-ground nesting birds and canopy-dwelling species of spiders and insects to insecticide drift. I retrieved the PSDs from the field ≤ 2.25 hours after insecticide spraying in the adjacent soybean field ended. At control sites, I also placed PSDs at ground and mid-canopy levels at each sampling station. I allowed the PSDs to be exposed to air for a similar amount of time as PSDs at treatment sites. Upon PSD collection, I wrapped the pieces of filter paper in aluminum foil, enclosed them in airtight plastic bags, and placed them in a cooler with dry ice in the field. This prevented chemical degradation by sunlight and heat. I then stored these bags in a -80 °C freezer until I shipped them to the laboratory for analysis.

During 2018 only, I deployed PSDs 1–3 days prior to spraying at mid-canopy and ground height at each 0-m sampling station. I conducted this sampling at both treatment and control sites. PSDs were exposed to the air for 1–3 hours. These samples served as a secondary field-based control to determine whether insecticides were present prior to known spraying events at treatment sites.

Measuring Insecticide Deposition on Arthropods

To assess the potential for birds and other insectivorous wildlife to be exposed to insecticides indirectly via consumption of contaminated arthropod prey, I collected arthropod samples ≤ 4 hours after insecticide application in adjacent soybean fields. In

each study site, I established secondary arthropod sampling transects beginning at the sampling stations at 0, 5, and 25 m from the soybean field edge along each of the 3 primary transects. The secondary arthropod sampling transects ran 30 m to the right (when facing the adjacent crop field) of each PSD sampling station and parallel to the field edge. Two observers simultaneously collected arthropod samples along each secondary transect: 1 observer used a sweep net while another used a vacuum sampler (Southwood and Henderson 2000). Observers walked each secondary transect down and back for a total of 60 m per collection method per sample. Observers walked unique paths 1.25 m apart to minimize disturbance from sampling and to maximize the likelihood that the arthropod communities being sampled were similar (Doxon et al. 2011).

I collected sweep-net samples using a standard 38-cm diameter canvas net that was swung 60 times per sample, and the same observer collected all sweep-net samples in this study. A second observer collected vacuum samples using a modified hand-held vacuum with a 15-cm long nozzle held 15 cm above the ground (BioQuip Products Inc., Rancho Dominguez, CA, U.S.A.). Sweep-net sampling collected canopy-dwelling arthropods whereas vacuum sampling collected ground-dwelling arthropods. I combined sweep-net and vacuum samples from each transect into 1 sample for a total of 9 samples per study site. I immediately placed arthropod samples in airtight plastic bags and froze them on dry ice to prevent chemical degradation by sunlight and heat. I then stored them in a -80 °C freezer until later analysis. I collected arthropod samples at control sites using the same transect layout and methods, with the timing of collection based on when I deployed PSDs.

Arthropod samples contained varying amounts of vegetation (e.g., grass seed heads and/or small pieces of stem). This plant material was not separated from arthropods before sending to the laboratory because post-sampling processing could have caused contamination or UV degradation of insecticides. I did not weigh samples before shipment and estimated the maximum mass of any arthropod + vegetation sample to be 10.6 g. This value is the maximum mass of 7 samples that I collected and weighed while evaluating arthropod sampling techniques (i.e., sweep netting and vacuum sampling) along 60-m transects at a WMA not used as one of my study sites.

Laboratory Analysis

I sent filter paper and arthropod samples to the U.S. Department of Agriculture - Agricultural Marketing Service National Science Laboratory (USDA-AMS NSL; Gastonia, NC, U.S.A.). Laboratory staff analyzed the samples using a solvent-based extraction method and tested for several insecticides and fungicides. They concentrated extracts by evaporation and then analyzed them using gas chromatography/mass spectrometry-negative chemical ionization (GC/MS-NCI) or another appropriate method (MET-104). The laboratory reported chemical residues of chlorpyrifos, cyhalothrin (total), and bifenthrin in parts per billion (ppb), which I converted to ng/cm^2 for PSDs and ng/g or mg/kg for arthropod samples (see Residue Conversions in the Data Analysis section). The laboratory's limit of detection (LOD) was 2 ppb for chlorpyrifos, 1 ppb for cyhalothrin, and 4 ppb for bifenthrin. As an additional control, I sent 5 filter paper samples to the USDA-AMS NSL for chemical residue analysis. I did not deploy these samples in the field, but I had attached them to hardware cloth frames and stored them in an airtight bin in the back of a field vehicle prior to shipment to the lab.

Weather Measurements

I used Kestrel 5500AG agricultural weather meters (Nielsen-Kellerman Co., Boothwyn, PA, U.S.A.) mounted on tripods and equipped with weather vanes to measure relevant weather data including ambient temperature, relative humidity, wind speed, and wind direction every 20 seconds during PSD deployment and arthropod sample collection. I also measured weather data while sampling at control sites. I placed the weather meters along the center primary transect at 0 m, 100 m, and 200 m from the field edge.

Vegetation Measurements

I measured ground cover, canopy cover, litter depth, maximum height of live and dead vegetation, vertical density, and species richness of vegetation in 30 by 55 cm plots at each PSD station and at the endpoints of arthropod sampling transects. I measured vegetation 1–3 d prior to the spraying event at treatment sites and 1–3 d prior to PSD deployment at control sites. Using a modified point-intercept method, I categorized ground cover into bare ground, litter, or other (e.g., woody debris, rock, or gopher mound; Bureau of Land Management 1996). I calculated canopy cover from nadir digital photographs taken of each plot from 1.5 m above the ground with the program SamplePoint (Booth et al. 2006). Canopy cover categories included grass, forb, dead vegetation, woody vegetation, and other. I measured litter depth to the nearest 0.1 cm at 1 point within the plot that subjectively represented the average condition of the plot. I recorded the maximum height of live and dead vegetation within each plot to the nearest 0.5 dm. I measured vertical vegetation density by placing a Robel pole in the center of each plot and estimating the visual obstruction reading (VOR) from 4 m away and 1 m

above the ground from each of the 4 cardinal directions (Robel et al. 1970). Finally, I counted the number of unique forb and grass species to determine species richness in the plot.

Researcher Safety

Long-term exposure to organophosphate and pyrethroid insecticides has been linked to detrimental health effects in humans, particularly chemical applicators. These chronic health risks include adverse respiratory effects (e.g., asthma and wheezing) and lung cancer (Lee et al. 2007, Hoppin et al. 2017). Bifenthrin is listed by the EPA as a possible human carcinogen (Johnson et al. 2010). Exposure to chlorpyrifos, lambda-cyhalothrin, and bifenthrin can cause short-term side effects including eye, skin, nose, and throat irritation, headaches, nausea, and dizziness (Dow AgroSciences LLC 2014a, Syngenta Crop Protection LLC 2014).

To reduce exposure to these insecticides, researchers followed the Personal Protective Equipment (PPE) recommendations listed on the specimen labels of mixes containing chlorpyrifos. This chemical is associated with the most severe health risks of insecticides used on soybeans in my study area. Researchers were equipped with more PPE than necessary because the PPE recommendations on specimen labels are intended for applicators who spend several days and many hours per year working with these chemicals (D. Herzfeld, University of Minnesota, personal communication). Our overall exposure levels were very low as researchers spent ≤ 4 hours in grasslands adjacent to sprayed fields on 1 day per treatment study site. Researchers did not enter the fields where insecticides were sprayed. We were equipped with Tychem® QC 127 series hooded coveralls (DuPont, Wilmington, DE, U.S.A.), StanSolv® 15 mil nitrile gloves

(MAPA Professional, Colombes, FR), and rubber boots while collecting samples in treatment sites immediately after insecticide application. Researchers had chemical-resistant goggles and half-mask air-purifying respirators on-hand in the event that they experienced eye, skin, nose, or throat irritation while in the field.

Data Analysis

Residue Conversions

To convert the ppb of chemicals reported by the laboratory to ng/cm² on PSDs or ng/g on arthropod samples, I used the following equations:

$$(Ca * Va) * \frac{Vtx}{Vxs} * \frac{Wt/Ws}{Ap} = \text{ng/cm}^2$$

or

$$(Ca * Va) * \frac{Vtx}{Vxs} * \frac{Wt/Ws}{Wa} = \text{ng/g}$$

where:

Ca = concentration of analytical sample (ppb, ng/mL)

Va = volume of analytical sample = 1 mL

Vtx = volume of total extract = 15 mL

Vxs = volume of extract subsample prior to concentration for analysis = 5 mL

Wt = weight of total frozen sample = 3.53 g for PSDs weighing ≥ 3 g;

or

= known weight (g) of PSD or arthropod samples weighing < 3 g;

or

= 3.0 g (minimum) or 10.6 g (maximum) for arthropod samples with unknown mass ≥ 3 g

W_s = weight of frozen subsample = 3 g for PSD and arthropod samples

weighing ≥ 3 g;

or

= W_t (g) when $W_t < 3$ g

A_p = surface area of filter paper from PSD prior to freezer mill processing =

354.75 cm²

W_a = weight of arthropod sample prior to freezer mill processing = 15 g

I did not weigh samples before shipping them to the laboratory. To calculate minimum residue estimates on arthropod samples with unknown weights, I used 3.0 g for the total frozen sample weight (W_t), because the laboratory provided me a list of any sample weighing < 3 g. Therefore, 3 g reflected their minimum sample weight threshold. To calculate maximum residue estimates on arthropod samples with unknown weights, I used 10.6 g. I obtained this value from the maximum weight of samples collected during practice arthropod sampling (see Measuring Insecticide Deposition on Arthropods section). The maximum residue estimates represent “worst-case scenarios” of the deposition of insecticides on arthropod samples.

I used insecticide residue estimates on arthropods to calculate the mass of insecticides that birds could consume in a day and the mass of contaminated arthropods required to reach the LD_{50} for several bird species. In these calculations, I used values of bird body mass, mass of food that birds eat in a day, and LD_{50} values reported in Solomon et al. (2001). I assumed that my samples were representative of food items birds could be consuming. Although samples contained arthropods and some vegetation from

sweep netting, birds in farmland regions include both invertebrate and plant materials in their diets (McNicol et al. 1982, Moreby 2004).

Model Covariate Selection

I measured multiple characteristics of weather and vegetation at study sites, but subsequently reduced the number of covariates used in models assessing insecticide deposition on PSDs and arthropod samples. To select weather covariates to use in models, I first considered the aspects of weather that may influence drift: air temperature, relative humidity, mean wind speed, maximum wind speed, and wind direction. I considered mean air temperature and mean wind speed in models of insecticide deposition because I observed no substantive fluctuations in these measures during each spraying event. I derived values of mean air temperature and wind speed by first calculating the average of simultaneous measurements at 3 weather meters; every weather meter was recording measurements at 20 s intervals. Then, I calculated the mean of values between the start and end time of the spraying event at treatment sites or PSD deployment at control sites. I deemed a site to be downwind when the average wind direction was within $\pm 62^\circ$ of the transect orientation. I then tested whether any of the remaining weather covariates were highly correlated with any other weather covariate (i.e., $|r| > 0.7$; Dormann et al. 2013). Temperature and relative humidity were highly correlated ($r = -0.78$) and I considered temperature and not relative humidity in models of insecticide deposition. Higher air temperatures are associated with higher measures of drift, as higher temperatures promote evaporation and result in smaller droplets that are readily transported by the wind (Nuyttens et al. 2017).

I reduced the list of all potential vegetation covariates to those that were associated with distinct aspects of vegetation structure above the ground level: height, density, and canopy cover. Although I measured the height and canopy cover of both live and dead vegetation separately, I only used measurements from live vegetation in my models, as the majority of plants within vegetation plots were alive. I then tested for correlations between the remaining covariates: maximum height of live vegetation, vertical obstruction readings, and canopy cover of live plants. These covariates were not highly correlated (i.e., $|r| < 0.7$; Dormann et al. 2013).

Models of Insecticide Deposition

I used linear mixed models to assess the potential effects of distance from field edge, spray method, and PSD height on insecticide deposition on PSDs. To assess insecticide deposition on arthropod samples, I used linear mixed models with all of the same covariates except PSD height. The response variables in these models were the summed residues of chlorpyrifos, lambda-cyhalothrin, and bifenthrin in each sample (hereafter, “target chemicals”). The units of these residues were ng/cm^2 for PSD samples and ng/g for arthropod samples. Because the arthropod samples that I collected had unknown weights, my calculations converting lab-reported parts per billion to ng/g resulted in estimates of target chemical residues per sample (see the Residue Conversions section). I used maximum residue estimates in models of insecticide deposition on arthropod samples.

Using data from treatment sites only, I used a hierarchical model selection approach similar to the methods of Daly et al. (2015). The first set of models incorporated weather conditions during the spraying event, the second set incorporated

vegetation covariates, and the final step incorporated my primary factors of interest (i.e., distance, spray method, and height). This approach allowed me to examine how these primary factors of interest influenced insecticide deposition after accounting for other environmental factors that I expected to affect drift based on the literature (Table 2). I constructed models using package nlme (Pinheiro et al. 2021) in program R (version 3.6.0, R Core Team 2021). I fitted models using the maximum likelihood method with study site as a random effect.

The first set of models included the reduced set of weather covariates: mean air temperature (TEMP; °C), mean wind speed (WSP; m/s), and a binary covariate for whether transects were downwind of the sprayed field (WDIR). I retained weather covariates from the model with the lowest Akaike's Information Criteria corrected for sample size (AIC_c ; Burnham and Anderson 2002) and included them in all models in the next step of analysis.

In the second step of model selection, I added vegetation covariates to the best-supported model considering weather covariates to account for additional variation in the data. I included continuous vegetation covariates for maximum height of live vegetation (MHL; dm), VOR reading from the direction of the sprayed field (VOR; dm), and percentage of the canopy consisting of live vegetation (CCLIVE; summed percent cover of grasses and forbs). I used vegetation measurements recorded at each PSD sampling station for models of insecticide deposition on PSDs. For models of insecticide deposition on arthropod samples, I used the averaged vegetation measurements from the start and end of arthropod collection transects. I retained weather and vegetation covariates from the model with the lowest AIC_c for inclusion in the final modeling step.

I incorporated distance from field edge (DIST; m), spray method (SPRAY; i.e., ground or airplane application), and PSD height (HT; i.e., ground or mid-canopy) in the final step of model selection of insecticide deposition on PSDs. I added DIST, SPRAY, and HT to the best-supported model of insecticide deposition on PSDs from step 2. I also included the interaction of spray method \times distance as a covariate in these models. I followed the same approach for models of insecticide deposition on arthropods except that I did not include HT. The inclusion of distance from field edge, spray method, and/or PSD height in the best-supported model would suggest that these factors influenced insecticide deposition, after accounting for other environmental factors (i.e., weather and vegetation).

RESULTS

Landowner Surveys

Of the 206 surveys I initially mailed to landowners who owned land adjacent to potential study sites in spring 2017, 24.4% were returned. I sent a second round of 164 letters and had a response rate of 6.1%. Not all landowners filled out the survey completely because many rented their land and did not have information on aphid insecticide spraying practices. Approximately 13.6% of landowners completed the survey in its entirety and 11 landowners indicated that they would be planting soybeans adjacent to a WMA in 2017. These landowners were willing to be contacted during the growing season; however, I did not deem these WMAs as feasible treatment sites after visiting them in-person. More landowners reported spraying their fields by airplanes ($n = 12$) than by ground sprayers ($n = 8$) in the previous 3-5 years. The majority of landowners that

sprayed aphid insecticides in past years reported using products with the trade names Lorsban® (chlorpyrifos; $n = 9$) and Warrior® (lambda-cyhalothrin; $n = 4$).

Insecticide Spraying

I collected samples at 5 treatment study sites and 4 control sites between 29 July – 24 August 2017 and 18 July – 18 August 2018, coinciding with peak activity for aphid insecticide spraying (Table 3). I collected 368 PSD samples and 81 arthropod samples combined across years. Additionally, I collected 30 pre-spraying PSD samples as secondary field-based controls in 2018. Soybean fields bordering my treatment study sites were treated with chlorpyrifos ($n = 4$) and cyhalothrin ($n = 3$; Table 4). No insecticide applicators used bifenthrin. One applicator reported using the insecticide thiamethoxam in their tank mix, but I did not detect this chemical on PSDs at treatment sites (Appendix B). Both airplanes ($n = 3$) and ground sprayers ($n = 2$) were used to apply aphid insecticides at treatment study sites. Two of 5 treatment study sites were downwind at the time of spraying.

Insecticide Deposition on Passive Sampling Devices

I detected target chemicals on PSDs at all distances (0–400 m) at both treatment and control sites (Table 5, Fig. 3). I detected chlorpyrifos and cyhalothrin at treatment sites, but did not detect bifenthrin (Fig. 3, Appendix B). The mean value of target chemical deposition on PSDs at treatment sites was greatest at 0 m from the field edge ($\bar{x} = 351.44 \text{ ng/cm}^2$, Table 5). At every distance from the field edge at treatment sites, mean target chemical deposition on mid-canopy PSDs was greater than deposition on ground level PSDs (Table 5). Treatment sites' target chemical residues at 400 m from the field edge had a greater mean ($\bar{x} = 6.96 \text{ ng/cm}^2$) than residues I measured at 50, 100, and 200

m from the field edge ($\bar{x} = 3.83, 0.37, \text{ and } 0.13 \text{ ng/cm}^2$, respectively), likely due to the high mean residue value I measured at 400 m at treatment sites bordered by fields treated by ground sprayers ($\bar{x} = 22.43 \text{ ng/cm}^2$, Table 5). At treatment sites, means and coefficients of variation (CVs) of target chemical deposition on PSDs were greater when bordering fields were sprayed by airplanes compared to ground sprayers at most distances, with the greatest differences occurring 0–50 m from the field edge (Table 5).

I detected all 3 target chemicals on PSDs at control sites (Fig. 3). Means of target chemical deposition were generally much lower at control sites compared to treatment sites; however, mean deposition at 200 m at control sites was 0.06 ng/cm^2 greater than at treatment sites (Table 5). At all distances from the field edge at control sites, target chemical deposition CVs were similar (CV = 1.85, 0.95, 0.90, 0.97, 1.03, 0.98, 1.02; Table 5).

Chlorpyrifos had the greatest mean deposition values of all the chemical residues I measured at both treatment and control sites, with the exception of diethyltoluamide (DEET; Appendix B). I summarized chlorpyrifos residues separately from target chemicals to compare residues to levels shown to be toxic to birds and pollinators. Mean chlorpyrifos residues exceeded 66.67 ng/cm^2 (the acute contact LD_{50} for honey bees, Table 1) on PSDs at 0, 5, and 25 m from the field edge at treatment sites ($\bar{x} = 346.99, 141.88, 263.58$, respectively; Table 6). Mean chlorpyrifos residues at treatment sites also exceeded 66.67 ng/cm^2 at sites bordered by soybean fields sprayed by airplane at 0–25 m from the field edge (Table 6). Mean chlorpyrifos residues on PSDs did not exceed 66.67 ng/cm^2 at any distance from the field edge at control sites (Table 6, Fig. 4).

There were consistent differences in mean chlorpyrifos deposition associated with PSD height at treatment sites, with greater deposition on mid-canopy PSDs (Table 6). The greatest differences in residues between mid-canopy and ground-height samplers occurred 0–25 m from field edges (Table 6). These differences were not statistically significant based on results of Welch’s two-sample *t*-tests, however, and did not exhibit a trend as distance from the field edge increased (Appendix C). PSDs from treatment study sites contained residue means > 66.67 ng/cm² at both ground and mid-canopy heights at 0 and 25 m from field edges; mid-canopy PSDs at 5 m from field edges also had a mean chlorpyrifos residue > 66.67 ng/cm² (Table 6).

To compare target chemical deposition on PSDs to the concentrations of insecticides applied in the soybean field at treatment sites, I used landowner-reported application rates (Table 4) and insecticide label information (Syngenta Canada Inc. 2012; Cheminova Inc. 2013; Dow AgroSciences LLC 2014*b, a*; Syngenta Crop Protection LLC 2014) to calculate the active ingredient concentrations of target chemicals applied in ng/cm². The landowner at site tC did not provide application rate information, so I used the label-recommended application rate for calculations pertaining to this site. PSDs captured very low percentages of active ingredients applied in the field, with the exception of site tB (Fig. 5). At site tB, 63.9% and 25.5% of the applied concentrations of chlorpyrifos and cyhalothrin, respectively, were deposited on PSDs at 0 m from the field edge.

The PSDs that I deployed pre-spraying in 2018 contained very low levels of the target chemicals in control sites (\bar{x} = 0.054 ng/cm², CV = 0.41) and treatment sites (\bar{x} = 0.071 ng/cm², CV = 0.61). Chlorpyrifos was the predominant insecticide detected in

these control samples. The samples that I sent to the lab that were not deployed in the field but had been attached to hardware cloth frames and stored in the back of a field vehicle contained detectable levels of bifenthrin ($\bar{x} = 0.35 \text{ ng/cm}^2$, $CV = 0.98$). Bifenthrin was the only target chemical detected in these samples.

Insecticide Deposition on Arthropod Samples

I detected target chemicals on arthropod samples at all distances (0–25 m) at both treatment and control sites (Fig. 6). I detected chlorpyrifos and cyhalothrin at treatment sites, but did not detect bifenthrin (Fig. 6, Appendix D). I detected all 3 target chemicals at control sites (Fig. 6, Appendix D).

Similar to PSD samples, I analyzed chlorpyrifos residues separately from other target chemicals. Chlorpyrifos was the insecticide with the greatest deposition values on arthropods (Appendix D). Mean chlorpyrifos residues on arthropods collected at 0 and 5 m from the field edge were greater at control sites than treatment sites (Table 7), and these values were different based on results of Welch's two-sample *t*-tests (Appendix E). Conversely, I found that mean chlorpyrifos residues on samples collected 25 m from the field edge were greater at treatment sites than control sites (Table 7) but Welch's two-sample *t*-tests did not indicate a statistically significant difference (Appendix E).

I compared chlorpyrifos residues on arthropods to the acute oral LD₅₀ values for house sparrows, ring-necked pheasants, and common grackles (Table 1). I also compared residues to the level at which sublethal exposure to chlorpyrifos has been shown to cause orientation impairment in white-crowned sparrows (Eng et al. 2017). Arthropods collected in grasslands 0–25 m from field edges at treatment sites contained mean chlorpyrifos residues lower than acute oral LD₅₀ values for all bird species I considered,

and levels were below those causing sublethal effects of orientation impairment in white-crowned sparrows (Table 7, Fig. 7). However, at some treatment sites at 25 m from field edges, samples contained residues greater than the acute oral LD₅₀ values for ring-necked pheasants and common grackles (Fig. 7). These samples also contained residues above the level associated with orientation impairment in white-crowned sparrows (Fig. 7).

I calculated the mass of chlorpyrifos a bird could consume in a day if every food item contained the amount of chlorpyrifos residues found in my arthropod samples. These values resulted from multiplying chlorpyrifos residues on arthropod samples (mg/kg) by the mass of food birds are reported to eat per day (kg; Solomon et al. 2001). When comparing the amount of chlorpyrifos that could be contained in birds' daily food to LD₅₀ values (mg/bird; Solomon et al. 2001), using maximum residue estimates resulted in values below oral LD₅₀ values for all bird species I considered: common grackles, house sparrows, northern bobwhites, red-winged blackbirds, and ring-necked pheasants (Table 8). When I used minimum residue estimates, the amounts of chlorpyrifos that could have been in birds' daily food intakes were further below the LD₅₀ values for every species (Table 9).

Using chlorpyrifos residue estimates on arthropod samples, I calculated the masses of contaminated arthropods that would be needed to reach LD₅₀ values for various bird species. I divided the LD₅₀ values for birds (mg/bird; Solomon et al. 2001) by the chlorpyrifos residues on arthropod samples (mg/kg). Using maximum residue estimates on arthropods resulted in arthropod masses much greater than what birds are reported to consume in a single day (Table 8). These calculated arthropod masses increased when I used minimum chlorpyrifos residue estimates in calculations (Table 9).

Model Selection: Insecticide Deposition on Passive Sampling Devices

The best-supported model of target chemical deposition on PSDs at treatment sites after accounting for weather and vegetation covariates included distance from the field edge and PSD height (Table 10). There was an inverse association between distance from grassland field edge and deposition ($\beta = -0.62$, 95% CI = -1.30 – 0.06). Deposition was greater on PSDs placed at the mid-canopy level than ground level ($\beta = 146.81$, 95% CI = -28.99 – 322.60). Spray method (i.e., ground or airplane) was not included in the best-supported model. Wind direction during insecticide spraying events, mean air temperature, and percent canopy cover of live vegetation were the environmental factors included in the best-supported models of insecticide deposition on PSDs. Sites downwind of sprayed fields had a positive association with target chemical deposition ($\beta = 123.19$, 95% CI = -298.89 – 545.27). Air temperature had an inverse association with deposition ($\beta = -54.14$, 95% CI = -112.21 – 3.94). Canopy cover of live vegetation also had an inverse association with deposition ($\beta = -6.02$, 95% CI = -12.16 – 0.12).

Model Selection: Insecticide Deposition on Arthropod Samples

The best-supported model of target chemical deposition on arthropod samples at treatment sites after accounting for weather and vegetation covariates did not include distance from grassland/field edge or spray method (Table 11). Mean air temperature and the maximum height of live vegetation were included in the best-supported models of insecticide deposition that considered weather and vegetation variables. Air temperature had an inverse association to deposition ($\beta = -544.19$, 95% CI = -937.41 – -150.98). The association between maximum height of vegetation and target chemical deposition was positive ($\beta = 272.97$, 95% CI = 2.10 – 543.84).

DISCUSSION

In the farmland region of Minnesota, I found detectable levels of target chemicals, particularly chlorpyrifos, within both treatment and control sites, suggesting that some background levels of insecticide drift were occurring across this landscape. Amounts of chemical drift occurring from agricultural fields can vary greatly, with one study finding foliar insecticide residues on PSDs located up to 2,000 m away from aerially-treated fields (Baio et al. 2019). Runquist et al. (2018) sampled grassland vegetation near sprayed fields in Minnesota and detected chlorpyrifos residues in all samples, with the greatest residue measuring 2,290 ppb along a grassland edge. Insecticide spraying was not observed in their vicinity when they collected their samples, indicating that chlorpyrifos residues were present in grasslands even in the absence of spraying on bordering fields (Runquist et al. 2018). Chlorpyrifos has been shown to persist in the environment after its initial application: its half-life is 4.2 hours in the atmosphere and 7-120 days in soils, and residues can remain on plant surfaces up to 14 days post-application, potentially accumulating through time (Solomon et al. 2001, Christensen et al. 2009).

The insecticide residues I measured at control study sites likely did not drift from the corn fields bordering these sites. Chlorpyrifos, cyhalothrin, and bifenthrin are used as foliar insecticides against corn pests in Minnesota; however, over 84% of the area planted to corn in Minnesota in 2018 contained seeds genetically modified to protect against insect pests (Potter et al. 2018). With these modifications, the need for foliar insecticides on corn has decreased considerably in recent years (L. Stahl, University of Minnesota Extension, personal communication). Thus, the likely sources of insecticide residues that

I measured on samples at control sites and the extremely low residues I detected on pre-spraying PSDs were sprayed soybean fields in the vicinity of my study sites.

Contaminated arthropods could also have immigrated to my control sites after receiving sub-lethal doses of insecticides from nearby treated fields (Longley et al. 1997). The bifenthrin residues I detected on PSDs that were not deployed were likely due to contamination from insecticide application in the Minnesota farmland landscape in which my study sites were located. Because these samples were stored in a vehicle used for fieldwork, exposure to bifenthrin could have occurred as I was driving through areas where this insecticide was recently applied.

Insecticide deposition on PSDs in my study decreased as distance from the soybean field edge increased. Several other studies have also documented edge effects of chemical drift from agricultural fields (Threadgill and Smith 1975, Bui et al. 1998, Langhof et al. 2005, Carlsen et al. 2006, Nsibande et al. 2015, Holterman et al. 2017, Baio et al. 2019). However, I found that distance from the field edge was not related to insecticide deposition on arthropods, likely because I collected arthropods only ≤ 25 m from the field edge. Twenty-five meters was likely insufficient for detecting trends in insecticide deposition over the gradient from field edge to grassland interior.

Alternatively, arthropods could have received lethal doses of insecticides and died before they could be captured by my sampling efforts.

Mean chlorpyrifos residues on PSDs exceeded the contact LD_{50} for honey bees 0–25 m from the field edge at treatment sites on mid-canopy height samplers and at sites bordered by fields sprayed via airplane (Table 6). Insecticides can also have sublethal effects that negatively impact arthropod physiology and behavior at levels below the

contact LD₅₀ (Desneux et al. 2007). Birds' dermal exposure to insecticides is not considered in the EPA's registration process, and contact LD₅₀ values for birds have not been established. Thus, I was unable to compare residues on PSDs to levels that may cause harmful effects to birds resulting from dermal exposure.

The mass of chlorpyrifos a bird could consume in a day (if every food item contained chlorpyrifos residues equivalent to those found in my arthropod samples) was below the acute oral LD₅₀ values for several farmland species including common grackles, house sparrows, northern bobwhites, red-winged blackbirds, and ring-necked pheasants (Table 8, Table 9). Arthropods in grasslands 0–25 m from sprayed fields contained chlorpyrifos levels lower than acute oral LD₅₀ values for all bird species I considered (Table 7, Fig. 7). Solomon et al.'s (2001) risk assessment also indicated that chlorpyrifos residues on invertebrates in agricultural systems were below oral acute LD₅₀ values for birds. Notably, although I collected chlorpyrifos residues from arthropod samples with unknown masses and made conversions from chlorpyrifos concentrations (ppb) using sample mass estimates, even using maximum estimates of chlorpyrifos residues per sample did not result in estimates of exposure that approached oral LD₅₀ values. My results, therefore, represent best estimates of worst-case scenarios of chlorpyrifos exposure to birds at my study sites.

The masses of contaminated arthropods in my study that would be needed to reach oral LD₅₀ values for various bird species were much greater than what birds could plausibly consume in a single day (Table 8, Table 9). I made these estimates with the assumption that birds would be consuming arthropods with chlorpyrifos residues on them, but Bennett, Jr. and Prince (1981) found that ring-necked pheasants avoided food

items treated with insecticides. In that study, pheasants detected the presence of insecticides on treated seeds, avoided treated food items when alternatives were available, and consumed less food when it was treated with insecticides (Bennett, Jr. and Prince 1981). Thus, the presence of insecticide residues could cause birds to avoid contaminated arthropod food items. Future studies considering birds' potential for indirect mortality from insecticides would benefit from measuring residues on individual food items and studying birds' avoidance of arthropods contaminated with chlorpyrifos.

Acute oral LD₅₀ values have several limitations in assessing potential effects of insecticide exposure in birds. First, acute oral LD₅₀ values do not reflect chronic exposure or multiple exposures to insecticides. Second, some mortality can still occur at exposures below LD₅₀ values. Third, the doses I considered were for adult animals, and in general, juveniles are more susceptible to negative effects from exposure to chemicals than adults (Solomon et al. 2001). Finally, negative effects can occur at sublethal doses, although such effects are difficult to quantify. Acute oral LD₅₀ values are determined by administering single doses to birds and evaluating mortality at increasing concentrations of insecticides in laboratory testing (U.S. EPA 2012). Effects of chronic or repeated oral exposure are less understood, and widespread use of soybean aphid insecticides in farmland landscapes could contribute to chronic exposure with direct and indirect effects on birds. Symptoms of exposure to sublethal doses of chlorpyrifos have been documented in birds in both lab and field studies (e.g., altered brain cholinesterase activity, altered behaviors, reduced weight gain, and impaired migratory ability; McEwen et al. 1986, Richards et al. 2000, Al-Badrany and Mohammad 2007, Moye 2008, Eng et al. 2017). Across breeding biomes, grassland birds have experienced the greatest population losses

in recent years with 74% of grassland species reported to be declining (Rosenberg et al. 2019). Chronic exposure to insecticides and detrimental sublethal effects could be contributing to these declines, and it would be beneficial to consider these potential population-level effects in future research.

Canopy cover of live vegetation had an inverse association to deposition on PSDs with higher deposition where vegetation was less dense. Other studies have also found that canopy cover is related to insecticide deposition resulting from drift (Praat et al. 2000, Donkersley and Nuyttens 2011, Holterman et al. 2017). Higher deposition in areas with less dense vegetation, in addition to mid-canopy PSDs having greater target chemical residues than ground-level PSDs, indicated that ground-nesting birds and other ground-dwelling wildlife may experience less direct exposure to insecticide drift than organisms in the upper canopy layer of grasslands. Height of vegetation had a positive association with deposition on arthropod samples. Taller vegetation causes greater air turbulence intensity that can prevent droplets from settling out of the air (Fogarty et al. 2018), which suggests that higher vegetation height would be associated with lower insecticide deposition. It is not clear why I did not observe this pattern in my study.

There is some evidence that higher temperatures promote drift by increasing evaporation rates and diminishing spray droplet size (Nuyttens et al. 2007, Donkersley and Nuyttens 2011). However, I observed an inverse association between mean air temperature and insecticide deposition on PSDs and arthropod samples. A previous study found that downwind spray deposition had a positive association with temperature up to 15° C whereas deposition appeared to decrease at temperatures above 15° C (Holterman et al. 2017). The mean air temperature at my treatment study sites ranged from 17–28° C

during spraying events. In temperatures $>15^{\circ}$ C, spray clouds can become more buoyant and spread vertically, causing dilution and decreasing deposition immediately downwind of the chemical application site (Holterman et al. 2017).

The method of insecticide application (i.e., airplane or ground sprayer) was not included in the best-supported models of insecticide deposition on PSDs and arthropods. I suspected that models including the interaction of spray method \times distance would best explain drift because my analyses showed that target chemical residues on PSDs had more variation (greater CVs) at treatment sites bordering fields sprayed by plane than those sprayed on the ground. However, this interaction term was not included in the best-supported model of insecticide deposition on PSDs or arthropods. Several other factors related to spraying equipment can impact drift, including spray droplet size, nozzle type, operating pressure, driving speed, boom height, and uncontrolled boom movements (Threadgill and Smith 1975; Nuyttens et al. 2007, 2017; Arvidsson et al. 2011; Donkersley and Nuyttens 2011; Nsibande et al. 2015). Furthermore, the application rate of insecticides and other pesticides has been shown to be an important predictor of drift (Donkersley and Nuyttens 2011, Nsibande et al. 2015, Baio et al. 2019). Although I requested information from cooperators regarding the spraying equipment and rate they used, I did not control for these variables in my study design. These factors likely influenced the amount of insecticides deposited on PSDs and arthropod samples.

Very low percentages of active ingredients applied in the field were deposited as drift on my PSDs at treatment sites. This is consistent with Carlsen et al. (2006), who showed 0.1–9% of applied amounts of herbicides were deposited on passive samplers near a field edge. However, PSDs contained very high percentages of applied ingredients

at site tB. The field bordering this site was sprayed by airplane and the site was downwind of the sprayed field. During the spraying event, the wind speed averaged 0.93 m/s and the ambient air temperature was 17 °C. The relative humidity was 90.53%, but high humidity values have been shown to correlate with lower drift rates due to low evaporation rates (Nuyttens et al. 2007). Thus, the spray method (i.e., via airplane) likely contributed more to these high drift measures than weather conditions.

Few field studies have documented direct mortality of birds or other grassland wildlife from insecticide exposure associated with foliar insecticide application in farmland landscapes. The ecotoxicological risk assessment of chlorpyrifos performed by Solomon et al. (2001) did not support the contention that the use of chlorpyrifos in agroecosystems results in extensive mortality of wildlife at the landscape level. However, my results indicate that insecticide deposition occurs in grasslands and on arthropods that inhabit these grasslands in a farmland landscape in Minnesota, suggesting that wildlife are being exposed to these insecticides in grasslands adjacent to row crops treated with foliar insecticides.

Management Implications

Although increasing the amount of grassland cover in the farmland region of Minnesota is a priority for natural resource managers, little is known about the exposure of grassland wildlife to insecticides applied to control insect pests in these landscapes. My results indicated that chemical deposition in grasslands was greatest ≤ 25 m from edges of soybean fields sprayed with foliar insecticides, suggesting that interior grassland cover ≥ 25 m from row crop edges may provide habitat with lower exposure to insecticides. Additionally, chlorpyrifos levels exceeded contact LD₅₀ values for honey

bees up to 25 m from the edges of treated soybean fields. Narrow strips of grassland cover (e.g., buffers created by the 2016 Minnesota Buffer Law) may be ecological traps or population sinks for organisms that inhabit them, in part due to potential exposure to agricultural insecticides. My results also suggest that management regimes that increase the percent canopy cover of vegetation have the potential to decrease exposure of grassland wildlife to insecticides. These results are relevant to tallgrass prairie systems beyond Minnesota to areas that share similar climates, topographies, and vegetation communities.

Table 1. LD₅₀ values for chlorpyrifos for various species of birds (Solomon et al. 2001) and pollinators. The acute contact LD₅₀ for honey bees (*Apis mellifera*) is reported as 70 ng/bee in Tomlin (2000) and was converted to ng/cm² by dividing this value by a honey bee's apparent exposure surface area (1.05 cm²; Poquet et al. 2014). The acute LD₅₀ is a common measure of acute toxicity and represents the lethal dose that causes death in 50% of animals from a single brief exposure. Exposure to chlorpyrifos in contaminated pollen and nectar of adult honey bees is also representative of non-*Apis* bee species (e.g., bumblebees; Cutler et al. 2014, U.S. EPA et al. 2014).

| Species | Acute oral LD₅₀ (mg/kg) | Acute oral LD₅₀ (mg/bird) | Acute toxicity classification | Mass of food eaten per day (kg) |
|---------------------------|---|--|--------------------------------------|--|
| <i>Avian species</i> | | | | |
| Common grackle | 8.5 | 0.97 | Very highly toxic | 0.034 |
| House sparrow | 29.5 | 0.83 | Highly toxic | 0.008 |
| Northern bobwhite | 32 | 5.70 | Highly toxic | 0.053 |
| Red-winged blackbird | 13.2 | 0.69 | Highly toxic | 0.016 |
| Ring-necked pheasant | 12.2 | 13.85 | Very highly toxic | 0.114 |
| | | Acute contact LD₅₀ (ng/cm²) | Acute toxicity classification | |
| <i>Pollinator species</i> | | | | |
| Honey bee | | 66.67 | Highly toxic | |

Table 2. Weather, vegetation, and primary factors of interest and their hypothesized relationships to chemical deposition. Each set of covariates constitute steps in the hierarchical modeling process used to assess insecticide deposition on passive sampling devices (PSDs) and arthropods in Minnesota’s farmland region. Height was included in models of insecticide deposition on PSDs but not in models of deposition on arthropods.

| Acronym | | Hypothesized relationship to chemical deposition | Rationale |
|------------------------------------|--|---|---|
| <i>Weather</i> | | | |
| TEMP | Ambient air temperature (°C) | Greater deposition associated with greater temperatures | Nuyttens et al. 2007, Arvidsson et al. 2011, Donkersley and Nuyttens 2011 |
| WDIR | Wind direction (whether the study site was downwind of the sprayed field or not) | Greater deposition associated with sites that were downwind of sprayed fields | Holterman et al. 2017 |
| WSP | Wind speed (m/s) | Greater deposition associated with faster wind speeds | Arvidsson et al. 2011, Nsibande et al. 2015, Holterman et al. 2017, Nuyttens et al. 2017, Baio et al. 2019 |
| <i>Vegetation</i> | | | |
| CCLIVE | Percentage of the canopy consisting of live vegetation (%) | Greater deposition associated with lesser percent canopy cover | Praat et al. 2000, Donkersley and Nuyttens 2011, Holterman et al. 2017 |
| MHL | Maximum height of live vegetation (dm) | Greater deposition associated with lower vegetation height | Fogarty et al. 2018 |
| VOR | Visual Obstruction Reading (VOR) | Greater deposition associated with lesser vegetation density | Donkersley and Nuyttens 2011 |
| <i>Primary factors of interest</i> | | | |
| DIST | Distance from grassland/field edge (m) | Greater deposition associated with shorter distance from edge | Threadgill and Smith 1975, Bui et al. 1998, Langhof et al. 2005, Carlsen et al. 2006, Nsibande et al. 2015, Holterman et al. 2017, Baio et al. 2019 |
| HT | Height of PSD (ground or mid-canopy) | Greater deposition associated with mid-canopy PSDs | Langhof et al. 2005 |
| SPRAY | Spray method (airplane or ground sprayer) | Greater deposition associated with airplane sprayers | Nuyttens et al. 2007 |

Table 3. Locations and sampling dates of study sites during the summers of 2017 and 2018 in Minnesota's farmland region. Filter paper and arthropod samples were collected to assess insecticide deposition from adjacent soybean fields. Regions of Minnesota sampled in this study include the southwest (SW), west central (WC), and central (C) regions. Treatment sites were grasslands adjacent to soybean fields that were sprayed with insecticides to control aphids; control sites were grasslands adjacent to corn fields that were not sprayed for aphids.

| Site ID | Region | County | Site type | Year | Dates when field sampling occurred |
|----------------|---------------|-----------------|------------------|-------------|---|
| tA | SW | Jackson | Treatment | 2017 | 29 July |
| tB | SW | Murray | Treatment | 2017 | 11 August |
| cA | SW | Jackson | Control | 2017 | 24 August |
| cB | SW | Lyon | Control | 2017 | 12 August |
| tC | WC | Lac qui Parle | Treatment | 2018 | 8–10 August |
| tD | C | Stearns | Treatment | 2018 | 27–28 July |
| tE | WC | Yellow Medicine | Treatment | 2018 | 6–7 August |
| cC | C | Kandiyohi | Control | 2018 | 17–18 August |
| cD | WC | Lac qui Parle | Control | 2018 | 18–21 July |

Table 4. Spraying methods and other application information for soybean aphid spraying events by cooperating landowners. Insecticide applications occurred on soybean fields adjacent to treatment sites that were sampled during the summers of 2017 and 2018 in Minnesota's farmland region. Formulated products are commercially available that contain active ingredients and inert ingredients, and their labels provide recommended application rates and percentages of active ingredients. Sprayer application rate refers to the rate of tank mix applied to the field; a tank mix could include combinations of insecticides, other pesticides, adjuvants, and solvents. Some landowners declined to provide some information.

| Site ID | Spray method | Trade name of formulated product | Insecticide active ingredients | Formulated product application rate (L/ha) | Active ingredient application rate (ng/cm ²) ^a | Sprayer application rate (L/ha) | Application speed (m/s) | Boom height (m) | Tank pressure (kPa) |
|---------|--------------|----------------------------------|-----------------------------------|--|---|---------------------------------|-------------------------|-----------------|---------------------|
| tA | Ground | Endigo™ | lambda-cyhalothrin + thiamethoxam | 0.26 | 271.1 + 360.6 | 140.3 | 4 | 0.2–0.3 | 275.8 |
| tB | Airplane | Bolton™ | chlorpyrifos + gamma-cyhalothrin | 0.88 | 2627.0 + 87.2 | 18.7 | 67.9 | 1.5 | 275.8 |
| tC | Ground | Lorsban®-4E | chlorpyrifos | NA | 8406.4 ^b | 93.5 | NA | NA | 137.9–206.8 |
| tD | Airplane | Lorsban® Advanced | chlorpyrifos | 1.17 | 5261.0 | 18.7 | 55.9 | 2.7–4.0 | 275.8 |
| tE | Airplane | Lorsban® Advanced; Warrior II® | chlorpyrifos; lambda-cyhalothrin | 0.44; 0.22 | 1972.9; 546.4 | NA | NA | NA | NA |

^a Values were calculated using landowner-reported formulated product application rates and product label information.

^b Value was calculated using an estimated formulated product application rate of 1.75 L/ha based on label recommendations.

Table 5. Means and coefficients of variation (\bar{x} [CV]) of target chemicals (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) detected on passive sampling devices (PSDs) by distance from soybean field edge to grassland interior. Samples were collected in Minnesota's farmland region in 2017 and 2018. Treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides; control sites were grasslands adjacent to unsprayed corn fields. PSD height refers to samplers placed at mid-canopy height (0.5 m above ground) or ground level (0 m above ground) and these calculations included samples from treatment sites with both spray methods. Spray method refers to whether the bordering soybean field was sprayed using an airplane or ground boom and these calculations included samples from both mid-canopy and ground height PSDs. Mean values are reported in ng/cm².

| Site type | Distance from soybean field edge | | | | | | |
|---------------------|----------------------------------|---------------|---------------|-------------|-------------|-------------|--------------|
| | 0 m | 5 m | 25 m | 50 m | 100 m | 200 m | 400 m |
| Treatment | 351.44 (4.11) | 143.86 (3.82) | 265.81 (3.48) | 3.83 (2.35) | 0.37 (1.64) | 0.13 (1.44) | 6.96 (5.02) |
| <i>PSD height</i> | | | | | | | |
| Mid-canopy | 616.68 (3.29) | 257.4 (2.99) | 383.14 (3.28) | 5.66 (2.11) | 0.47 (1.56) | 0.18 (1.35) | 13.8 (3.58) |
| Ground | 86.2 (2.8) | 30.31 (2.36) | 148.47 (2.68) | 2 (2.16) | 0.27 (1.68) | 0.09 (1.38) | 0.11 (0.96) |
| <i>Spray method</i> | | | | | | | |
| Airplane | 569.15 (3.25) | 239.57 (2.93) | 442.84 (2.65) | 6.26 (1.77) | 0.47 (1.61) | 0.07 (1.31) | 0.08 (0.99) |
| Ground | 24.89 (2.22) | 0.28 (1.05) | 0.25 (1.05) | 0.19 (1.1) | 0.23 (1.18) | 0.22 (1.17) | 22.43 (2.81) |
| Control | 0.41 (1.85) | 0.2 (0.95) | 0.2 (0.9) | 0.21 (0.97) | 0.22 (1.03) | 0.19 (0.98) | 0.29 (1.02) |

Table 6. Means and coefficients of variation (\bar{x} [CV]) of chlorpyrifos residues detected on passive sampling devices (PSDs) by distance from soybean field edge to grassland interior. Samples were collected in Minnesota's farmland region in 2017 and 2018. Treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides; control sites were grasslands adjacent to unsprayed corn fields. PSD height refers to samplers placed at mid-canopy height (0.5 m above ground) or ground level (0 m above ground) and these calculations included samples from treatment sites with both spray methods. Spray method refers to whether the bordering soybean field was sprayed using an airplane or ground boom and these calculations included samples from both mid-canopy and ground height PSDs. Mean values are reported in ng/cm².

| Site type | Distance from soybean field edge | | | | | | |
|---------------------|----------------------------------|---------------|---------------|-------------|-------------|-------------|--------------|
| | 0 m | 5 m | 25 m | 50 m | 100 m | 200 m | 400 m |
| Treatment | 346.99 (4.15) | 141.88 (3.86) | 263.58 (3.5) | 3.71 (2.36) | 0.36 (1.62) | 0.13 (1.44) | 6.96 (5.02) |
| <i>PSD height</i> | | | | | | | |
| Mid-canopy | 611.42 (3.31) | 254.2 (3.02) | 380.84 (3.29) | 5.48 (2.12) | 0.46 (1.53) | 0.18 (1.35) | 13.8 (3.58) |
| Ground | 82.55 (2.91) | 29.56 (2.37) | 146.33 (2.7) | 1.93 (2.16) | 0.26 (1.65) | 0.09 (1.38) | 0.11 (0.96) |
| <i>Spray method</i> | | | | | | | |
| Airplane | 561.72 (3.29) | 236.28 (2.95) | 439.14 (2.66) | 6.05 (1.78) | 0.45 (1.59) | 0.07 (1.31) | 0.08 (0.99) |
| Ground | 24.88 (2.22) | 0.28 (1.05) | 0.25 (1.05) | 0.19 (1.1) | 0.23 (1.18) | 0.22 (1.17) | 22.43 (2.81) |
| Control | 0.37 (1.93) | 0.19 (1.02) | 0.19 (1) | 0.21 (0.99) | 0.21 (1.1) | 0.18 (1.05) | 0.23 (0.93) |

Table 7. Means and coefficients of variation (\bar{x} [CV]) of chlorpyrifos residues detected on arthropod samples by distance from soybean field edge to grassland interior in Minnesota's farmland region in the summers of 2017 and 2018. Treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides either by airplanes or ground sprayers; control sites were grasslands adjacent to unsprayed corn fields. Means are reported in mg/kg. Residues were collected from arthropod samples with unknown masses, and calculations converting parts per billion to mg/kg included the maximum estimates of target chemical residues per sample.

| Site type | Distance from soybean field edge | | |
|------------------|---|--------------|-------------|
| | 0 m | 5 m | 25 m |
| Treatment | 0.044 (1.14) | 0.034 (0.73) | 2.15 (2.64) |
| Control | 0.44 (1.11) | 0.50 (1.22) | 0.55 (1.32) |

Table 8. Minimums, maximums, and coefficients of variation (CV) of the masses of chlorpyrifos that would be in a birds' daily food intake if consuming items with arthropod sample residue amounts estimated in this study, and masses of arthropods needed to reach the LD₅₀ values for various bird species. Chlorpyrifos residues were collected from arthropod samples with unknown masses and calculations included the **maximum** estimates of chlorpyrifos residues per sample. Chlorpyrifos residues (mg/kg) were multiplied by the masses of food that various bird species typically consume in one day (kg; Solomon et al. 2001) to calculate masses of chlorpyrifos in daily food intake. LD₅₀ values for birds (mg/bird; Solomon et al. 2001) were divided by the chlorpyrifos residues on arthropod samples (mg/kg) to calculate masses of arthropods needed to reach LD₅₀ values. Treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides either by airplanes or ground sprayers; control sites were grasslands adjacent to unsprayed corn fields. Arthropod samples were collected during the summers of 2017 and 2018 in Minnesota's farmland region.

| Species | Mass of food eaten/day (kg) | LD₅₀ (mg/bird) | Mass of chlorpyrifos in daily food (mg) [min-max (CV)] | Mass of arthropods to reach LD₅₀ (kg) [min-max (CV)] |
|------------------------|------------------------------------|----------------------------------|---|--|
| <i>Treatment sites</i> | | | | |
| Common grackle | 0.034 | 0.97 | 0–0.65 (4.51) | 0.05–162 (0.97) |
| House sparrow | 0.008 | 0.83 | 0–0.16 (4.51) | 0.04–138 (0.97) |
| Northern bobwhite | 0.053 | 5.70 | 0–1.01 (4.51) | 0.3–949 (0.97) |
| Red-winged blackbird | 0.016 | 0.69 | 0–0.29 (4.51) | 0.04–114 (0.97) |
| Ring-necked pheasant | 0.114 | 13.85 | 0–2.14 (4.51) | 0.73–2,308 (0.97) |
| <i>Control sites</i> | | | | |
| Common grackle | 0.034 | 0.97 | 0–0.08 (1.2) | 0.41–969 (2.21) |
| House sparrow | 0.008 | 0.83 | 0–0.02 (1.2) | 0.35–826 (2.21) |
| Northern bobwhite | 0.053 | 5.70 | 0–0.13 (1.2) | 2.4–5,696 (2.21) |
| Red-winged blackbird | 0.016 | 0.69 | 0–0.04 (1.2) | 0.29–686 (2.21) |
| Ring-necked pheasant | 0.114 | 13.85 | 0–0.27 (1.2) | 5.83–13,847 (2.21) |

Table 9. Minimums, maximums, and coefficients of variation (CV) of the masses of chlorpyrifos that would be in a birds' daily food intake if consuming items with arthropod sample residue amounts estimated in this study, and masses of arthropods needed to reach the LD₅₀ values for various bird species. Chlorpyrifos residues were collected from arthropod samples with unknown masses, and calculations included the **minimum** estimates of chlorpyrifos residues per sample. Chlorpyrifos residues (mg/kg) were multiplied by the masses of food that various bird species typically consume in one day (kg; Solomon et al. 2001) to calculate masses of chlorpyrifos in daily food intake. LD₅₀ values for birds (mg/bird; Solomon et al. 2001) were divided by the chlorpyrifos residues on arthropod samples (mg/kg) to calculate masses of arthropods needed to reach LD₅₀ values. Treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides either by airplanes or ground sprayers; control sites were grasslands adjacent to unsprayed corn fields. Arthropod samples were collected during the summers of 2017 and 2018 in Minnesota's farmland region.

| Species | Mass of food eaten/day (kg) | LD ₅₀ (mg/bird) | Mass of chlorpyrifos in daily food (mg) [min-max (CV)] | Mass of arthropods to reach LD ₅₀ (kg) [min-max (CV)] |
|------------------------|-----------------------------|----------------------------|--|--|
| <i>Treatment sites</i> | | | | |
| Common grackle | 0.034 | 0.97 | 0–0.18 (4.43) | 0.18–346 (0.86) |
| House sparrow | 0.008 | 0.83 | 0–0.04 (4.43) | 0.15–295 (0.86) |
| Northern bobwhite | 0.053 | 5.70 | 0–0.29 (4.43) | 1.07–2,034 (0.86) |
| Red-winged blackbird | 0.016 | 0.69 | 0–0.08 (4.43) | 0.13–245 (0.86) |
| Ring-necked pheasant | 0.114 | 13.85 | 0–0.61 (4.43) | 2.59–4,945 (0.86) |
| <i>Control sites</i> | | | | |
| Common grackle | 0.034 | 0.97 | 0–0.02 (1.17) | 1.44–1,211 (1.78) |
| House sparrow | 0.008 | 0.83 | 0–0.01 (1.17) | 1.23–1,033 (1.78) |
| Northern bobwhite | 0.053 | 5.70 | 0–0.04 (1.17) | 8.48–7,120 (1.78) |
| Red-winged blackbird | 0.016 | 0.69 | 0–0.01 (1.17) | 1.02–858 (1.78) |
| Ring-necked pheasant | 0.114 | 13.85 | 0–0.08 (1.17) | 20.61–17,309 (1.78) |

Table 10. Number of parameters (K), Akaike's Information Criterion corrected for sample size (AIC_c ; $n = 206$), conditional R^2 value (R^2 ; variation explained by the entire model including random effects), deviance (d), and model weight (ω) for models of target chemical deposition (ng/cm^2) onto passive sampling devices (PSDs) at treatment study sites in the farmland region of Minnesota during the summers of 2017 and 2018. PSDs were used to assess direct exposure of wildlife to drift from target insecticides (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) sprayed to control soybean aphids. A hierarchical model selection approach was used in which the first set of models assessed weather conditions during the spraying event: whether the study site was downwind of the sprayed field (WDIR), ambient air temperature (TEMP), and wind speed (WSP). The best-supported weather model was then used as a base model to assess vegetation covariates in step 2: percentage of the canopy consisting of live vegetation (CCLIVE), maximum height of live vegetation (MHL), and the vertical density (visual obstruction reading) from the direction of the sprayed field (VOR). The best-supported weather + vegetation model was then used in step 3 to assess primary factors of interest: distance of the PSD from the grassland/soybean edge (DIST), whether the PSD was placed at mid-canopy or ground level (HT), and whether insecticides were applied via airplane or ground sprayer (SPRAY). The column ΔAIC_c compares models within each step of model development. Models were linear mixed models, included site as a random effect, and were fitted using the maximum likelihood method.

| Model | K | AIC_c | ΔAIC_c | R^2 | d | ω |
|-------------------------------|----------|---------------------------|----------------------------------|-------------------------|----------|----------------------------|
| Step one: | | | | | | |
| WDIR + TEMP | 5 | 3,266.75 | 0 | 0.096 | 3,256.45 | 0.44 |
| TEMP | 4 | 3,267.55 | 0.81 | 0.083 | 3,259.35 | 0.29 |
| WSP + WDIR + TEMP | 6 | 3,268.86 | 2.12 | 0.096 | 3,256.44 | 0.15 |
| WSP + TEMP | 5 | 3,269.56 | 2.82 | 0.083 | 3,259.26 | 0.11 |
| WDIR | 4 | 3,274.32 | 7.58 | 0.072 | 3,266.12 | 0.0099 |
| WSP + WDIR | 5 | 3,276.42 | 9.68 | 0.072 | 3,266.12 | 0.0034 |
| WSP | 4 | 3,276.73 | 9.99 | 0.072 | 3,268.54 | 0.0029 |
| Step two: | | | | | | |
| WEATHER ^a + CCLIVE | 6 | 3,264.79 | 0 | 0.11 | 3,252.37 | 0.30 |
| WEATHER + MHL + CCLIVE | 7 | 3,265.97 | 1.18 | 0.12 | 3,251.40 | 0.17 |
| WEATHER + MHL | 6 | 3,266.37 | 1.58 | 0.11 | 3,253.95 | 0.14 |
| WEATHER | 5 | 3,266.75 | 1.95 | 0.096 | 3,256.45 | 0.11 |

| | | | | | | |
|---------------------------------|----|----------|------|-------|----------|-------|
| WEATHER + VOR + CCLIVE | 7 | 3,266.92 | 2.13 | 0.11 | 3,252.35 | 0.10 |
| WEATHER + MHL + VOR + CCLIVE | 8 | 3,267.62 | 2.83 | 0.12 | 3,250.89 | 0.074 |
| WEATHER + MHL + VOR | 7 | 3,268.39 | 3.60 | 0.11 | 3,253.82 | 0.050 |
| WEATHER + VOR | 6 | 3,268.54 | 3.75 | 0.097 | 3,256.12 | 0.047 |
| Step three: | | | | | | |
| VEG ^b + DIST + HT | 8 | 3,263.28 | 0 | 0.14 | 3,246.55 | 0.21 |
| VEG + DIST | 7 | 3,263.81 | 0.53 | 0.13 | 3,249.24 | 0.16 |
| VEG + HT | 7 | 3,264.28 | 1.00 | 0.13 | 3,249.71 | 0.13 |
| VEG | 6 | 3,264.79 | 1.51 | 0.11 | 3,252.37 | 0.10 |
| VEG + HT + SPRAY * DIST | 10 | 3,265.07 | 1.79 | 0.15 | 3,243.94 | 0.087 |
| VEG + DIST + SPRAY + HT | 9 | 3,265.17 | 1.89 | 0.14 | 3,246.25 | 0.083 |
| VEG + SPRAY * DIST | 9 | 3,265.59 | 2.31 | 0.14 | 3,246.67 | 0.067 |
| VEG + DIST + SPRAY | 8 | 3,265.68 | 2.40 | 0.13 | 3,248.95 | 0.064 |
| VEG + SPRAY + HT | 8 | 3,266.19 | 2.91 | 0.13 | 3,249.46 | 0.050 |
| VEG + SPRAY | 7 | 3,266.68 | 3.40 | 0.11 | 3,252.11 | 0.039 |

^a WEATHER = covariates in the top-ranked Weather model (WDIR + TEMP) from step 1.

^b VEG = covariates in the top-ranked Weather and Vegetation model (WDIR + TEMP + CCLIVE) from step 2.

Table 11. Number of parameters (K), Akaike's Information Criterion corrected for sample size (AIC_c ; $n = 45$), conditional R^2 value (R^2 ; variation explained by the entire model including random effects), deviance (d), and model weight (ω) for models of target chemical deposition (ng/g) on arthropod samples collected from treatment study sites in the farmland region of Minnesota during the summers of 2017 and 2018. Arthropods were used to assess potential for indirect exposure of wildlife to drift from target insecticides (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) sprayed to control soybean aphids. A hierarchical model selection approach was used in which the first set of models assessed weather conditions during the spraying event: whether the study site was downwind of the sprayed field (WDIR), ambient air temperature (TEMP), and wind speed (WSP). The best-supported weather model was then used as a base model in step 2 to assess vegetation covariates: percentage of the canopy consisting of live vegetation (CCLIVE), maximum height of live vegetation (MHL), and vertical density (visual obstruction reading) from the direction of the sprayed field (VOR). The best-supported weather + vegetation model was then used in step 3 to assess primary factors of interest: distance from the grassland/soybean edge (DIST) and whether insecticides were applied via airplane or ground sprayer (SPRAY). The column ΔAIC_c compares models within each step of model development. Models were linear mixed models, included site as a random effect, and were fitted using the maximum likelihood method. Insecticide residues were collected from arthropod samples with unknown masses, and calculations converting parts per billion to ng/g included the maximum estimates of target chemical residues per sample.

| Model | K | AIC_c | ΔAIC_c | R^2 | d | ω |
|----------------------------|----------|---------------------------|----------------------------------|-------------------------|----------|----------------------------|
| Step one: | | | | | | |
| TEMP | 4 | 855.21 | 0 | 0.25 | 846.21 | 0.41 |
| WDIR + TEMP | 5 | 855.57 | 0.36 | 0.29 | 844.03 | 0.35 |
| WSP + TEMP | 5 | 857.68 | 2.47 | 0.25 | 846.14 | 0.12 |
| WSP + WDIR + TEMP | 6 | 858.23 | 3.02 | 0.29 | 844.02 | 0.092 |
| WDIR | 4 | 861.67 | 6.46 | 0.19 | 852.67 | 0.016 |
| WSP | 4 | 863.93 | 8.72 | 0.19 | 854.93 | 0.0053 |
| WSP + WDIR | 5 | 864.21 | 9.00 | 0.19 | 852.67 | 0.0046 |
| Step two: | | | | | | |
| WEATHER ^a + MHL | 5 | 853.77 | 0 | 0.31 | 842.24 | 0.32 |
| WEATHER + MHL + VOR | 6 | 854.11 | 0.34 | 0.35 | 839.9 | 0.27 |
| WEATHER | 4 | 855.21 | 1.44 | 0.25 | 846.21 | 0.15 |

| | | | | | | |
|---------------------------------|---|--------|------|------|--------|-------|
| WEATHER + MHL + CCLIVE | 6 | 856.21 | 2.44 | 0.32 | 842.00 | 0.094 |
| WEATHER + MHL + VOR + CCLIVE | 7 | 856.92 | 3.15 | 0.35 | 839.90 | 0.066 |
| WEATHER + VOR | 5 | 857.72 | 3.95 | 0.25 | 846.19 | 0.044 |
| WEATHER + CCLIVE | 5 | 857.74 | 3.97 | 0.25 | 846.20 | 0.044 |
| WEATHER + VOR + CCLIVE | 6 | 860.37 | 6.60 | 0.25 | 846.16 | 0.012 |
| Step three: | | | | | | |
| VEG ^b | 5 | 853.77 | 0 | 0.31 | 842.24 | 0.46 |
| VEG + DIST | 6 | 854.95 | 1.18 | 0.34 | 840.74 | 0.25 |
| VEG + SPRAY | 6 | 856.10 | 2.33 | 0.32 | 841.89 | 0.14 |
| VEG + DIST + SPRAY | 7 | 857.29 | 3.52 | 0.34 | 840.27 | 0.078 |
| VEG + SPRAY * DIST | 8 | 857.46 | 3.68 | 0.38 | 837.46 | 0.072 |

^a WEATHER = covariates in the top-ranked Weather model (TEMP) from step 1.

^b VEG = covariates in the top-ranked Weather and Vegetation model (TEMP + MHL) from step 2.

Figure 1. Locations of treatment (purple symbols) and control sites (green symbols) in the farmland region of Minnesota during 2017 (square symbols) and 2018 (circle symbols) field sampling efforts. Treatment sites were grasslands adjacent to soybean fields sprayed for aphids; control sites were grasslands adjacent to corn fields that were not sprayed with insecticides to control soybean aphids. Regions shown include: SW = southwest, SC = south central, WC = west central, and C = central.

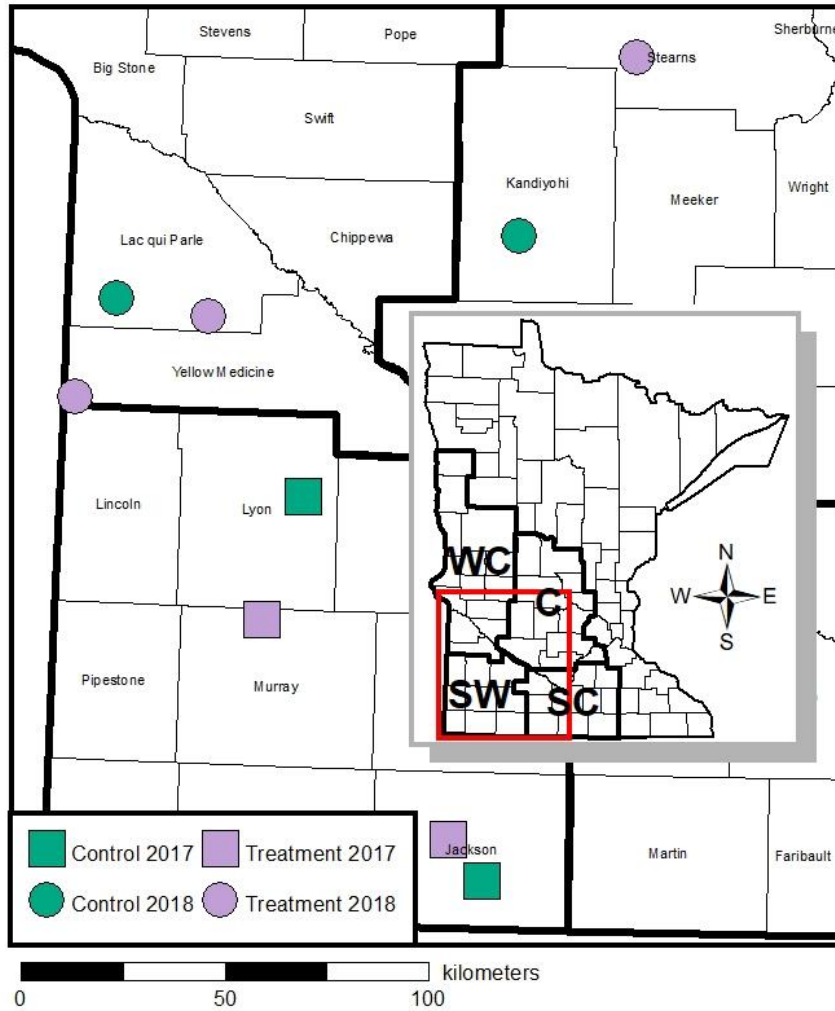


Figure 2. Field sampling design used to assess the exposure of grassland wildlife to soybean aphid insecticides in the farmland region of Minnesota during the summers of 2017 and 2018. Sampling was conducted on grasslands adjacent to privately owned soybean fields sprayed for aphid infestations. Black lines indicate primary sampling transects established perpendicular to the field edge (orange line) and extending into the grassland. Sampling stations (white circles) were placed 0, 5, 25, 50, 100, and 200 m from the field edge. An additional station at 400 m was added if the size of the grassland allowed.

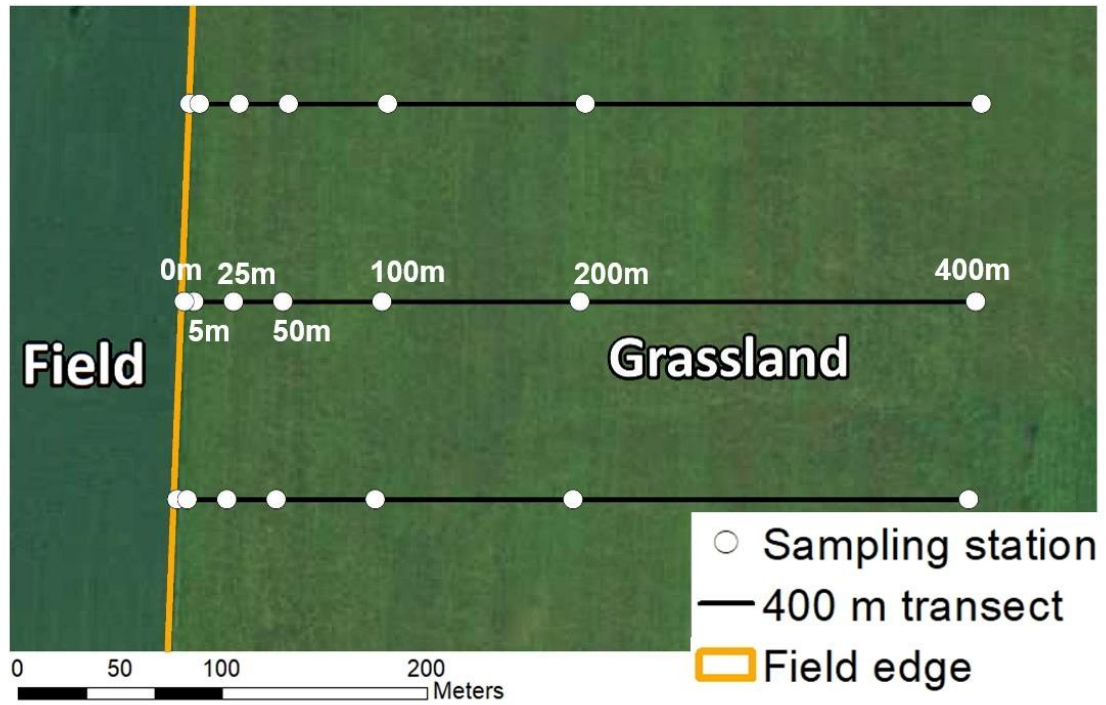


Figure 3. Target chemical (i.e., chlorpyrifos, cyhalothrin, and bifenthrin) deposition on passive sampling devices (PSDs; $n = 368$) by distance from field edge to grassland interior at treatment sites and control sites. Sampling was conducted during the summers of 2017 and 2018 in Minnesota's farmland region. Control sites were grasslands adjacent to corn fields that were not treated with insecticides during sampling; treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides either by airplanes or ground sprayers. Negative values on the y-axis resulted when calculating the logarithm of values between 0 and 1.

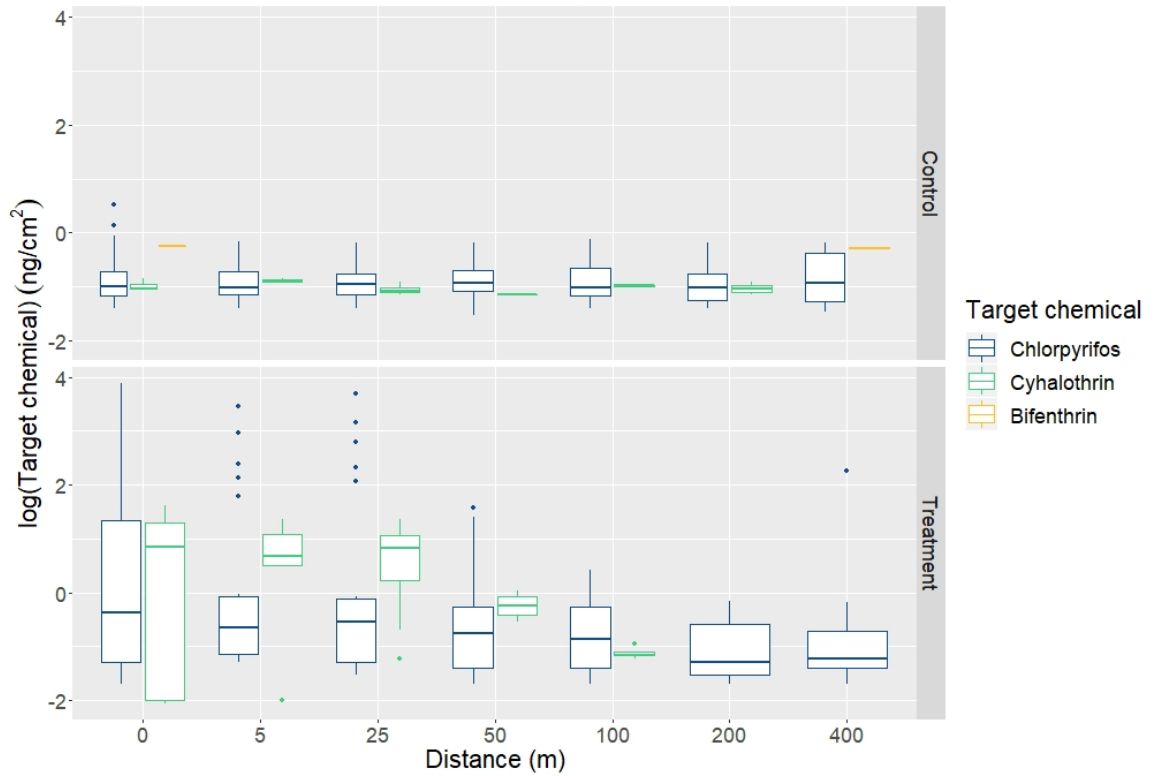


Figure 4. Chlorpyrifos deposition on passive sampling devices (PSDs; $n = 368$) by distance from field edge to grassland interior at treatment sites and control sites. White bars represent PSDs deployed at mid-canopy height (0.5 m above ground); gray bars represent PSDs deployed at ground level (0 m above ground). The horizontal dashed line represents the contact LD₅₀ for honey bees (*Apis mellifera*; 66.67 ng/cm², see Table 1). Sampling was conducted during the summers of 2017 and 2018 in Minnesota's farmland region. Treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides either by airplanes or ground sprayers; control sites were grasslands adjacent to unsprayed corn fields. Negative values on the y-axis resulted when calculating the logarithm of values between 0 and 1.

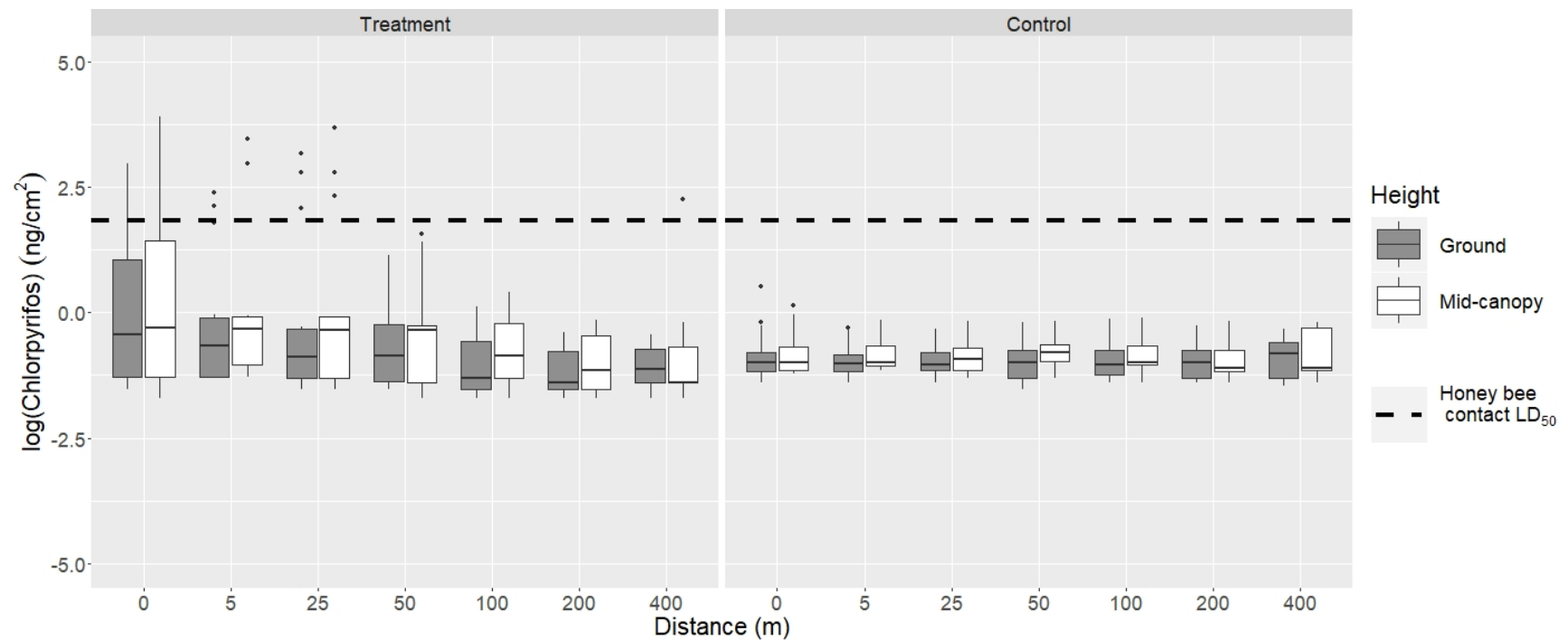


Figure 5. Percentages of applied active ingredients captured as drift on passive sampling devices (PSDs; $n = 206$) at treatment sites. Sampling was conducted during the summers of 2017 and 2018 in Minnesota's farmland region. Treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides either by airplanes or ground sprayers. Codes in upper right corners of plots correspond to site IDs (see Table 4). At treatment site tA, the landowner reported using thiamethoxam but this insecticide was not detected on PSDs. Note that y-axes differ among plots.

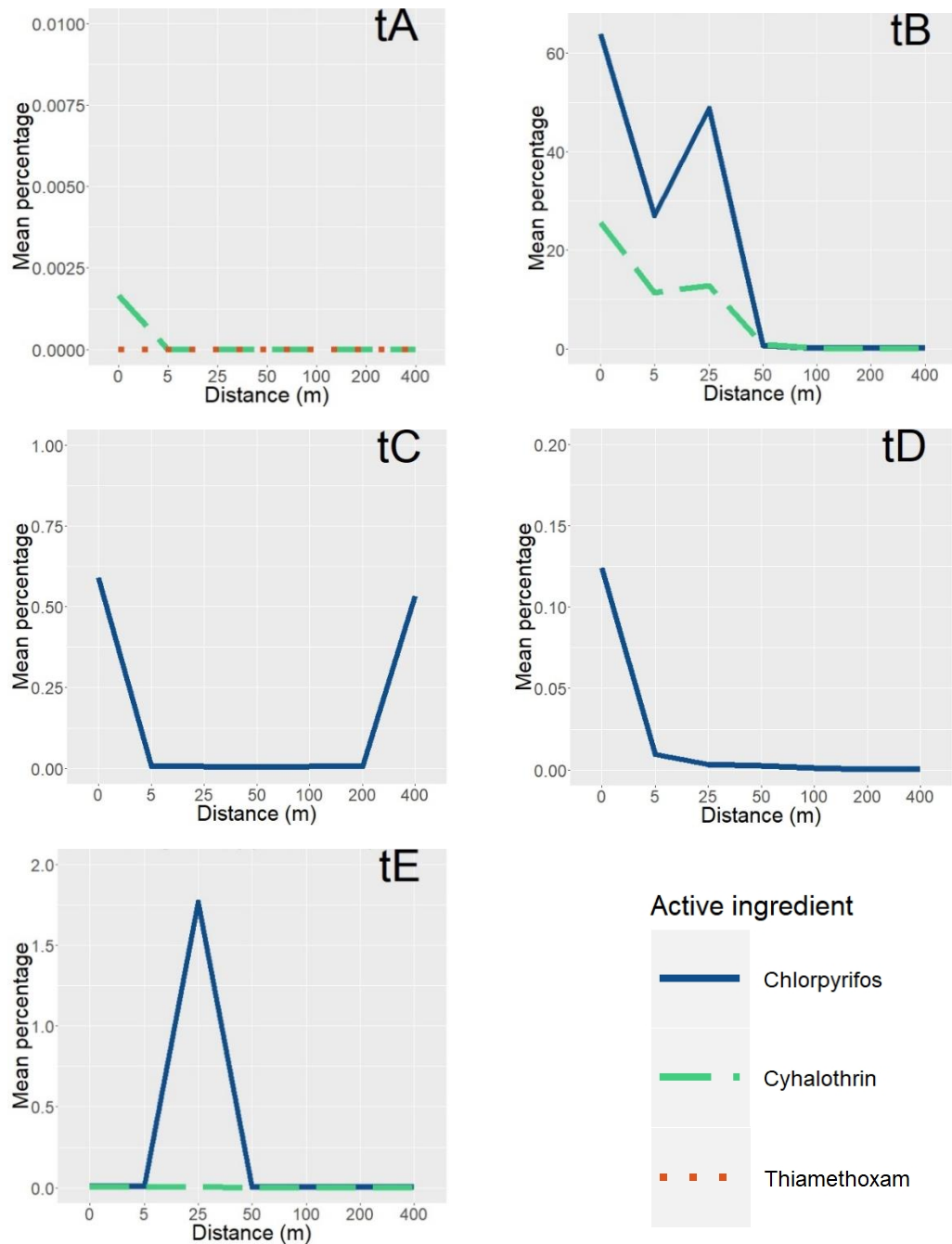


Figure 6. Target chemical (i.e., chlorpyrifos, cyhalothrin, and bifenthrin) deposition on arthropod samples ($n = 81$) by distance from field edge to grassland interior at treatment sites and control sites. Sampling was conducted during the summers of 2017 and 2018 in Minnesota's farmland region. Control sites were grasslands adjacent to corn fields that were not treated with insecticides during sampling; treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides either by airplanes or ground sprayers. Residues were collected from arthropod samples with unknown masses, and calculations converting parts per billion to ng/g included the maximum estimates of target chemical residues per sample.

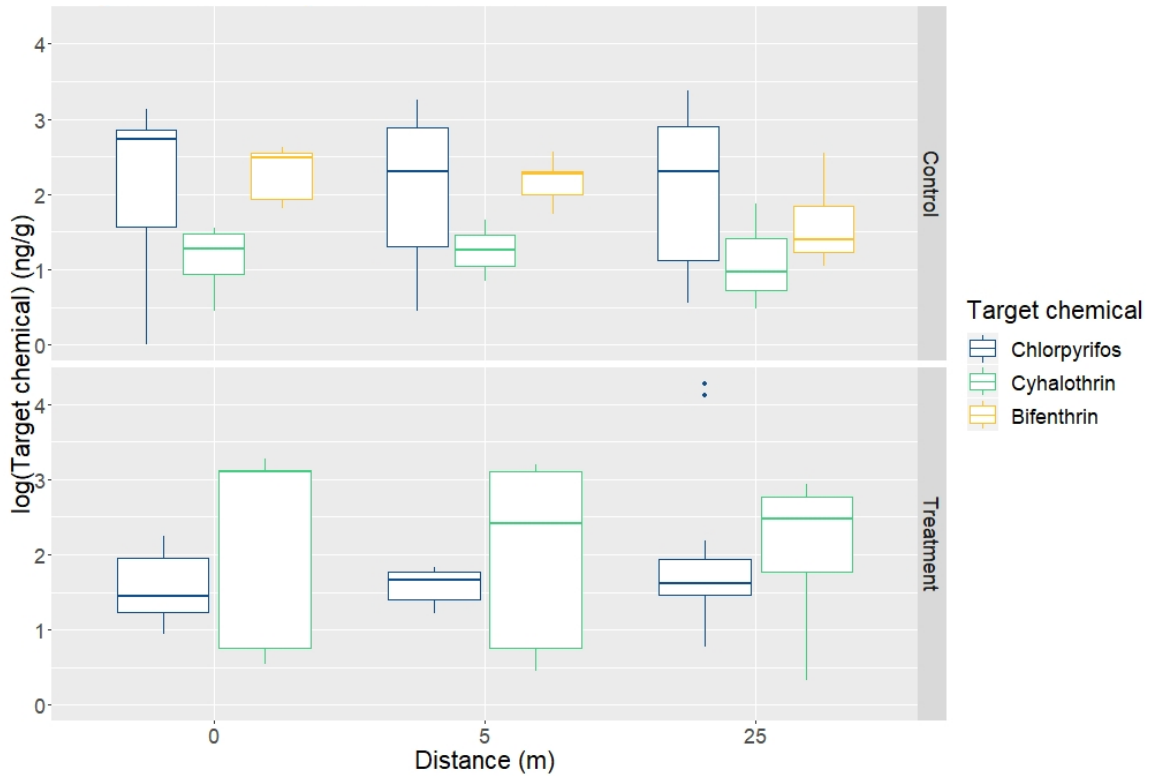
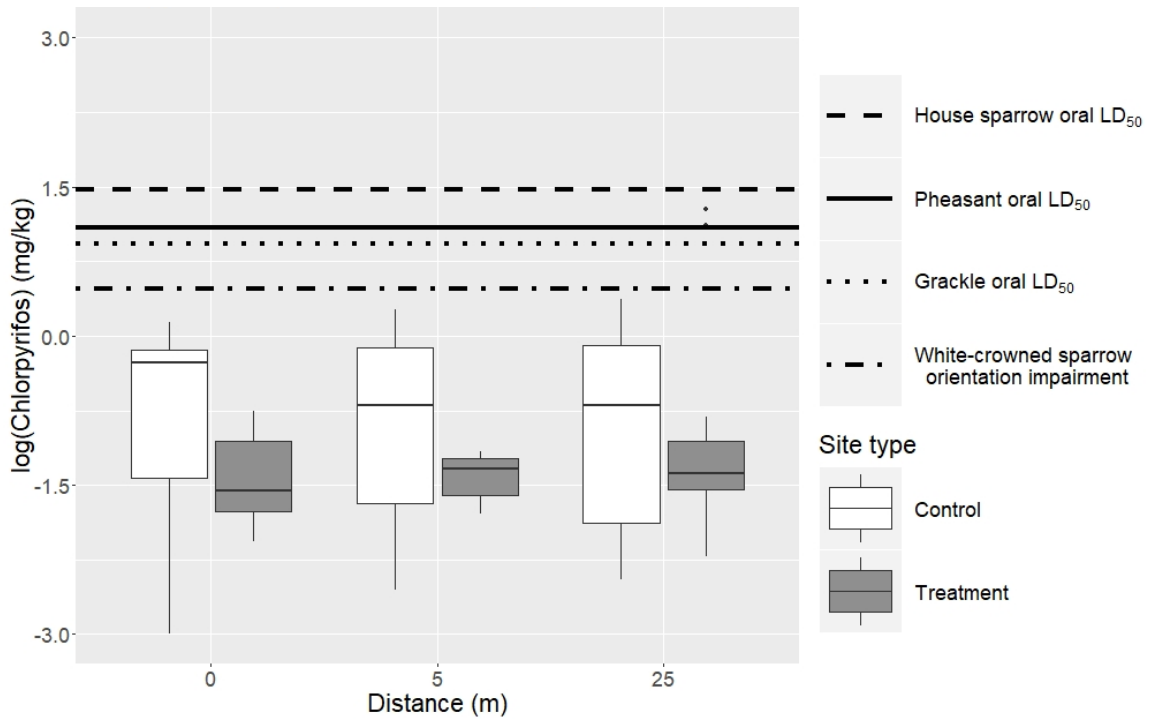


Figure 7. Chlorpyrifos deposition on arthropod samples ($n = 81$) by distance from field edge to grassland interior at treatment sites and control sites. White bars represent samples collected at control sites (grasslands adjacent to unsprayed corn fields); gray bars represent samples collected at treatment sites (grasslands adjacent to soybean fields that were treated with insecticides). The horizontal lines represent the acute oral LD₅₀ for house sparrows (*Passer domesticus*), acute oral LD₅₀ for ring-necked pheasants (*Phasianus colchicus*), acute oral LD₅₀ for common grackles (*Quiscalus quiscula*), and acute oral dose causing orientation impairment in white-crowned sparrows (*Zonotrichia leucophrys*; Eng et al. 2017). Acute oral LD₅₀ values are reported in Solomon et al. (2001). Sampling was conducted during the summers of 2017 and 2018 in Minnesota's farmland region. Chlorpyrifos residues were collected from arthropod samples with unknown masses, and calculations converting parts per billion to mg/kg included the maximum estimates of chlorpyrifos residues per sample. Negative values on the y-axis resulted when calculating the logarithm of values between 0 and 1.



Appendix A. Survey sent to landowners with fields immediately adjacent to potential study sites in March and April 2017 to assess soybean aphid spraying practices and to solicit cooperation for summer 2017 sampling efforts.

Print your name here _____

Spraying Practices Survey

PART I

1. **Have you planted soybeans on your land in the past 3-5 years?**
 Yes
 No → *please continue to Part II*

2. **Were your soybeans treated with foliar insecticides in the past 3-5 years?**
 Yes
 No → *please continue to Part II*

3. **On what date(s) were foliar insecticides applied on your soybeans?**

4. **How was the majority of foliar insecticides sprayed on your soybeans in the past 3-5 years?**
 Ground boom
 Aerial
 Other (please specify):

5. **Please list the foliar insecticide trade names and/or the application logistics used on your soybeans in the past 3-5 years to control aphids.**
Example: "2016: Lorsban - 20 gpa through 8004 nozzles @ 50-60 psi from a 854 Rogator traveling at 6 mph to apply a 90' swath"

6. **Did you hire an applicator (e.g. agricultural consultant company) to treat your soybeans with foliar insecticides in the past 3-5 years?**
 Yes (please specify company or individual):

 No, I applied insecticides myself

PART II

1. **Will you be planting soybeans on your land that borders a Wildlife Management Area (WMA) or other protected grassland in 2017?**
 Yes
 No → *end of survey - thank you*
 I'm not sure

2. **Will you be treating these soybeans with foliar insecticides in 2017 if significant numbers of aphids occur?**
 Yes
 No → *end of survey - thank you*
 I'm not sure

3. **How will foliar insecticides likely be sprayed on these soybeans in 2017?**
 Ground boom
 Aerial
 Other (please specify):

 I'm not sure

4. **Please list the foliar insecticide trade names and/or the application logistics that will likely be used on these soybeans in 2017 to control aphids.**
Example: "Lorsban - 20 gpa through 8004 nozzles @ 50-60 psi from a 854 Rogator traveling at 6 mph to apply a 90' swath"

5. **Will you hire an applicator (e.g. agricultural consultant company) to treat these soybeans with foliar insecticides in 2017 if chemical treatment is needed?**
 Yes (please specify company or individual):

 No, I will apply insecticides myself
 I'm not sure

Please return to Katelin Goebel in the envelope provided. Thank you.

Print your name here _____

Contact Information Form

1. **May we contact you to identify foliar insecticide spraying date(s) in the summer of 2017?**

- Yes
- No

2. **What is the best way to reach you?**

Home phone

- -

Cell phone

- -

Both home & cell phones

3. **In order to identify the exact date(s) of spraying, how often are you comfortable with us contacting you during the late summer of 2017?**

- Weekly
- Semi-weekly
- As often as necessary as the spraying date approaches (no more than once daily)

4. **Would you like to receive a paper copy of the LCCMR work plan for our project?**

This can also be found at: http://www.lccmr.leg.mn/projects/2016/work_plans_may/_2016_03n.pdf

- Yes
- No

5. **Would you like to receive a paper copy of your responses to the Spraying Practices Survey and Contact Information Form?**

- Yes
- No

6. **If you rent your land, please provide the name and address of your renter so we may send them a letter and survey:**

Please return to Katelin Goebel in the envelope provided. Thank you.

Appendix B. Minimums, medians, maximums, means, and coefficients of variation (CV) of all chemical residues on passive sampling devices deployed during the summers of 2017 and 2018 in Minnesota's farmland region. Control sites were grasslands adjacent to unsprayed corn fields; treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides either by airplanes or ground sprayers. Minimums, medians, maximums, and means are reported in ng/cm².

| Site type | Chemical | Minimum | Median | Maximum | Mean | CV |
|----------------|-----------------|----------|--------|---------|---------|-------|
| Control | Acephate | 0 | 0 | 0 | 0 | NA |
| | Azoxystrobin | 0 | 0 | 0.02 | 0.00018 | 9.46 |
| | Bifenthrin | 0 | 0 | 0.57 | 0.01 | 7.31 |
| | Chlorothalonil | 0 | 0 | 0 | 0 | NA |
| | Chlorpyrifos | 0.03 | 0.1 | 3.38 | 0.23 | 1.48 |
| | Clothianidin | 0 | 0 | 0 | 0 | NA |
| | Cyfluthrin | 0 | 0 | 0 | 0 | NA |
| | Cyhalothrin | 0 | 0 | 0.14 | 0.0087 | 3.36 |
| | Cypermethrin | 0 | 0 | 0 | 0 | NA |
| | DEET | 1.84 | 26.32 | 97.62 | 32.2 | 0.76 |
| | Deltamethrin | 0 | 0 | 0 | 0 | NA |
| | Dimethoate | 0 | 0 | 0 | 0 | NA |
| | Dinotefuran | 0 | 0 | 0 | 0 | NA |
| | Esfenvalerate | 0 | 0 | 0 | 0 | NA |
| | Fluoxastrobin | 0 | 0 | 0 | 0 | NA |
| | Fluxapyroxad | 0 | 0 | 0 | 0 | NA |
| | Imidacloprid | 0 | 0 | 0 | 0 | NA |
| | Metconazole | 0 | 0 | 0 | 0 | NA |
| | Methomyl | 0 | 0 | 0 | 0 | NA |
| | Propiconazole | 0 | 0 | 0 | 0 | NA |
| | Pyraclostrobin | 0 | 0 | 0 | 0 | NA |
| | Sulfoxaflor | 0 | 0 | 0 | 0 | NA |
| | Tebuconazole | 0 | 0 | 0 | 0 | NA |
| | Tefluthrin | 0 | 0 | 0 | 0 | NA |
| | Tetraconazole | 0 | 0 | 0.03 | 0.0015 | 3.7 |
| | Thiamethoxam | 0 | 0 | 0 | 0 | NA |
| | Trifloxystrobin | 0 | 0 | 0 | 0 | NA |
| | Treatment | Acephate | 0 | 0 | 0 | 0 |
| Azoxystrobin | | 0 | 0 | 0.025 | 0.00092 | 3.76 |
| Bifenthrin | | 0 | 0 | 0 | 0 | NA |
| Chlorothalonil | | 0 | 0 | 1.14 | 0.0055 | 14.35 |
| Chlorpyrifos | | 0 | 0.06 | 7841.13 | 111.07 | 6.2 |
| Clothianidin | | 0 | 0 | 0 | 0 | NA |
| Cyfluthrin | | 0 | 0 | 0 | 0 | NA |
| Cyhalothrin | | 0 | 0 | 40.8 | 1.28 | 4.11 |

| | | | | | |
|-----------------|------|-------|--------|----------|-------|
| Cypermethrin | 0 | 0 | 0 | 0 | NA |
| DEET | 1.82 | 32.29 | 157.22 | 39.84 | 0.87 |
| Deltamethrin | 0 | 0 | 0 | 0 | NA |
| Dimethoate | 0 | 0 | 0 | 0 | NA |
| Dinotefuran | 0 | 0 | 0 | 0 | NA |
| Esfenvalerate | 0 | 0 | 0 | 0 | NA |
| Fluoxastrobin | 0 | 0 | 0 | 0 | NA |
| Fluxapyroxad | 0 | 0 | 0.15 | 0.0036 | 4.43 |
| Imidacloprid | 0 | 0 | 0 | 0 | NA |
| Metconazole | 0 | 0 | 1.52 | 0.036 | 4.64 |
| Methomyl | 0 | 0 | 0 | 0 | NA |
| Propiconazole | 0 | 0 | 0.059 | 0.0012 | 5.07 |
| Pyraclostrobin | 0 | 0 | 3.71 | 0.097 | 4.35 |
| Sulfoxaflor | 0 | 0 | 0 | 0 | NA |
| Tebuconazole | 0 | 0 | 0 | 0 | NA |
| Tefluthrin | 0 | 0 | 0 | 0 | NA |
| Tetraconazole | 0 | 0 | 0 | 0 | NA |
| Thiamethoxam | 0 | 0 | 0 | 0 | NA |
| Trifloxystrobin | 0 | 0 | 0.0085 | 0.000041 | 14.35 |

Appendix C. Welch's two-sample *t*-test results comparing chlorpyrifos residues (ng/cm²) on passive sampling devices deployed at mid-canopy height (0.5 m above ground) and ground level (0 m above ground). Samples were collected during the summers of 2017 and 2018 in Minnesota's farmland region. Treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides either by airplanes or ground sprayers; control sites were grasslands adjacent to unsprayed corn fields.

| Distance from field edge (m) | t | df | p-value |
|-------------------------------------|----------|-----------|----------------|
| <i>Treatment sites</i> | | | |
| 0 | 1.01 | 14.39 | 0.331 |
| 5 | 1.13 | 14.23 | 0.277 |
| 25 | 0.69 | 16.76 | 0.499 |
| 50 | 1.12 | 17.56 | 0.280 |
| 100 | 0.94 | 23.07 | 0.358 |
| 200 | 1.27 | 20.83 | 0.216 |
| 400 | 1.00 | 12.00 | 0.337 |
| <i>Control sites</i> | | | |
| 0 | -0.44 | 15.22 | 0.669 |
| 5 | 0.68 | 20.15 | 0.503 |
| 25 | 0.59 | 19.93 | 0.562 |
| 50 | 0.95 | 20.78 | 0.353 |
| 100 | 0.23 | 22.00 | 0.821 |
| 200 | 0.42 | 19.83 | 0.680 |
| 400 | 0.90 | 12.89 | 0.383 |

Appendix D. Minimums, medians, maximums, means, and coefficients of variation (CV) of all chemical residues on arthropod samples collected during the summers of 2017 and 2018 in Minnesota’s farmland region. Control sites were grasslands adjacent to unsprayed corn fields; treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides either by airplanes or ground sprayers. Residues were collected from arthropod samples with unknown masses, and calculations converting parts per billion to ng/g included the maximum estimates of chemical residues per sample. Minimums, medians, maximums, and means are reported in ng/g.

| Site type | Chemical | Minimum | Median | Maximum | Mean | CV |
|-----------------|----------------|---------|--------|----------|--------|------|
| Control | Acephate | 0 | 0 | 0 | 0 | NA |
| | Azoxystrobin | 0.71 | 19.79 | 124.37 | 31.35 | 1.02 |
| | Bifenthrin | 0 | 0 | 418.35 | 72.63 | 1.76 |
| | Chlorothalonil | 0 | 0 | 0 | 0 | NA |
| | Chlorpyrifos | 0 | 254.75 | 2,374.40 | 497.07 | 1.2 |
| | Clothianidin | 0 | 0 | 0 | 0 | NA |
| | Cyfluthrin | 0 | 0 | 0 | 0 | NA |
| | Cyhalothrin | 0 | 0 | 75.61 | 6.11 | 2.6 |
| | Cypermethrin | 0 | 0 | 0 | 0 | NA |
| | DEET | 0 | 49.2 | 375.95 | 88.18 | 1.17 |
| | Deltamethrin | 0 | 0 | 0 | 0 | NA |
| | Dimethoate | 0 | 0 | 0 | 0 | NA |
| | Dinotefuran | 0 | 0 | 0 | 0 | NA |
| | Esfenvalerate | 0 | 0 | 0 | 0 | NA |
| | Fluoxastrobin | 0 | 0 | 0 | 0 | NA |
| | Fluxapyroxad | 0 | 0 | 37.45 | 2.34 | 2.84 |
| | Imidacloprid | 0 | 0 | 0 | 0 | NA |
| | Metconazole | 0 | 0 | 0 | 0 | NA |
| | Methomyl | 0 | 0 | 0 | 0 | NA |
| | Propiconazole | 0 | 12.72 | 174.55 | 27.89 | 1.33 |
| | Pyraclostrobin | 0 | 6.58 | 47.35 | 8.81 | 1.16 |
| | Sulfoxaflor | 0 | 0 | 0 | 0 | NA |
| | Tebuconazole | 0 | 0 | 11.31 | 0.31 | 6 |
| | Tefluthrin | 0 | 0 | 0 | 0 | NA |
| | Tetraconazole | 0 | 0 | 92.57 | 10.17 | 2.1 |
| | Thiamethoxam | 0 | 0 | 0 | 0 | NA |
| Trifloxystrobin | 0 | 0.8 | 4.95 | 1.15 | 1.19 | |
| Treatment | Acephate | 0 | 0 | 0 | 0 | NA |
| | Azoxystrobin | 0 | 9.19 | 52.29 | 13.39 | 0.92 |
| | Bifenthrin | 0 | 0 | 0 | 0 | NA |
| | Chlorothalonil | 0 | 0 | 0 | 0 | NA |
| | Chlorpyrifos | 0 | 30.39 | 18,868 | 744.29 | 4.51 |
| | Clothianidin | 0 | 0 | 0 | 0 | NA |
| | Cyfluthrin | 0 | 0 | 0 | 0 | NA |

| | | | | | |
|-----------------|---|-------|----------|--------|------|
| Cyhalothrin | 0 | 0 | 1,858.53 | 202.06 | 2.37 |
| Cypermethrin | 0 | 0 | 0 | 0 | NA |
| DEET | 0 | 61.83 | 496.79 | 117.49 | 1.25 |
| Deltamethrin | 0 | 0 | 0 | 0 | NA |
| Dimethoate | 0 | 0 | 1.6 | 0.16 | 2.69 |
| Dinotefuran | 0 | 0 | 0 | 0 | NA |
| Esfenvalerate | 0 | 0 | 0 | 0 | NA |
| Fluoxastrobin | 0 | 0 | 0 | 0 | NA |
| Fluxapyroxad | 0 | 0 | 17.67 | 0.94 | 3.28 |
| Imidacloprid | 0 | 0 | 18.37 | 1.16 | 3.32 |
| Metconazole | 0 | 0 | 243.8 | 21.59 | 2.36 |
| Methomyl | 0 | 0 | 0 | 0 | NA |
| Propiconazole | 0 | 9.19 | 99.6 | 15.22 | 1.27 |
| Pyraclostrobin | 0 | 9.89 | 608.44 | 60.76 | 1.91 |
| Sulfoxaflor | 0 | 0 | 0 | 0 | NA |
| Tebuconazole | 0 | 0 | 0 | 0 | NA |
| Tefluthrin | 0 | 0 | 0 | 0 | NA |
| Tetraconazole | 0 | 0 | 3.53 | 0.079 | 6.71 |
| Thiamethoxam | 0 | 0 | 0 | 0 | NA |
| Trifloxystrobin | 0 | 1.41 | 4.95 | 1.48 | 0.96 |

Appendix E. Welch’s two-sample *t*-test results comparing chlorpyrifos residues (ng/g) on arthropod samples at treatment sites and control sites. Samples were collected during the summers of 2017 and 2018 in Minnesota’s farmland region. Treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides either by airplanes or ground sprayers; control sites were grasslands adjacent to unsprayed corn fields. Chlorpyrifos residues were collected from arthropod samples with unknown masses, and calculations converting parts per billion to ng/g included the maximum estimates of chlorpyrifos residues per sample. Bold values indicate significant differences ($p < 0.05$).

| Distance from field edge (m) | t | df | p-value |
|-------------------------------------|----------|-----------|----------------|
| 0 | -2.80 | 11.19 | 0.017 |
| 5 | -2.65 | 11.03 | 0.022 |
| 25 | 1.08 | 14.57 | 0.297 |

Appendix F. Minimums, medians, maximums, means, and coefficients of variation (CV) of target chemical (i.e., chlorpyrifos, cyhalothrin, and bifenthrin) residues on passive sampling devices (PSDs) deployed during the summers of 2017 and 2018 in Minnesota's farmland region. Control sites were grasslands adjacent to unsprayed corn fields; treatment sites consisted of grasslands adjacent to soybean fields that were treated with insecticides either by airplanes or ground sprayers. PSDs were deployed at mid-canopy height (0.5 m above ground) and ground level (0 m above ground). Minimums, medians, maximums, and means are reported in ng/cm².

| Site type | Distance | Minimum | Median | Maximum | Mean | CV |
|---------------------|-----------------|----------------|---------------|----------------|-------------|-----------|
| Control | 0 | 0.04 | 0.1 | 3.38 | 0.41 | 1.85 |
| | 5 | 0.04 | 0.096 | 0.7 | 0.2 | 0.95 |
| | 25 | 0.05 | 0.12 | 0.66 | 0.2 | 0.9 |
| | 50 | 0.03 | 0.14 | 0.67 | 0.21 | 0.97 |
| | 100 | 0.04 | 0.12 | 0.78 | 0.22 | 1.03 |
| | 200 | 0.04 | 0.11 | 0.67 | 0.19 | 0.98 |
| | 400 | 0.034 | 0.12 | 0.93 | 0.29 | 1.02 |
| Treatment | 0 | 0 | 0.44 | 7,860.53 | 351.44 | 4.11 |
| | 5 | 0 | 0.22 | 2,907.74 | 143.86 | 3.82 |
| | 25 | 0 | 0.18 | 4,884.19 | 265.81 | 3.48 |
| | 50 | 0 | 0.046 | 38.07 | 3.83 | 2.35 |
| | 100 | 0 | 0.05 | 2.67 | 0.37 | 1.64 |
| | 200 | 0 | 0.032 | 0.7 | 0.13 | 1.44 |
| | 400 | 0 | 0.046 | 178.12 | 6.96 | 5.02 |
| <i>PSD height</i> | | | | | | |
| Mid-canopy | 0 | 0 | 0.51 | 7,860.53 | 616.68 | 3.29 |
| Ground | | 0 | 0.37 | 936.51 | 86.2 | 2.8 |
| Mid-canopy | 5 | 0 | 0.47 | 2,907.74 | 257.4 | 2.99 |
| Ground | | 0 | 0.22 | 249.02 | 30.31 | 2.36 |
| Mid-canopy | 25 | 0 | 0.46 | 4,884.19 | 383.14 | 3.28 |
| Ground | | 0 | 0.06 | 1,456.19 | 148.47 | 2.68 |
| Mid-canopy | 50 | 0 | 0.042 | 38.07 | 5.66 | 2.11 |
| Ground | | 0 | 0.05 | 14.14 | 2 | 2.16 |
| Mid-canopy | 100 | 0 | 0.13 | 2.67 | 0.47 | 1.56 |
| Ground | | 0 | 0.05 | 1.36 | 0.27 | 1.68 |
| Mid-canopy | 200 | 0 | 0.05 | 0.7 | 0.18 | 1.35 |
| Ground | | 0 | 0.03 | 0.41 | 0.088 | 1.38 |
| Mid-canopy | 400 | 0 | 0.04 | 178.12 | 13.8 | 3.58 |
| Ground | | 0 | 0.07 | 0.36 | 0.11 | 0.96 |
| <i>Spray method</i> | | | | | | |
| Airplane | 0 | 0.02 | 0.44 | 7,860.53 | 569.15 | 3.25 |
| Ground | | 0 | 0.3 | 146.27 | 24.89 | 2.22 |
| Airplane | 5 | 0.05 | 0.51 | 2,907.74 | 239.57 | 2.93 |
| Ground | | 0 | 0.14 | 0.81 | 0.28 | 1.05 |
| Airplane | 25 | 0.03 | 0.33 | 4,884.19 | 442.84 | 2.65 |
| Ground | | 0 | 0.17 | 0.83 | 0.25 | 1.05 |
| Airplane | 50 | 0 | 0.045 | 38.07 | 6.26 | 1.77 |
| Ground | | 0 | 0.11 | 0.54 | 0.19 | 1.1 |
| Airplane | 100 | 0.02 | 0.05 | 2.67 | 0.47 | 1.61 |
| Ground | | 0 | 0.13 | 0.79 | 0.23 | 1.18 |
| Airplane | 200 | 0 | 0.03 | 0.34 | 0.072 | 1.31 |
| Ground | | 0 | 0.1 | 0.7 | 0.22 | 1.17 |

| | | | | | | |
|----------|-----|---|-------|--------|-------|------|
| Airplane | 400 | 0 | 0.045 | 0.25 | 0.082 | 0.99 |
| Ground | | 0 | 0.11 | 178.12 | 22.43 | 2.81 |

Chapter 2

Impacts of insecticide spray drift on arthropod prey resources of birds in grasslands in Minnesota

OVERVIEW

Soybean aphid (*Aphis glycines*) insecticides are used throughout the farmland region of Minnesota to combat insect pests. However, these foliar spray insecticides have the potential to drift beyond target fields into nearby grassland cover where birds and other insectivores forage. Arthropods serve important roles in grassland ecology and are susceptible to mortality from exposure to broad-spectrum insecticides. My objective was to assess impacts of soybean aphid insecticides on arthropods in grasslands, especially those that are important in grassland bird diets. I measured the abundance, consumable biomass, and family richness of insects and spiders in grasslands adjacent to soybean fields that were treated with chlorpyrifos, lambda-cyhalothrin, and bifenthrin—the 3 most common insecticides used to treat soybean aphids in Minnesota. I compared these measures to samples collected at control sites adjacent to corn fields not sprayed for aphids during 3 periods: 1–3 days before spraying, 3–5 days post-spraying, and 19–21 days post-spraying. Short-term reductions in total arthropod abundance, bird prey abundance, and Coleopteran family richness occurred in grasslands bordered by fields sprayed with foliar insecticides. The total abundance of arthropods in grasslands bordering sprayed soybean fields was lower 3–5 days after insecticide applications ($\beta = -49.06$, 95% CI = $-89.84 - -8.28$). The abundance of arthropods important in grassland bird diets (specifically, Araneae, Coleoptera, Orthoptera, and Lepidoptera larvae) was also lower after nearby spraying, with lower abundance measured in treatment sites

19–21 days post-spraying ($\beta = -23.94$, 95% CI = -44.99 – -2.88). Coleopteran family richness at treatment sites was lower than control sites 3–5 days after insecticide applications ($\beta = -0.94$, 95% CI = -1.82 – -0.06). Measures of total consumable dry biomass, bird prey biomass, family richness of Araneae, family richness of Hemipterans, and family richness of Orthopterans were not different between treatment and control sites post-spraying. My results suggest that reductions in arthropod food abundance for grassland birds are associated with insecticide spraying up to 21 days after the spraying event.

Key Words: insecticides, farmland landscape, row crops, grasslands, non-target arthropods, grassland bird diets

INTRODUCTION

Insecticides are used on soybeans throughout the farmland region of Minnesota to control insect pest populations. Over 3 million ha of soybeans are planted in Minnesota annually (U.S. Department of Agriculture [USDA] 2019a), and in 2015–2018, approximately 41% of the area planted to soybeans was treated with insecticides (U.S. Department of Agriculture National Agricultural Statistics Service 2016, 2018, 2019). Producers apply broad-spectrum foliar insecticides on their soybeans to control soybean aphids (*Aphis glycines*). If left untreated, these pests can decrease crop yields by 40% (Ragsdale et al. 2011). Grasslands in Minnesota’s farmland regions are highly fragmented and often share borders with row crop fields (Minnesota Prairie Plan Working Group 2018). Thus, grassland wildlife, including beneficial insects and spiders, have the potential to be exposed to soybean aphid insecticides due to chemical drift in these landscapes (see Chapter 1).

Chlorpyrifos, lambda-cyhalothrin, and bifenthrin are the most commonly used foliar insecticides applied to soybeans in Minnesota (USDA National Agricultural Statistics Service [NASS] 2016). When soybean aphids reach threshold levels, these chemicals are applied in a liquid form using airplanes or ground sprayers. These insecticides kill insects by disrupting nervous system function and are designed to be effective by direct contact, ingestion, and inhalation (National Pesticide Information Center [NPIC] 2001, Christensen et al. 2009, Johnson et al. 2010). The modes of action of these chemicals are similar for both target and non-target organisms (Christensen et al. 2009, Johnson et al. 2010). Thus, these insecticides can kill beneficial arthropods including pollinators and predators of soybean aphids (Minnesota Department of

Agriculture [MDA] 2018). Sublethal doses of insecticides can also be harmful to beneficial arthropods. Symptoms of sublethal exposure include increased susceptibility to predation, depressed immune system capacity, reduced fecundity, impaired development of offspring, loss of mobility, and impaired feeding and breeding (Desneux et al. 2007).

Although insecticide applications target arthropods in row crops, insecticides can drift beyond fields into nearby grassland cover. Chemical drift occurs when liquid foliar pesticides are sprayed on crops and wind or other environmental factors transport them beyond the application site. Foliar pesticides have been shown to drift beyond target crop fields under typical application conditions (see Chapter 1; Threadgill and Smith 1975, Bui et al. 1998, Langhof et al. 2005, Carlsen et al. 2006, Nsibande et al. 2015, Holterman et al. 2017, Baio et al. 2019). Drift can occur over large distances, even up to 2,000 m beyond targeted areas (Baio et al. 2019). Many factors can influence drift, including environmental factors (e.g., wind speed, wind direction, and air temperature) and spraying equipment (e.g., boom height, tank pressure, and nozzle design). Insecticide product labels contain best management practices with recommendations for equipment that reduces chemical drift, but it is the applicator's responsibility to choose appropriate methods. Labels also include information on suitable wind, temperature, and humidity conditions for insecticide application (Dow AgroSciences LLC 2014a).

Grassland areas near row crop fields are especially susceptible to insecticide deposit via drift and are important to non-target arthropods in farmland regions. Insecticide product labels contain information on buffer zones where insecticides must not be applied around water bodies and residential areas, but no such regulations exist around grasslands or other natural areas (Dow AgroSciences LLC 2014a). Grassland

cover in farmland landscapes is important to non-target arthropods because it provides diverse vegetation composition and structure that is non-existent in monoculture row crops, serves as a refuge during tilling and harvest, and harbors source populations that contribute to the recolonization of insecticide-treated fields (Tschamntke and Greiler 1995, Longley et al. 1997).

Arthropods represent a large proportion of the total biodiversity in tallgrass prairie ecosystems and maintaining or enhancing their populations is an important conservation goal (Dietrich et al. 1998, Harper et al. 2000). Minnesota's Prairie Conservation Plan aims to establish and enhance grassland cover within the farmland region of the state to support diverse populations of birds, arthropods, and other wildlife (Minnesota Prairie Plan Working Group 2018). Beyond their importance to biodiversity, diverse arthropod communities are crucial for their role in ecosystem function (e.g., pollination and nutrient cycling; Harper et al. 2000). With insects being the primary pollinators of native plants, management that maintains a diverse arthropod fauna is crucial to the conservation of tallgrass prairie ecosystems (Dietrich et al. 1998). Thus, reduced arthropod populations resulting from insecticide application may pose a threat to grasslands and the wildlife they support.

Reductions in arthropod abundance and biomass due to foliar insecticide drift could negatively affect insectivorous grassland birds by reducing their food supply. The majority of breeding grassland birds' diets incorporate insects, and insects are the primary item fed to nestlings (Wiens and Rotenberry 1979, Kaspari and Joern 1993). Protein-rich arthropods are especially important for breeding grassland birds during egg-laying, nestling, and fledgling periods. Insect diversity and abundance have been shown

to be lower in crop fields exposed to lambda-cyhalothrin (Langhof et al. 2003, Galvan et al. 2005). There is correlative evidence that reduced insect food supplies are associated with reduced nesting success for birds in fragmented habitats surrounded by cultivated fields (Zanette et al. 2000). High pesticide use on landscapes has also been shown to correlate with insectivorous bird population declines, with reduced insect food supplies cited as a cause of this relationship (Hallmann et al. 2014).

My objective was to assess the effects of soybean aphid insecticides on arthropod prey of grassland nesting birds and other insectivorous wildlife. Specifically, I measured the abundance, consumable biomass, and family richness of insects and spiders in grasslands adjacent to row crop fields in Minnesota's farmland region. I hypothesized that arthropod samples at treatment sites where bordering soybean fields were sprayed with foliar insecticides would have lower abundance, biomass, and richness compared to control sites where foliar insecticides were not applied to the adjacent field. I predicted that these measures of arthropod communities would be lower at the field edge than in the grassland interior after insecticide spraying events at treatment sites.

METHODS

Study Area

I selected study sites in the southwest, west-central, and central regions of Minnesota (Fig. 1). Corn and soybeans accounted for approximately 90%, 67%, and 71% of the landscape in these 3 regions, respectively (USDA 2019*a, b*). Grasslands covered 6.9%, 10.0%, and 5.6% of the landscape in these regions on public and private land (Messinger and Davros 2018). These areas have experienced some of the greatest

estimated uses of chlorpyrifos and lambda-cyhalothrin in Minnesota since 2003 (MDA 2005, 2012, 2014, 2016).

My study sites consisted of public Wildlife Management Areas (WMAs) managed by the Minnesota Department of Natural Resources (MNDNR) as habitat for grassland and wetland wildlife. I first selected study sites using ArcGIS (version 10.6.1, ESRI 2021) and chose WMAs consisting of grasslands or grassland/wetland complexes bordered by row crops. I chose potential treatment sites that were predicted to be downwind (east or north) from soybean fields based on typical wind direction patterns determined from archived National Weather Service data (TWC Product and Technology LLC 2015). I then visited these WMAs to observe their plant diversity and to identify the crops planted in adjacent fields. I chose sites dominated by a diverse mesic tallgrass prairie mix containing warm-season grasses and forbs. This assemblage is increasingly used by MNDNR managers and agency partners to restore grassland habitat for birds and pollinators. The predominant grass species in my sites were big bluestem (*Andropogon gerardii*), smooth brome (*Bromus inermis*), Canada wild rye (*Elymus Canadensis*), and Kentucky bluegrass (*Poa pratensis*). Dominant forb species included wild bergamot (*Monarda fistulosa*), smooth oxeye (*Heliopsis helianthoides*), and Canada goldenrod (*Solidago canadensis*). Canada thistle (*Cirsium arvense*) was also common in my sites, but this forb is not planted by the MNDNR.

Landowner Contact

Landowner cooperation was vital to timing my field sampling efforts. I visited landowner residences and contacted them via telephone to request their cooperation with my study, learn whether they would hire an applicator from a farming cooperative to

spray their field or if they would do it themselves, and ask permission to call them bi-weekly during peak aphid spraying months. I contacted combinations of landowners, renters, agronomists, farmer cooperative representatives, and pilots to learn the exact time of spraying and the insecticide product(s) they applied.

Experimental Design

I sampled study sites in July–September 2017 and 2018, coinciding with when soybeans were treated with insecticides to control soybean aphids. Each treatment study site consisted of a WMA containing upland grassland cover adjacent to a soybean field. The soybean field adjacent to each treatment study site was treated with foliar insecticides to control soybean aphids, and applicators treated fields using ground sprayers or airplanes. I contacted landowners to learn the exact dates of spraying and chemical formulations applied to their fields. Control study sites had similar features with the exception of being adjacent to corn fields. I did not contact the landowners of these corn fields and did not observe foliar pesticide spraying on these fields during sampling efforts.

I collected arthropod samples to assess the effects of insecticides on their communities. I established primary transects 90–100 m apart that extended perpendicular from the adjacent soybean field edge to the grassland interior at each treatment site. Along each of these transects, I established secondary arthropod collection transects at 0, 25, and 100 m from the field edge, except at the first treatment site I sampled in 2017, where I established secondary transects 0, 5, and 25 m from the field edge. The secondary transects ran parallel to the field edge (Fig. 2). At control sites, I established the primary transects and secondary arthropod collection transects starting at the grassland edge that

was east or north of a corn field. I collected samples on 1 day in each of 3 periods: 1–3 days before the spraying event, 3–5 days after spraying, and 19–21 days after spraying. At control sites, I timed sampling based on an arbitrary “spray day” near dates when treatment sites received insecticide applications. Each time I returned to a site after the spraying event, I began sampling from the endpoint of the previous secondary transect at each distance from the field edge to avoid repeat sampling of the same area.

I established extra reference transects at every treatment and control site and collected samples from each one during 3–5 or 19–21 days post-spraying to assess the effect of repeated sampling at my study sites. These transects were located > 60 m from the primary transects, and I established secondary arthropod collection transects at 0, 25, and 100 m from the field edge along these reference transects (Fig. 2). At 1 control site, the field edge was not long enough to accommodate all transects and I therefore established a reference transect along the closest adjacent cornfield edge.

Arthropod Sampling

To quantify and compare the effects of insecticide spraying on abundance, consumable biomass, and family richness of arthropods, I collected insects and spiders via vacuum and sweep-net sampling at treatment and control sites. I collected samples between 10:00 – 16:30 CDT in conditions with wind speeds < 6 m/s, temperatures ranging from 15–35 °C, and no precipitation. Two observers simultaneously collected arthropod samples along each secondary transect: 1 observer used a sweep net while another used a vacuum sampler (Southwood and Henderson 2000). Observers walked down and back along each secondary transect for a total of 40 m per collection method per sample, with the exception of 1 study site. At a single treatment site, observers

collected pre-spraying samples along secondary transects that were 30-m long (60 m when sampled in both directions) and I later modified my protocol to use 20-m secondary transects instead. I included these data in my analyses. Observers walked unique paths 1.25 m apart to minimize sampling disturbance and to maximize the likelihood that the arthropod communities being sampled were similar (Doxon et al. 2011). An observer collected sweep-net samples by swinging a standard 38-cm-diameter canvas net 40 times per sample at 1 m above the ground. The same observer collected all sweep-net samples throughout this study to ensure consistent sampling. The other observer collected vacuum samples using a modified hand-held vacuum with a 15-cm-long nozzle held 15 cm above the ground (BioQuip Products Inc., Rancho Dominguez, CA, U.S.A.).

Using sweep netting and vacuum sampling enabled me to capture canopy-dwelling arthropods and ground-dwelling arthropods, respectively. Sweep-net sampling provides measures of relative abundance and species richness across areas with similar vegetation structure (Siemann et al. 1998). Vacuum sampling complements sweep netting by collecting smaller and ground-dwelling arthropods (Doxon et al. 2011). I combined sweep-net and vacuum samples that were collected on the same secondary transect during the same sampling period into a single sample. Thus, I collected a total of 9 samples per study site prior to spraying. I collected 12 samples at each period 3-5 and 19-21 days post-spraying with the inclusion of reference transect samples. I immediately placed samples in 70% ethanol in airtight plastic bags until later analysis.

I sorted arthropod samples and retained individuals > 1 mm in body length. Entomologists with expertise in arthropod identification sorted and identified arthropods in the orders Araneae (spiders), Coleoptera (beetles), Hemiptera (true bugs), and

Orthoptera (grasshoppers, crickets, and katydids) to family and distinguished Lepidoptera larvae (caterpillars) from adults. I placed emphasis on Araneae, Coleoptera, Orthoptera, and Lepidoptera larvae (hereafter, collectively referred to as “bird prey”) because these are the most common arthropod groups preyed on by grassland birds (Wiens and Rotenberry 1979, Rotenberry 1980, Kobal et al. 1998, Linn 2004). I also placed emphasis on Hemipterans because insects in this order have a great diversity of body forms and occupy multiple trophic levels. All other insects were identified to order. I calculated family richness for orders Araneae, Coleoptera, Hemiptera, and Orthoptera by counting the number of unique families in each order.

I counted and measured the body lengths of arthropods to the nearest 0.01 mm and calculated consumable dry biomass with formulas reported by Straus and Avilés (2018). These equations were unique to taxonomic order and used body length to predict consumable dry biomass. I chose to calculate consumable dry biomass because arthropods’ chitinous exoskeletons are not easily digested by birds (e.g., American robin [*Turdus migratorius*] and northern bobwhite [*Colinus virginianus*] chitin digestibility has been estimated to be only 7-14%; Weiser et al. 1997).

Vegetation Measurements

I measured ground cover, canopy cover, litter depth, maximum height of live and dead vegetation, vertical vegetation density, and species richness in 30 × 55 cm plots at the start and endpoints of each secondary arthropod sampling transect. I collected vegetation data on the same day that I collected arthropod samples. I categorized ground cover into bare ground, litter, or other (e.g., woody debris, rock, or gopher mound) using a modified point-intercept method (Bureau of Land Management 1996). To determine

percent cover of grasses, forbs, dead vegetation, woody vegetation, and other objects in the canopy layer, I used nadir digital photographs taken of each plot from 1.5 m above the ground in the program SamplePoint (Booth et al. 2006). I measured litter depth to the nearest 0.1 cm at 1 point within the plot that represented the average condition of the plot. I recorded the maximum height of live and dead vegetation to the nearest 0.5 dm. I measured vertical vegetation density by placing a Robel pole in the center of each plot and determining visual obstruction readings (VOR) from 4 m away and 1 m above the ground from each of the 4 cardinal directions (Robel et al. 1970). Finally, I counted the number of unique forb and grass species in each plot for a measure of species richness.

Data Analysis

I used linear mixed models to assess the potential effects of drift from soybean aphid insecticide application on the abundance, consumable biomass, and family richness of arthropods. I constructed models using package nlme (Pinheiro et al. 2021) in program R (version 3.6.0, R Core Team 2021) using the maximum likelihood method. I used data that I collected from the secondary transects at each site and excluded samples from the reference transects. I implemented this model-building process with 8 response variables: total abundance, total consumable biomass, abundance of bird prey, consumable biomass of bird prey, family richness of order Araneae, family richness of order Coleoptera, family richness of order Hemiptera, and family richness of order Orthoptera.

Model Covariate Selection

To select vegetation covariates to use in models, I first conducted a principal component analysis (PCA) to identify covariates that were distinct in terms of the variation in the data that they explained and the characteristics of grassland vegetation

that they represented (i.e., ground cover, canopy cover, or vertical structure). I used the mean of vegetation measurements recorded at the start and end of each secondary arthropod collection transect in the PCA. After centering and scaling the vegetation data, I examined the variables included in the top principal components. I considered variables with loading scores with absolute values >0.32 to be members of the same proxy set (Booth et al. 1994). Next, I tested the correlation between covariates in each proxy set and selected vegetation covariates that were not highly correlated (i.e., $|r| < 0.7$; Dormann et al. 2013) and that had a biological basis for influencing arthropod abundance (Table 1). I included percent canopy cover of forbs, maximum height of live vegetation, grass and forb species richness, and percent cover of litter in models of arthropod abundance, biomass, and family richness.

Modeling

To assess differences in arthropod abundance, biomass, and family richness between control and treatment sites after insecticide spraying events, I used a hierarchical model selection approach similar to the methods of Daly et al. (2015). The first set of models incorporated vegetation covariates and year, the second set incorporated site type (i.e., treatment or control) and sample timing (i.e., pre-spraying, 3-5 days post spraying, or 19-21 days post spraying), and the final step incorporated distance from the field edge. This approach allowed me to examine how site type and sample timing influenced arthropod abundance, biomass, and family richness after accounting for other environmental factors that I expected to affect these measures.

In the first model-building step, I assessed whether vegetation characteristics and/or year influenced arthropod abundance, biomass, and family richness at treatment

and control sites at all distances from the field edge prior to spraying. For each response variable, I constructed a model containing vegetation covariates and year as fixed effects. I deemed covariates to have significant effects if the 95% confidence intervals around their parameter estimates did not include zero, and I incorporated covariates with significant effects in the next step of model-building.

In the second model-building step, I constructed models that included the significant covariates identified in step 1 and fixed effects of site type (i.e., treatment or control), sample collection timing (i.e., pre-spraying, 3-5 days post spraying, or 19-21 days post spraying), and the interaction of site type \times sample timing. I used data collected at treatment and control sites at 0 and 25 m from the field edge to construct these models. I excluded samples collected 100 m from the field edge because I measured a small difference in residues of chlorpyrifos, lambda-cyhalothrin, and bifenthrin between treatment and control sites at 100 m from the field edge (see Table 5 in Chapter 1). Thus, any differences in arthropod measures detected at 100 m from the field edge would not be expected to be caused by nearby insecticide application.

If site type or an interaction of site type \times sample timing was significant in step 2 (i.e., 95% confidence intervals around parameter estimates did not include zero), I then tested whether distance from the field edge influenced measures of arthropod abundance, biomass, and richness at treatment sites. I subset the data to include only samples that were relevant to the significant predictor in step 2. For example, if I found that samples from treatment sites collected between days 3–5 post-spraying were significantly different than measures at control sites in step 2, then I subset the data to include samples from treatment sites collected between days 3–5 post-spraying at 0 and 25 m from the

field edge. Using these data, I constructed models including distance from the field edge as a continuous fixed effect in addition to any significant covariates from step 1.

RESULTS

Insecticide Spraying

I collected samples at 5 treatment study sites and 4 control sites between 28 July – 14 September 2017 and 18 July – 5 September 2018, coinciding with peak activity for aphid insecticide spraying (Table 2). Cooperating insecticide applicators used airplanes ($n = 3$) and ground sprayers ($n = 2$) to apply aphid insecticides on soybean fields adjacent to treatment study sites (see Table 4 in Chapter 1 for additional information). Two of 5 treatment sites were downwind at the time of spraying (i.e., the average wind direction during spraying was within $\pm 62^\circ$ of the transect orientation).

Arthropod Sampling

I collected 297 arthropod samples across both years: 243 samples from secondary transects and 54 samples from reference transects. I collected 34,247 individuals representing 26 orders and 104 families in 2017 and 2018. By order, Hemipterans constituted 26% of the total number of individuals, followed by Coleopterans (23%), Dipterans (19%), Hymenopterans (15%), Araneae (7.2%), Orthopterans (3.5%), and Lepidoptera (2.12%, Appendix A). All other orders combined constituted 3.9% of the total number of individuals (Appendix A). Coleopterans and Orthopterans made up the greatest percentage of total dry consumable biomass (26% each), followed by Hemipterans (16%), Dipterans (8.1%), Lepidoptera (7.6%), Hymenopterans (5.8%), and Araneae (4.8%, Appendix A). All other orders combined constituted 6.4% of the total biomass (Appendix A).

I compared abundance and consumable dry biomass measurements between samples collected on the secondary and reference transects for Araneae, Coleoptera, Hemiptera, Lepidoptera larvae, and Orthoptera. These comparisons assessed whether repeated visits to the secondary transects influenced my measurements. Most abundance and biomass measurements from treatment and control sites did not show significant differences by arthropod order between often-visited secondary transects and less-disturbed reference transects (Fig. 3, Fig. 4). The abundance of Hemiptera was significantly different between secondary and reference transects in samples collected at treatment sites 3–5 days post-spraying (Table 3). The abundance of Lepidoptera larvae was different between secondary and reference transects at treatment sites 19–21 days post-spraying (Table 3). The total dry consumable biomass of Araneae was significantly different between secondary and reference transects 19–21 days after spraying at both treatment and control sites (Table 4). The biomass of Hemiptera was significantly different between secondary and reference transects 19–21 days post-spraying at control sites (Table 4). See Appendices B-E for descriptive statistics (means [\bar{x}] and standard deviations [SD]) of arthropod abundance on secondary transects (Appendix B), abundance on reference transects (Appendix C), biomass on secondary transects (Appendix D), and biomass on reference transects (Appendix E).

Arthropod Total Abundance Models

Total arthropod abundance was strongly and inversely associated with percent cover of litter at all study sites prior to spraying (modeling step 1; $\beta = -1.11$, 95% CI = -2.2 – -0.02). Arthropod abundance was not associated with percent cover of forbs, maximum height of live vegetation, species richness, or year. Arthropod abundance on

days 19-21 post-spraying at both treatment and control sites was significantly lower than pre-spraying abundance ($\beta = -46.04$, 95% CI = -76.81 – -15.26, Table 5). Samples collected at treatment sites 3–5 days post-spraying had significantly lower measures of abundance than samples collected in this same timeframe at control sites ($\beta = -49.06$, 95% CI = -89.84 – -8.28, Table 5). The abundance of arthropods 3–5 days post-spraying at treatment sites was not related to distance from the field edge ($\beta = -0.38$, 95% CI = -1.12 – 0.36, model conditional $R^2 = 0.83$).

Arthropod Total Biomass Models

Total consumable dry biomass of arthropods was strongly and inversely related to the percent cover of litter at all study sites prior to spraying (modeling step 1; $\beta = -2.35$, 95% CI = -4.15 – -0.55). Total biomass was not associated with percent cover of forbs, maximum height of live vegetation, species richness, or year. Arthropod biomass on days 3–5 and 19–21 post-spraying at both treatment and control sites was significantly lower than pre-spraying biomass ($\beta = -99.78$, 95% CI = -169.09 – -30.48; $\beta = -75.91$, 95% CI = -145.28 – -6.55; Table 5). Consumable biomass was not different between treatment and control sites in samples collected during post-spraying periods (Table 5).

Bird Prey Abundance Models

The abundance of bird prey at all sites before spraying was strongly and inversely related to the percent cover of litter (modeling step 1; $\beta = -0.62$, 95% CI = -1.07 – -0.17). I measured lower abundance of bird prey arthropods in 2018 than in 2017 (modeling step 1; $\beta = -32.70$, 95% CI = -52.75 – -12.64). Bird prey arthropod abundance was not associated with percent cover of forbs, maximum height of live vegetation, or species richness. Samples collected at treatment sites 19–21 days post-spraying contained lower

abundances than samples collected at control sites in this same timeframe ($\beta = -23.94$, 95% CI = $-44.99 - -2.88$, Table 5). Bird prey abundance at treatment sites 19–21 days post-spraying was not related to distance from the field edge ($\beta = -0.02$, 95% CI = $-0.44 - 0.39$, model conditional $R^2 = 0.16$).

Bird Prey Biomass Models

The consumable biomass of bird prey at all sites before spraying was not strongly associated with percent cover of litter, percent cover of forbs, maximum height of live vegetation, species richness, or year. Samples collected 3–5 days post-spraying at both treatment and control sites contained significantly lower biomass measures than samples collected before spraying ($\beta = -62.36$, 95% CI = $-123.91 - -0.81$, Table 5). Consumable biomass of bird prey arthropods did not differ between treatment and control sites in samples collected after spraying (Table 5).

Araneae Family Richness Models

The family richness of Araneae was not strongly related to percent cover of litter, percent cover of forbs, maximum height of live vegetation, species richness, or year. Family richness did not differ between treatment and control sites (Table 5).

Coleoptera Family Richness Models

The family richness of Coleopterans was strongly and positively associated with percent cover of forbs (modeling step 1; $\beta = 0.02$, 95% CI = $0.004 - 0.03$). Coleopteran family richness was not strongly related to percent cover of litter, maximum height of live vegetation, species richness, or year. Samples collected at treatment sites 3–5 days post-spraying contained lower Coleopteran family richness than samples collected at control sites in this same timeframe ($\beta = -0.94$, 95% CI = $-1.82 - -0.06$, Table 5). Family

richness of Coleopterans was not related to distance from field edge ($\beta = -0.003$, 95% CI = $-0.04 - 0.03$, model conditional $R^2 = 0.29$).

Hemiptera Family Richness Models

The family richness of Hemipterans was not strongly related to percent cover of litter, percent cover of forbs, maximum height of live vegetation, species richness, or year. Hemipteran family richness on days 3–5 and 19–21 post-spraying at treatment and control sites was significantly lower than pre-spraying biomass ($\beta = -1.04$, 95% CI = $-2.03 - -0.06$; $\beta = -1.33$, 95% CI = $-2.32 - -0.33$; Table 5). Family richness of Hemipterans was not significantly different between treatment and control sites post-spraying (Table 5).

Orthoptera Family Richness Models

Orthopteran family richness was not strongly related to percent cover of litter, percent cover of forbs, maximum height of live vegetation, species richness, or year. Richness on days 3–5 and 19–21 post-spraying at treatment and control sites was significantly lower than pre-spraying biomass ($\beta = -0.46$, 95% CI = $-0.83 - -0.1$; $\beta = -0.56$, 95% CI = $-0.95 - -0.17$; Table 5). Family richness of Orthopterans was not significantly different between treatment and control sites after spraying (Table 5).

DISCUSSION

The decline of arthropods that are important in bird diets has become an increasing conservation concern in farmland landscapes where insecticides are widely used (Campbell et al. 1997, Barker 2004, Devine and Furlong 2007, Goulson 2014). Abundance and biomass of prey items of birds are important to consider, as arthropods constitute the majority of grassland songbirds' diets (Wiens and Rotenberry 1979) and

areas with high arthropod biomass have been shown to have a strong relationship with gamebird brood use (Jamison et al. 2002, Hagen et al. 2005). My results indicated that there were measurable impacts after insecticide spraying events on arthropods that serve as prey items for grassland birds. I measured short-term reductions in total arthropod and bird prey abundance in grasslands bordered by fields sprayed with foliar insecticides to control soybean aphids. The total abundance of arthropods in grasslands bordering sprayed soybean fields was lower 3–5 days after nearby insecticide applications compared to abundance observed before spraying. The abundance of arthropods important in grassland bird diets was also lower after nearby spraying, with lower abundance measured in treatments sites 19–21 days post-spraying. Previous studies have also found short-term reductions in non-target arthropod abundance and richness measures following insecticide application (Barrett 1968, Vickerman and Sunderland 1977, Longley et al. 1997, Galvan et al. 2005, Langhof et al. 2005, Devotto et al. 2007).

Although total abundance of arthropods was lower 3–5 days after insecticide application, measures were similar to pre-spraying levels 19–21 days post-application, indicating that arthropod populations rebounded during this period. Arthropods could have recolonized these grasslands from nearby areas that were not treated with insecticides during this time (Longley et al. 1997). Barrett (1968) reported that biomass of arthropods in insecticide-treated areas fully recovered after 7 weeks following application of foliar insecticides. Arthropod communities' recovery rates can vary widely among areas and among arthropods with differing dispersal capabilities, reproductive potentials, and life stages (Campbell et al. 1997, Longley et al. 1997). My measures of Coleopteran family richness showed this same trend of declining 3–5 days after

insecticide application then rebounding by 19–21 days post-application, indicating that this highly diverse and relatively vagile taxon may have been particularly susceptible to insecticide drift but also capable of recolonizing areas near treated fields fairly quickly.

My measures of consumable dry biomass were not different between treatment and control sites 3–5 or 19–21 days post-spraying. This result contradicts a previous study indicating that the total biomass of arthropods was reduced in insecticide-treated areas soon after spraying (Barrett 1968). Larger arthropods may have been more likely than smaller arthropods to survive spraying events at my study sites, which would have contributed to my observed pattern of reduced abundance but not biomass. Orthopterans comprised only 3.5% of total arthropod abundance but 26% of the total dry biomass in my samples (Appendix A). Thus, Orthopterans surviving nearby spraying events could have contributed to this finding. Alternatively, arthropods could be experiencing a landscape-level dosage of drift resulting in lethal or sublethal effects from insecticides regardless of whether application occurred on the nearest field. The widespread use of insecticides across the landscape throughout the timeframe of my sampling resulted in detectable chemical residues at both treatment and control sites up to 400 m from field edges (see Chapter 1), and other studies report drift occurring even farther (e.g., up to 2,000 m; Baio et al. 2019).

I observed declines in arthropod total abundance, total biomass, bird prey biomass, family richness of Hemipterans, and family richness of Orthopterans 3–5 days and/or 19–21 days post-spraying, regardless of whether samples were collected at treatment or control sites. These trends were surprising, as I expected to see differences between treatment and control sites attributed to effects of insecticide spraying. Although

the availability of arthropods to be sampled can be affected by weather (Southwood and Henderson 2000), I controlled for these environmental factors in my study design. I collected arthropod samples at consistent times of day and in similar weather conditions throughout this study and accounted for variation due to vegetation measurements in models. Repeated sampling disturbance was not a factor in the lower arthropod measures that I measured post-spraying, as indicated by my findings that biomass and abundance measurements of most taxa were not significantly different between secondary and reference transects (Tables 3 and 4). Thus, the declines in arthropod measures that I observed post-spraying at treatment and control sites were likely due to factors that I did not account for in this study, or were statistical artifacts of conducting multiple independent comparisons. However, the widespread use of insecticides on the landscape may have contributed to these trends at both treatment and control sites.

I predicted that measures of arthropod populations would be lower in samples collected at the field edge than samples from the grassland interior at treatment sites. However, I did not find distance from the field edge to be important in explaining total arthropod abundance, bird prey abundance, or Coleopteran family richness in samples collected post-spraying at treatment sites. This could be because I only considered samples collected ≤ 25 m from the field edge where the effects of insecticide drift would likely be highest (see Chapter 1), and this distance may have been insufficient for detecting trends in arthropod populations over the gradient from field edge to grassland interior.

The percent cover of litter was strongly and inversely associated with arthropod total abundance, total biomass, bird prey abundance, and family richness of Coleopterans

in treatment and control sites before spraying (model step 1). This finding was counter to many previous assessments of these relationships. Past studies have shown that arthropod abundance was positively associated with litter cover, as litter provides important nesting and overwintering habitat for several arthropod species, especially Lepidopterans (Vogel et al. 2010, Debinski et al. 2011). However, Lepidopterans constituted only < 3% of the total abundance of arthropods I collected. Prescribed burning can be used to manage grasslands and to remove the litter layer, and this technique is used by the MNDNR to manage WMAs. Burning has been shown to affect abundance and richness of Coleopterans, Hymenopterans, and Lepidopterans even years later (Debinski et al. 2011).

The effects of insecticides on non-target arthropods depend on a wide variety of factors. Toxicity of insecticides to arthropods varies widely by species (Sánchez-Bayo 2011). Different life stages of arthropods can also have different susceptibilities to insecticides, and decreases of arthropods in varying life stages can result in various effects on population growth rates (Stark et al. 2004). Insecticides can also have sublethal effects that negatively affect arthropod physiology and behavior (Desneux et al. 2007). Future studies would benefit from incorporating assessments of sublethal effects and arthropod life stages to obtain a more complete understanding of impacts of insecticides on arthropods in farmland areas. Even so, my results indicate that short-term reductions in total arthropod and bird prey abundance in grasslands may be associated with applications of foliar insecticides.

Management Implications

Arthropods are important in grassland bird diets but may be influenced by insecticide drift in farmland regions. In a row-crop-agriculture landscape in Minnesota,

reductions in arthropod food abundance for grassland birds were associated with nearby insecticide spraying, and these effects were measured up to 21 days after spraying events. However, bird prey biomass was not reduced in grasslands post-spraying, indicating that larger arthropods (e.g., Orthopterans) may have survived spraying events even when overall arthropod abundance was reduced. Overall, applications of insecticides in farmland areas have the potential to negatively affect arthropod populations in nearby grasslands. Grassland cover in farmland landscapes that is protected from insecticide drift is important to provide refugia from which beneficial arthropods can recolonize treated row crop fields and grasslands where drift has occurred. These areas can continue to provide food resources for grassland birds even in the presence of local insecticide spraying events.

Table 1. Vegetation covariates and their hypothesized relationships to arthropod population metrics. These covariates were used in models assessing effects of soybean aphid insecticide spraying on arthropod abundance, consumable biomass, and richness in grasslands in Minnesota’s farmland region.

| Vegetation covariate | Hypothesized relationship to arthropod population metrics | Rationale |
|--|--|---|
| Percent canopy cover of forbs (%) | Positively associated with species richness | Martinko et al. 2006 |
| Maximum height of live vegetation (dm) | Positively associated with species richness and abundance | Kruess and Tschardtke 2002, Pöyry et al. 2006, Debinski et al. 2011 |
| Species richness | Positively associated with species richness and abundance | Siemann et al. 1998, Kruess and Tschardtke 2002 |
| Percent ground cover of litter (%) | Positively associated with abundance | Harper et al. 2000, Debinski et al. 2011 |

Table 2. Locations and sampling dates of study sites during the summers of 2017 and 2018 in Minnesota's farmland region. Arthropod samples were collected to assess impacts of insecticide spraying on arthropod abundance, biomass, and richness in grasslands. Regions of Minnesota sampled in this study include the southwest (SW), west central (WC), and central (C) regions. Treatment sites were grasslands adjacent to soybean fields that were sprayed with insecticides to control aphids; control sites were grasslands adjacent to corn fields that were not sprayed for aphids.

| Site ID | Region | County | Site type | Year | Dates when field sampling occurred |
|----------------|---------------|-----------------|------------------|-------------|---|
| tA | SW | Jackson | Treatment | 2017 | 28 July – 18 August |
| tB | SW | Murray | Treatment | 2017 | 9 August – 30 August |
| cA | SW | Jackson | Control | 2017 | 21 August – 14 September |
| cB | SW | Lyon | Control | 2017 | 7 August – 31 August |
| tC | WC | Lac qui Parle | Treatment | 2018 | 10 August – 29 August |
| tD | C | Stearns | Treatment | 2018 | 28 July – 16 August |
| tE | WC | Yellow Medicine | Treatment | 2018 | 7 August – 28 August |
| cC | C | Kandiyohi | Control | 2018 | 17 August – 5 September |
| cD | WC | Lac qui Parle | Control | 2018 | 18 July – 8 August |

Table 3. Welch's two-sample *t*-test results comparing total abundance of arthropods collected on secondary sampling transects and reference transects at treatment and control sites during the summers of 2017 and 2018 in the farmland region of Minnesota. Treatment sites consisted of grasslands adjacent to soybean fields that were sprayed with soybean aphid insecticides; control sites were grasslands adjacent to unsprayed corn fields. Secondary transects were located parallel to field edges and extended from the field edges to grassland interiors; reference transects were located > 60 m from other transects to minimize sampling disturbance. Bold values indicate significant differences ($p < 0.05$).

| | t | df | p-value |
|------------------------|----------|-----------|----------------|
| <i>Treatment sites</i> | | | |
| 3–5 d post-spray | | | |
| Araneae | 1.70 | 27.56 | 0.099 |
| Coleoptera | 0.71 | 19.94 | 0.484 |
| Hemiptera | 2.32 | 15.84 | 0.034 |
| Lepidoptera larvae | 0.61 | 7.40 | 0.563 |
| Orthoptera | 0.57 | 13.25 | 0.579 |
| 19–21 d post-spray | | | |
| Araneae | -1.21 | 18.08 | 0.241 |
| Coleoptera | -1.33 | 41.74 | 0.189 |
| Hemiptera | -0.06 | 31.18 | 0.950 |
| Lepidoptera larvae | -3.14 | 15.64 | 0.006 |
| Orthoptera | 0.80 | 6.94 | 0.452 |
| <i>Control sites</i> | | | |
| 3–5 d post-spray | | | |
| Araneae | -1.03 | 40.17 | 0.309 |
| Coleoptera | -0.16 | 16.34 | 0.874 |
| Hemiptera | 0.40 | 15.61 | 0.694 |
| Lepidoptera larvae | -0.19 | 7.17 | 0.854 |
| Orthoptera | 1.36 | 9.76 | 0.203 |
| 19–21 d post-spray | | | |
| Araneae | -1.61 | 33.64 | 0.116 |
| Coleoptera | -1.37 | 25.74 | 0.182 |
| Hemiptera | -0.26 | 18.31 | 0.797 |
| Lepidoptera larvae | 1.30 | 3.42 | 0.276 |
| Orthoptera | 1.70 | 12.18 | 0.114 |

Table 4. Welch's two-sample *t*-tests results comparing total dry consumable biomass (mg) of arthropods collected on secondary sampling transects and reference transects at treatment and control sites during the summers of 2017 and 2018 in the farmland region of Minnesota. Treatment sites consisted of grasslands adjacent to soybean fields that were sprayed with soybean aphid insecticides; control sites were grasslands adjacent to unsprayed corn fields. Secondary transects were located parallel to field edges and extended from the field edges to grassland interiors; reference transects were located > 60 m from other transects to minimize sampling disturbance. Bold values indicate significant differences ($p < 0.05$).

| | t | df | p-value |
|------------------------|----------|-----------|----------------|
| <i>Treatment sites</i> | | | |
| 3–5 d post-spray | | | |
| Araneae | 1.09 | 18.74 | 0.288 |
| Coleoptera | 1.54 | 19.09 | 0.139 |
| Hemiptera | 1.73 | 22.16 | 0.097 |
| Lepidoptera larvae | 0.84 | 4.79 | 0.443 |
| Orthoptera | 0.87 | 18.43 | 0.397 |
| 19–21 d post-spray | | | |
| Araneae | -3.91 | 47.73 | 0.0003 |
| Coleoptera | -0.32 | 42.68 | 0.749 |
| Hemiptera | 1.09 | 17.97 | 0.292 |
| Lepidoptera larvae | 0.45 | 4.76 | 0.672 |
| Orthoptera | 0.55 | 7.72 | 0.595 |
| <i>Control sites</i> | | | |
| 3–5 d post-spray | | | |
| Araneae | -1.08 | 32.92 | 0.286 |
| Coleoptera | -0.17 | 31.59 | 0.867 |
| Hemiptera | 0.05 | 19.48 | 0.961 |
| Lepidoptera larvae | 0.06 | 9.01 | 0.954 |
| Orthoptera | 2.06 | 8.85 | 0.070 |
| 19–21 d post-spray | | | |
| Araneae | -2.08 | 34.35 | 0.045 |
| Coleoptera | -0.02 | 20.24 | 0.986 |
| Hemiptera | -2.31 | 39.07 | 0.026 |
| Lepidoptera larvae | 1.54 | 9.72 | 0.155 |
| Orthoptera | 1.15 | 9.82 | 0.278 |

Table 5. Coefficient estimates and 95% confidence intervals for covariates included in model step 2 used to assess arthropod total abundance, total consumable dry biomass, bird prey (i.e., insects in orders Araneae, Coleoptera, and Orthoptera and Lepidoptera larvae) abundance, bird prey consumable biomass, Araneae family richness, Coleoptera family richness, Hemiptera family richness, and Orthoptera family richness. Bold estimates indicate significance based on 95% confidence intervals not overlapping zero. The covariate coefficient estimates of percent canopy cover, percent forb cover, and year are listed only if these coefficients were included in best-supported models using data collected prior to spraying at both treatment and control sites (model step 1). Covariates that were included in best-supported models in step 1 were included in models using data collected at treatment and control sites at 0 and 25 m from the field edge during pre-spray, 3–5 days post-spray, and 19–21 days post-spray sampling periods (model step 2). Arthropod samples were collected in grasslands in the farmland region of Minnesota during the summers of 2017 and 2018.

| Response variable | Cond R ^{2a} | Canopy cover % forb | Ground cover % litter | Year (2018) ^b | Site type (Treatment) ^c | Sample timing ^d | | Treatment sites ^e | |
|--------------------------|----------------------|---------------------|---------------------------------|--------------------------|------------------------------------|------------------------------------|-----------------------------------|----------------------------------|----------------------------------|
| | | | | | | 3-5 d post-spraying | 19-21 d post-spraying | 3-5 d post-spraying | 19-21 d post-spraying |
| Abundance: total | 0.43 | - | -0.71 | - | -17.57 | -25.64 | -46.04 | -49.06 | -18.71 |
| | | - | (-1.47, 0.06) | - | (-75.6, 40.46) | (-56.35, 5.06) | (-76.81 , -15.26) | (-89.84 , -8.28) | (-59.64, 22.22) |
| Biomass: total | 0.20 | - | -0.97 | - | -44.43 | -99.78 | -75.91 | 14.62 | -28.42 |
| | | - | (-2.09, 0.15) | - | (-127.27, 38.42) | (-169.09 , -30.48) | (-145.28 , -6.55) | (-77.44, 106.69) | (-120.85, 64.01) |
| Abundance: bird prey | 0.29 | - | -0.41 | -15.96 | 7.53 | -11.20 | -13.08 | -19.38 | -23.94 |
| | | - | (-0.71 , -0.11) | (-31.95, 0.03) | (-13.42, 28.49) | (-26.9, 4.49) | (-28.97, 2.81) | (-40.22, 1.47) | (-44.99 , -2.88) |
| Biomass: bird prey | 0.14 | - | - | - | -17.53 | -62.36 | -58.97 | -0.43 | -22.26 |
| | | - | - | - | (-97.68, 62.61) | (-123.91 , -0.81) | (-121.2, 3.26) | (-82.21, 81.36) | (-104.56, 60.04) |
| Family richness: Araneae | 0.05 | - | - | - | -0.24 | -0.01 | 0.01 | -0.26 | -0.22 |
| | | - | - | - | (-0.86, 0.39) | (-0.57, 0.55) | (-0.56, 0.57) | (-1.02, 0.49) | (-0.99, 0.55) |

| | | | | | | | | | |
|-----------------------------|------|------------------------|---|---|------------------------|---------------------------------------|---------------------------------------|---------------------------------------|------------------------|
| Family richness: Coleoptera | 0.21 | 0.01 (-0.002, 0.01) | - | - | -0.21 (-1.12, 0.7) | -0.33 (-0.99, 0.33) | -0.34 (-1.01, 0.33) | -0.94 (-1.82, -0.06) | 0.03 (-0.85, 0.92) |
| Family richness: Hemiptera | 0.47 | - | - | - | -1.12 (-3.55, 1.32) | -1.04 (-2.03, -0.06) | -1.33 (-2.32, -0.33) | -0.55 (-1.87, 0.77) | 0.16 (-1.16, 1.48) |
| Family richness: Orthoptera | 0.34 | - | - | - | -0.37 (-0.97, 0.23) | -0.46 (-0.83, -0.1) | -0.56 (-0.95, -0.17) | 0.02 (-0.48, 0.52) | -0.17 (-0.72, 0.38) |

^a Conditional R²: the amount of variation explained by the entire model including random effects.

^b Difference from the estimate from samples collected in 2017.

^c Difference from the estimate from samples collected at control sites. Treatment sites were grasslands bordered by soybean fields sprayed with foliar insecticides to control soybean aphids; control sites were bordered by corn fields that were not treated with insecticides during sampling.

^d Difference from the estimate from samples collected prior to spraying at both treatment and control sites.

^e Difference from the estimate from samples collected in the same timeframe at control sites.

Figure 1. Locations of treatment (purple symbols) and control sites (green symbols) during summer 2017 (square symbols) and 2018 (circle symbols) field sampling efforts in the farmland region of Minnesota. Treatment sites were grasslands adjacent to soybean fields sprayed for aphids; control sites were grasslands adjacent to corn fields that were not sprayed with insecticides to control soybean aphids. Regions shown include: SW = southwest, SC = south central, WC = west central, and C = central.

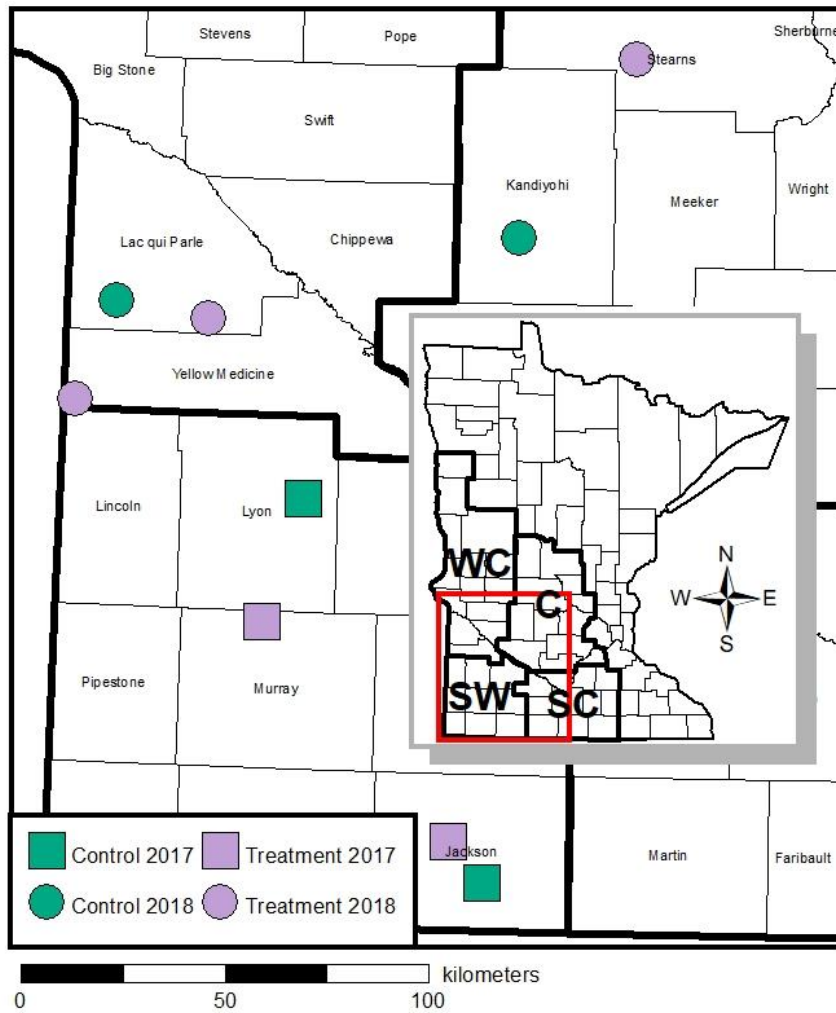


Figure 2. Field sampling design used to assess the effects of soybean aphid insecticides on arthropods in the farmland region of Minnesota. Sampling was conducted in grasslands adjacent to row crop fields during the summers of 2017 and 2018. Treatment sites were bordered by soybean fields sprayed with insecticides to combat soybean aphids; control sites were bordered by corn fields that were not treated with insecticides during sampling efforts. Secondary arthropod collection transects were 20 m long and were located 0, 25, and 100 m from the field edge. Reference transects were 20 m long and were located > 60 m from other transects to minimize sampling disturbance. Observers collected arthropods during 3 periods: before spraying (blue lines), 3–5 days post-spraying (green lines) and 19–21 days post-spraying (yellow lines).

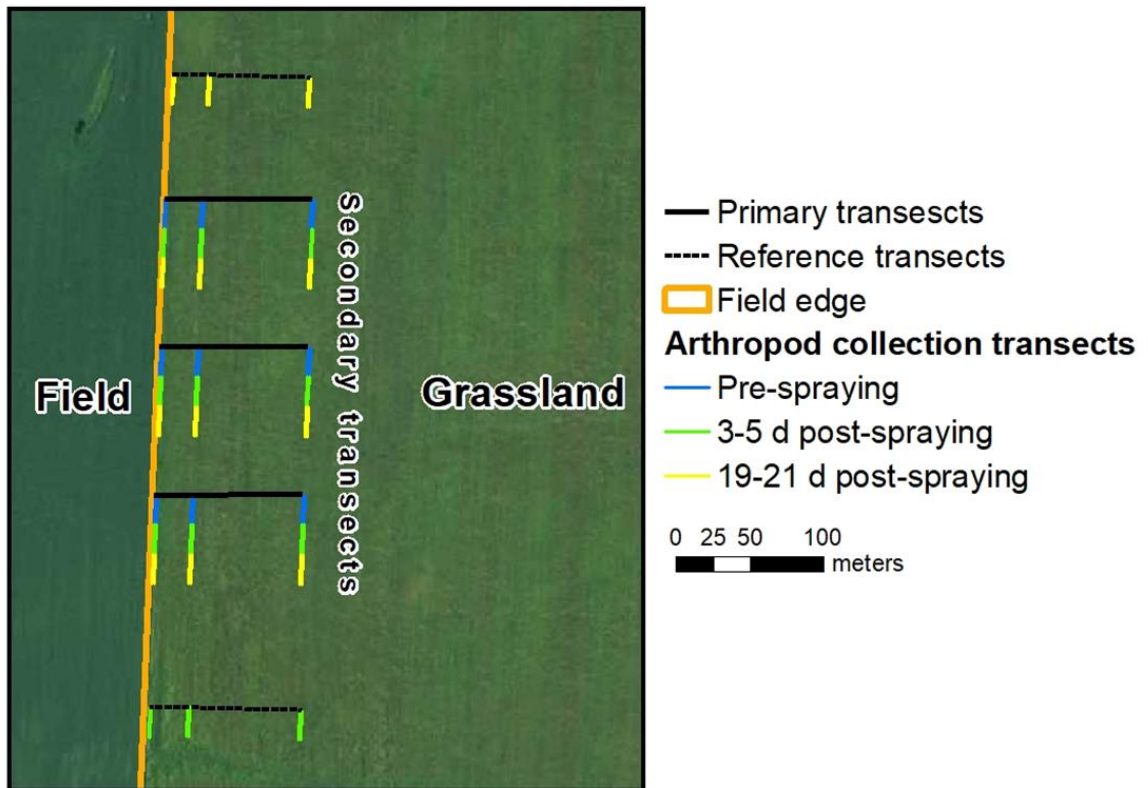


Figure 3. Mean arthropod abundances through time at treatment and control sites. Red circles indicate samples from control site secondary arthropod collection transects and blue triangles indicate samples from treatment site secondary arthropod collection transects. Black circles indicate samples from reference transects at control sites and black triangles indicate samples from reference transects at treatment sites. Error bars represent standard errors. Treatment sites were grasslands bordered by soybean fields sprayed with insecticides to combat soybean aphids; control sites were grasslands bordered by corn fields that were not treated with insecticides. Secondary arthropod collection transects were parallel to the field edge and were visited by researchers repeatedly; reference transects were located > 60 m from other transects to minimize sampling disturbance. Arthropod samples were collected during the summers of 2017 and 2018 in Minnesota’s farmland region.

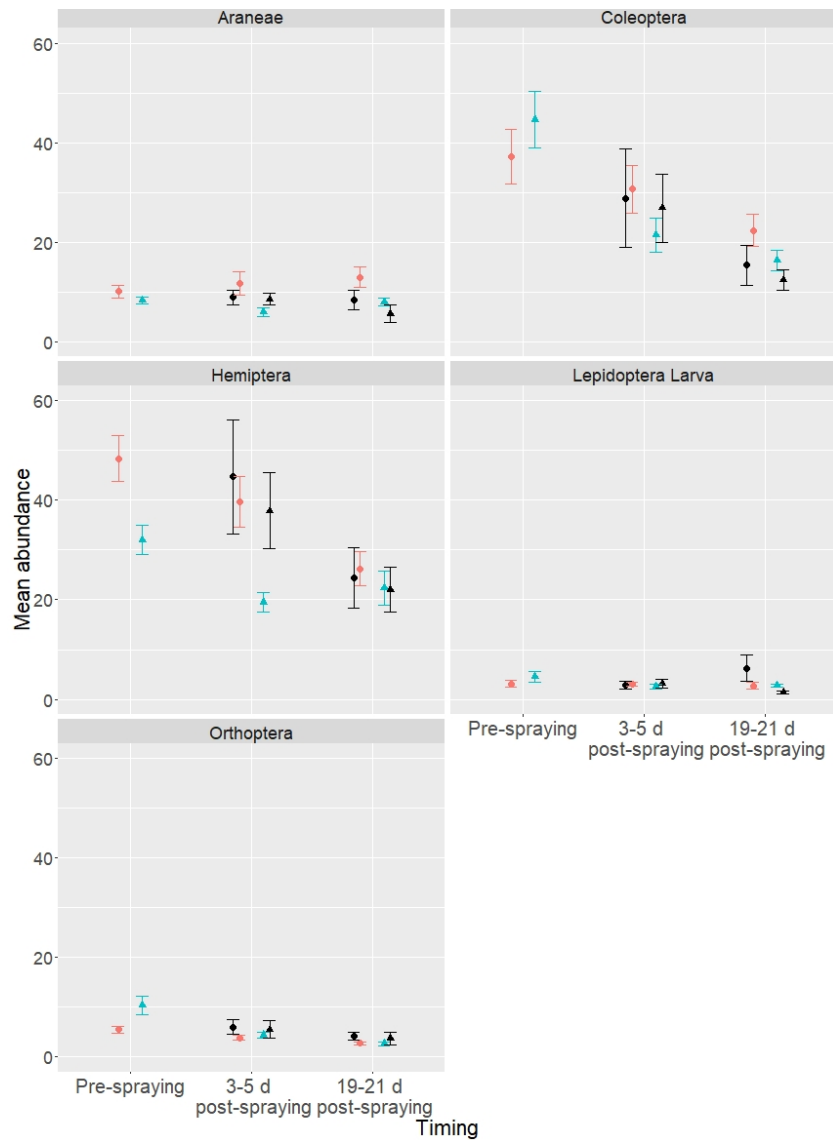
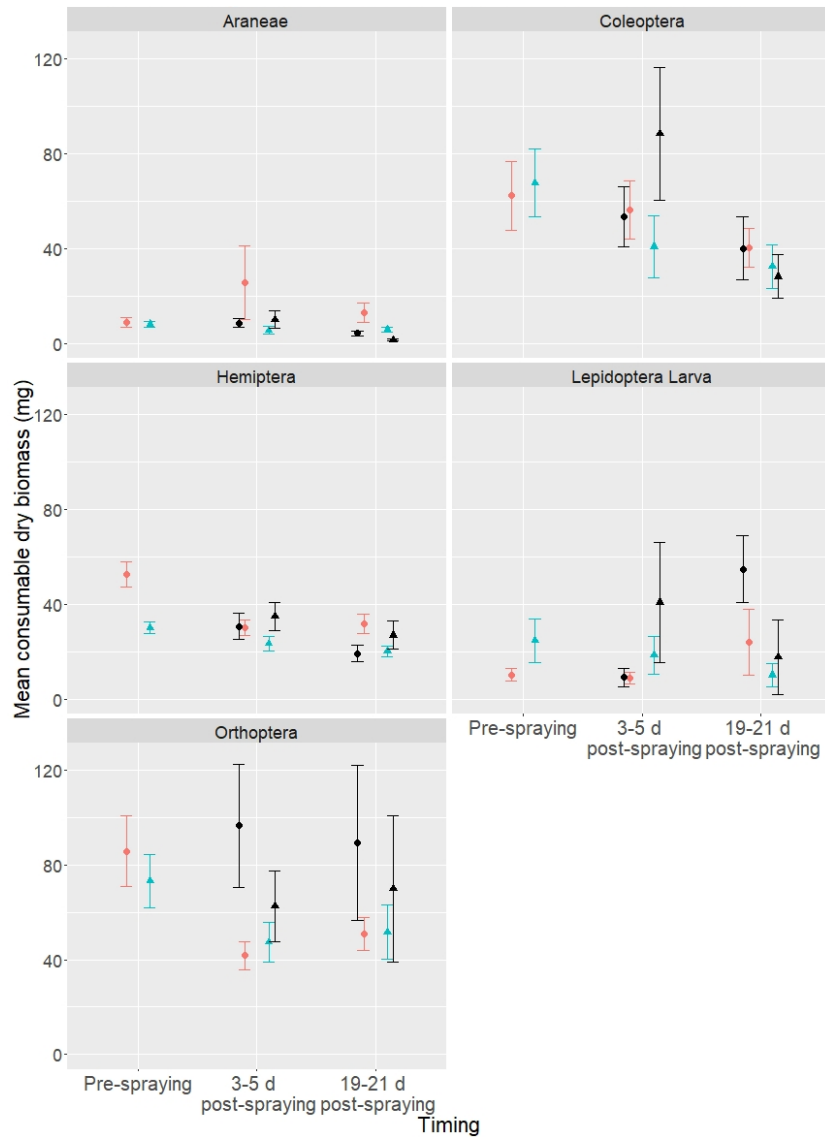


Figure 4. Mean arthropod consumable dry biomasses (mg) through time at treatment and control sites. Red circles indicate samples from control site secondary arthropod collection transects and blue triangles indicate samples from treatment site secondary arthropod collection transects. Black circles indicate samples from reference transects at control sites and black triangles indicate samples from reference transects at treatment sites. Error bars represent standard errors. Treatment sites were grasslands bordered by soybean fields sprayed with insecticides to combat soybean aphids; control sites were grasslands bordered by corn fields that were not treated with insecticides. Secondary arthropod collection transects were parallel to the field edge and were visited by researchers repeatedly; reference transects were located > 60 m from other transects to minimize sampling disturbance. Arthropod samples were collected during the summers of 2017 and 2018 in Minnesota’s farmland region.



Appendix A. Percentages of total arthropod abundance and dry consumable biomass by taxon for all samples collected at treatment and control study sites during the summers of 2017 and 2018 in the farmland region of Minnesota. Treatment sites were grasslands bordered by soybean fields sprayed with insecticides to combat soybean aphids; control sites were grasslands bordered by corn fields that were not treated with insecticides.

| Arthropod taxon | % of total abundance | % of total dry consumable biomass |
|-------------------------|-----------------------------|--|
| <i>Bird prey groups</i> | | |
| Araneae | 7.2 | 4.8 |
| Anyphaenidae | 0.0088 | 0.0012 |
| Araneidae | 0.2 | 0.57 |
| Clubionidae | 0.091 | 0.0057 |
| Ctenidae | 0.0058 | 0.000048 |
| Dictynidae | 0.0058 | 0.000052 |
| Eutichuridae | 0.012 | 0.0011 |
| Linyphiidae | 0.023 | 0.0049 |
| Liocranidae | 0.0029 | 0.0012 |
| Lycosidae | 0.044 | 0.0093 |
| Oxyopidae | 0.27 | 0.15 |
| Philodromidae | 0.48 | 0.42 |
| Pholcidae | 0.0058 | 0.011 |
| Pisauridae | 0.038 | 0.025 |
| Salticidae | 2.5 | 2.3 |
| Tetragnathidae | 0.43 | 0.76 |
| Theridiidae | 0.0029 | 0.000065 |
| Thomisidae | 0.5 | 0.097 |
| Unidentified | 2.6 | 0.41 |
| Coleoptera | 23.0 | 26.0 |
| Anthicidae | 0.0058 | 0.00092 |
| Bruchidae | 0.035 | 0.0023 |
| Cantharidae | 1.4 | 15 |
| Carabidae | 0.34 | 0.36 |
| Cerambycidae | 0.0029 | 0.039 |
| Chrysomelidae | 12 | 7.1 |
| Cleridae | 0.0088 | 0.0058 |
| Coccinellidae | 3.1 | 0.29 |
| Corylophidae | 0.0058 | 0.0001 |
| Cryptophagidae | 0.4 | 0.032 |
| Curculionidae | 4.4 | 1.8 |
| Elateridae | 0.0058 | 0.049 |
| Erotylidae | 0.0029 | 0.005 |
| Lampyridae | 0.029 | 0.079 |
| Meloidae | 0.14 | 1.1 |
| Mordellidae | 0.029 | 0.003 |
| Nitidulidae | 0.07 | 0.025 |
| Phalacridae | 1.3 | 0.14 |

| | | |
|---------------------|--------|---------|
| Ptiliidae | 0.018 | 0.00033 |
| Scirtidae | 0.0058 | 0.00084 |
| Silphidae | 0.0029 | 0.037 |
| Sphaeriusidae | 0.0029 | 0.00019 |
| Staphylinidae | 0.13 | 0.024 |
| Unidentified | 0.11 | 0.059 |
| Lepidoptera larvae | 0.92 | 3.4 |
| Orthoptera | 3.5 | 26.0 |
| Acrididae | 1.6 | 13 |
| Gryllacrididae | 0.015 | 0.12 |
| Gryllidae | 1.4 | 5 |
| Tettigoniidae | 0.55 | 7.3 |
| <i>Other groups</i> | | |
| Diptera | 19.0 | 8.1 |
| Hemiptera | 26.0 | 16.0 |
| Acanaloniidae | 1.2 | 0.63 |
| Aleyrodidae | 0.0058 | 0.00045 |
| Alydidae | 0.27 | 0.64 |
| Anthocoridae | 0.55 | 0.028 |
| Aphididae | 4.1 | 0.25 |
| Berytidae | 0.015 | 0.016 |
| Caliscelidae | 0.25 | 0.026 |
| Cercopidae | 2.6 | 1.0 |
| Cicadellidae | 6.0 | 3.0 |
| Cixiidae | 0.0058 | 0.00058 |
| Coccidae | 0.0029 | 0.00042 |
| Delphacidae | 1 | 0.15 |
| Derbidae | 0.023 | 0.0031 |
| Dictyopharidae | 0.22 | 0.37 |
| Fulgoridae | 0.023 | 0.002 |
| Issidae | 0.012 | 0.0033 |
| Lygaeidae | 0.77 | 0.61 |
| Membracidae | 0.18 | 0.15 |
| Miridae | 2.8 | 1.3 |
| Nabidae | 0.87 | 1.5 |
| Pentatomidae | 2.3 | 3.3 |
| Phymatidae | 0.29 | 0.7 |
| Psyllidae | 1.5 | 0.19 |
| Reduviidae | 0.35 | 1.6 |
| Rhopalidae | 0.058 | 0.033 |
| Scutelleridae | 0.032 | 0.022 |
| Thyreocoridae | 0.015 | 0.0026 |
| Tingidae | 0.68 | 0.11 |
| Unidentified | 0.14 | 0.022 |
| Hymenoptera | 15 | 5.8 |
| Lepidoptera adults | 1.2 | 4.2 |
| Other | 3.9 | 6.4 |

Appendix B. Means (\bar{x}) and standard deviations (SD) for abundance of arthropods collected via sweep-net and vacuum sampling during 3 sampling periods at treatment and control study sites on secondary arthropod collection transects. Arthropod samples were collected in grasslands in the farmland region of Minnesota during the summers of 2017 and 2018. Treatment sites were grasslands bordered by soybean fields sprayed with foliar insecticides to control soybean aphids; control sites were bordered by corn fields that were not treated with insecticides during sampling. Pre-spray samples were collected 1–3 days prior to the spraying event. Arthropods in orders important in the diets of grassland nesting birds are listed under “Bird prey groups”; arthropods less important in their diets are listed under “Other groups.” “Other” includes arthropods in any order not listed in addition to arthropods not identified to order.

| | Treatment sites | | | | | | Control sites | | | | | |
|-------------------------|-----------------------|------|---------------------------------|------|-----------------------------------|------|-----------------------|------|---------------------------------|------|-----------------------------------|------|
| | Pre-spray (n = 45) | | 3–5 d post-spray (n = 45) | | 19–21 d post-spray (n = 45) | | Pre-spray (n = 36) | | 3–5 d post-spray (n = 36) | | 19–21 d post-spray (n = 36) | |
| | \bar{x} | SD | \bar{x} | SD | \bar{x} | SD | \bar{x} | SD | \bar{x} | SD | \bar{x} | SD |
| <i>Bird prey groups</i> | | | | | | | | | | | | |
| Araneae | 3.14 | 2.56 | 2.88 | 3.19 | 3.58 | 3.48 | 3.69 | 3.6 | 4.35 | 5.23 | 4.4 | 5.06 |
| Anyphaenidae | - | NA | 1 | NA | - | NA | - | NA | - | NA | 1 | NA |
| Araneidae | 1.14 | 0.38 | 1 | 0 | 1 | NA | 1.2 | 0.45 | 3.44 | 4.48 | 1.83 | 0.75 |
| Clubionidae | 1 | NA | 1.5 | 0.71 | - | NA | - | NA | 27 | NA | - | NA |
| Ctenidae | - | NA | - | NA | - | NA | 1 | NA | 1 | NA | - | NA |
| Dictynidae | - | NA | - | NA | - | NA | - | NA | - | NA | 1 | 0 |
| Eutichuridae | - | NA | 2 | NA | - | NA | 1 | 0 | - | NA | - | NA |
| Linyphiidae | - | NA | 1 | NA | 1 | NA | 1.5 | 0.71 | 1.5 | 0.71 | - | NA |
| Liocranidae | - | NA | - | NA | - | NA | 1 | NA | - | NA | - | NA |
| Lycosidae | 1 | NA | 2.25 | 0.96 | 2 | NA | 1 | 0 | - | NA | - | NA |
| Oxyopidae | 1.67 | 1.12 | 1 | 0 | 1 | 0 | 2.17 | 1.17 | 1.38 | 0.74 | 1.67 | 0.98 |
| Philodromidae | 1 | 0 | 3.55 | 2.98 | 1.9 | 1.1 | 2.9 | 1.79 | 3.83 | 1.94 | 2 | 1.49 |
| Pholcidae | 2 | NA | - | NA | - | NA | - | NA | - | NA | - | NA |
| Pisauridae | 1 | NA | - | NA | 1.33 | 0.58 | 1.67 | 0.58 | 2 | NA | - | NA |
| Salticidae | 3.24 | 2.44 | 2 | 1.13 | 4.04 | 2.7 | 4.29 | 2.78 | 4.72 | 3.94 | 5.17 | 4.33 |

| | | | | | | | | | | | | |
|--------------------|-------|-------|-------|-------|------|-------|-------|-------|-------|-------|-------|-------|
| Tetragnathidae | 3.47 | 2.07 | 2.1 | 1.1 | 1.75 | 0.89 | 1.71 | 1.25 | 1.83 | 1.17 | 2.17 | 1.17 |
| Theridiidae | - | NA | - | NA | - | NA | 1 | NA | - | NA | - | NA |
| Thomisidae | 3.42 | 2.47 | 2.7 | 2.41 | 2.18 | 1.08 | 1.29 | 0.49 | 1.42 | 1.16 | 2.44 | 1.74 |
| Unidentified | 4.15 | 3.33 | 5.65 | 5.75 | 7 | 4.94 | 8 | 5.44 | 8.86 | 7.41 | 9.21 | 7.63 |
| Coleoptera | 12.1 | 18.74 | 7.63 | 12.49 | 4.87 | 7.62 | 9.42 | 13.9 | 8.26 | 14.51 | 6.46 | 10.21 |
| Anthicidae | 2 | NA | - | NA | - | NA | - | NA | - | NA | - | NA |
| Bruchidae | - | NA | - | NA | - | NA | 3.67 | 1.53 | 1 | NA | - | NA |
| Cantharidae | 5.35 | 6.1 | 3.67 | 6.06 | 3.64 | 4.27 | 5.42 | 6.64 | 5.29 | 5.39 | 3.77 | 2.74 |
| Carabidae | 1.5 | 0.58 | 1 | NA | 1.6 | 0.89 | 2 | 2 | 4.29 | 2.56 | 2.33 | 1.73 |
| Chrysomelidae | 21.3 | 23.41 | 16.16 | 18.76 | 7.97 | 10.21 | 15.97 | 17.1 | 18.67 | 24.18 | 15.78 | 15.56 |
| Cleridae | - | NA | - | NA | - | NA | - | NA | 1 | NA | 1 | NA |
| Coccinellidae | 30.44 | 28.92 | 15.09 | 15.33 | 4.29 | 3.52 | 9.92 | 19.12 | 2.88 | 2.3 | 1.22 | 0.44 |
| Corylophidae | - | NA | - | NA | - | NA | 2 | NA | - | NA | - | NA |
| Cryptophagidae | 2.67 | 2.1 | 2.18 | 1.47 | 2.26 | 1.91 | 1.57 | 0.53 | 1.2 | 0.45 | 1 | 0 |
| Curculionidae | 7.08 | 8.22 | 3.78 | 3.33 | 6.84 | 10.53 | 14.03 | 14.48 | 8.34 | 7.09 | 5 | 5.36 |
| Elateridae | 1 | NA | - | NA | - | NA | - | NA | 1 | NA | - | NA |
| Erotylidae | - | NA | - | NA | - | NA | - | NA | 1 | NA | - | NA |
| Lampyridae | 1.5 | 0.58 | - | NA | - | NA | - | NA | 1 | 0 | - | NA |
| Meloidae | 2.43 | 0.98 | 1.67 | 1.21 | 1.33 | 0.58 | 1.25 | 0.5 | 1 | 0 | 1 | NA |
| Mordellidae | 1 | 0 | - | NA | - | NA | 1 | 0 | 1.67 | 0.58 | - | NA |
| Nitidulidae | 2.5 | 1.29 | 2.5 | 2.12 | 1 | 0 | - | NA | 2 | NA | 1 | 0 |
| Phalacridae | 9.68 | 12.44 | 6.07 | 7.86 | 2.12 | 1.41 | 2.56 | 1.94 | 1.44 | 0.53 | 1.89 | 0.93 |
| Ptiliidae | - | NA | - | NA | - | NA | - | NA | - | NA | 3 | 1.41 |
| Silphidae | - | NA | 1 | NA | - | NA | - | NA | - | NA | - | NA |
| Sphaeriidae | - | NA | - | NA | - | NA | - | NA | - | NA | 1 | NA |
| Staphylinidae | 2 | 1.55 | 1 | 0 | 1.5 | 0.58 | 1.88 | 1.36 | 1 | NA | - | NA |
| Unidentified | 1.67 | 1.15 | 1.17 | 0.41 | 3.5 | 3.54 | 1 | 0 | 1 | 0 | 1.29 | 0.49 |
| Lepidoptera larvae | 4.5 | 4.1 | 2.58 | 1.88 | 2.77 | 1.3 | 3.08 | 2.53 | 3 | 1.15 | 2.73 | 2.66 |

| | | | | | | | | | | | | |
|---------------------|-------|-------|------|------|------|-------|-------|-------|-------|-------|-------|-------|
| Orthoptera | 5.41 | 7 | 2.9 | 2.23 | 1.85 | 1.16 | 2.85 | 2.16 | 2.18 | 1.55 | 1.79 | 1 |
| Acrididae | 8.07 | 10.28 | 2.5 | 2.48 | 2 | 1.22 | 2.54 | 1.45 | 2.08 | 1.73 | 1.56 | 0.7 |
| Gryllacrididae | - | NA | 1 | NA | 1 | NA | - | NA | 1 | NA | - | NA |
| Gryllidae | 5.52 | 3.92 | 3.61 | 2.15 | 2 | 1.35 | 3.77 | 2.94 | 2.45 | 1.54 | 2.19 | 1.33 |
| Tettigoniidae | 1.86 | 1.15 | 2.73 | 1.68 | 1.38 | 0.52 | 2.17 | 1.54 | 2 | 1.18 | 1.5 | 0.53 |
| <i>Other groups</i> | | | | | | | | | | | | |
| Diptera | 15.08 | 29.15 | 5.67 | 8.48 | 9.53 | 11.86 | 13.31 | 18.26 | 12.48 | 19.02 | 10.64 | 16.11 |
| Hemiptera | 4.65 | 6.24 | 3.56 | 4.21 | 3.83 | 9.05 | 6.2 | 8.62 | 5.77 | 8.35 | 4.03 | 4.59 |
| Acanaloniidae | 2.5 | 0.71 | 1.33 | 0.58 | 1.33 | 0.58 | 8.71 | 10.29 | 7.14 | 8.71 | 7.88 | 9.14 |
| Aleyrodidae | - | NA | - | NA | - | NA | - | NA | - | NA | 2 | NA |
| Alydidae | 2.5 | 1.58 | 1.4 | 0.89 | 1.17 | 0.41 | 3.17 | 2.32 | 1.67 | 0.58 | 2.17 | 1.33 |
| Anthocoridae | 3.92 | 2.81 | 3.78 | 4.24 | 2.54 | 2.02 | 1 | 0 | 1.33 | 0.58 | 1.67 | 0.58 |
| Aphididae | 7.97 | 11.4 | 6.53 | 6.82 | 8.77 | 21.82 | 10.41 | 15.08 | 7 | 8.19 | 3.35 | 3.6 |
| Berytidae | - | NA | 1 | NA | - | NA | 2 | NA | - | NA | 1 | 0 |
| Caliscelidae | 2.2 | 0.84 | 1 | 0 | 2.29 | 1.5 | 2.75 | 2.22 | 1.25 | 0.5 | 6 | 5.14 |
| Cercopidae | 4 | 3.43 | 2.2 | 2.1 | 6.38 | 9.4 | 9.13 | 9.37 | 12.74 | 16.22 | 3 | 3.16 |
| Cicadellidae | 8.23 | 6.28 | 5.93 | 4.71 | 3.92 | 2.79 | 14.3 | 12.58 | 11.21 | 10.65 | 6.09 | 4.95 |
| Cixiidae | - | NA | - | NA | - | NA | 2 | NA | - | NA | - | NA |
| Delphacidae | 4.09 | 3.83 | 2.2 | 1.37 | 2.36 | 1.08 | 3.93 | 2.05 | 2.67 | 1.97 | 3.14 | 1.79 |
| Derbidae | 1 | 0 | 1.5 | 0.71 | 1 | NA | - | NA | - | NA | - | NA |
| Dictyopharidae | 1.67 | 0.82 | 1 | 0 | 1.25 | 0.5 | 3.5 | 6.28 | 1.33 | 0.82 | 2 | 1.15 |
| Fulgoridae | - | NA | - | NA | - | NA | - | NA | - | NA | 4 | 1.41 |
| Issidae | - | NA | - | NA | - | NA | 3 | NA | 1 | NA | - | NA |
| Lygaeidae | 1.56 | 0.86 | 2.64 | 2.25 | 2.06 | 2.21 | 3.6 | 3.92 | 3.29 | 1.98 | 3.6 | 3.6 |
| Membracidae | 1.22 | 0.44 | 3.33 | 2.31 | 1.67 | 1.15 | 1.67 | 1.12 | 1 | 0 | 1.29 | 0.76 |
| Miridae | 4.87 | 5.85 | 4.5 | 5.18 | 3.21 | 4.34 | 4.39 | 5.12 | 5.16 | 7.62 | 5.52 | 6.34 |
| Nabidae | 4.44 | 4.16 | 3.21 | 3.08 | 1.6 | 1.35 | 2.83 | 2.83 | 2.18 | 2.04 | 2.15 | 1.95 |
| Pentatomidae | 3.28 | 2.33 | 2.31 | 1.84 | 3.21 | 2.25 | 4.35 | 3.13 | 4 | 2.62 | 3.89 | 2.59 |

| | | | | | | | | | | | | |
|--------------------|------|-------|------|------|-------|-------|------|------|-------|-------|-------|-------|
| Phymatidae | 1.17 | 0.41 | 1.67 | 1.21 | 1 | 0 | 2.38 | 2.69 | 1.31 | 0.48 | 2 | 1.41 |
| Psyllidae | 7.94 | 11.07 | 4.44 | 4.75 | 12 | 24.47 | 7.17 | 7.39 | 3 | 4.92 | 2.25 | 1.89 |
| Reduviidae | 1.73 | 1.49 | 1.27 | 0.59 | 2.36 | 2.34 | 2.2 | 0.84 | 1.2 | 0.45 | 1.2 | 0.45 |
| Rhopalidae | 1.5 | 1 | 1.33 | 0.58 | 1 | 0 | 2 | 1.41 | 2 | NA | - | NA |
| Scutelleridae | 1 | 0 | 1 | 0 | - | NA | 1.5 | 0.71 | - | NA | 1 | NA |
| Thyreocoridae | - | NA | 2 | 1.41 | 1 | NA | - | NA | - | NA | - | NA |
| Tingidae | 1.75 | 0.5 | 2 | NA | - | NA | 2.36 | 2.66 | 8 | 4.95 | 2.25 | 1.89 |
| Unidentified | 1 | 0 | 2.5 | 0.71 | 2 | NA | 1.33 | 0.58 | 2.67 | 4.08 | 1 | NA |
| Hymenoptera | 9.47 | 13.45 | 5.76 | 6.99 | 10.95 | 13.42 | 15.6 | 20.3 | 17.86 | 27.53 | 14.08 | 15.74 |
| Lepidoptera adults | 2.05 | 1.4 | 2.55 | 2.63 | 2.28 | 1.37 | 1.54 | 0.83 | 3.43 | 3.23 | 3.83 | 4.33 |
| Other | 2.88 | 4.97 | 4.75 | 9.34 | 2.81 | 5.26 | 4.14 | 8.66 | 4.33 | 11.8 | 4.51 | 13.34 |

Appendix C. Means (\bar{x}) and standard deviations (SD) for abundance of arthropods collected via sweep-net and vacuum sampling during 2 sampling periods at treatment and control study sites on additional reference transects. Arthropod samples were collected in grasslands in Minnesota’s farmland region during the summers of 2017 and 2018. Treatment sites were grasslands bordered by soybean fields sprayed with foliar insecticides to control soybean aphids; control sites were bordered by corn fields that were not treated with insecticides during sampling. Secondary arthropod collection transects were parallel to the field edge and were visited by researchers repeatedly; reference transects were located > 60 m from other transects to minimize sampling disturbance. Pre-spray samples were collected 1–3 days prior to the spraying event. Arthropods in orders important in the diets of grassland nesting birds are listed under “Bird prey groups”; arthropods less important in their diets are listed under “Other groups.” “Other” includes arthropods in any order not listed in addition to arthropods not identified to order.

| | Treatment sites | | | | Control sites | | | |
|-------------------------|------------------------------|-------|--------------------------------|------|------------------------------|-------|--------------------------------|-------|
| | 3–5 d post-spray (n = 15) | | 19–21 d post-spray (n = 15) | | 3–5 d post-spray (n = 12) | | 19–21 d post-spray (n = 12) | |
| | \bar{x} | SD | \bar{x} | SD | \bar{x} | SD | \bar{x} | SD |
| <i>Bird prey groups</i> | | | | | | | | |
| Araneae | 2.98 | 2.18 | 2.57 | 3.17 | 3.18 | 3.24 | 3.29 | 2.73 |
| Coleoptera | 7.37 | 12.48 | 3.96 | 4.01 | 7.69 | 11.94 | 5.26 | 7.86 |
| Lepidoptera larvae | 3.2 | 1.92 | 1.4 | 0.55 | 2.83 | 1.94 | 6.25 | 5.25 |
| Orthoptera | 3.88 | 3.62 | 2.36 | 2.38 | 2.84 | 2.22 | 2.47 | 2 |
| <i>Other groups</i> | | | | | | | | |
| Diptera | 5.9 | 7.12 | 7.81 | 8.39 | 18.14 | 23.76 | 14.64 | 21.39 |
| Hemiptera | 5.92 | 10.95 | 3.59 | 5.11 | 6.03 | 8.99 | 4.44 | 7.29 |
| Hymenoptera | 7 | 8.3 | 8.25 | 9.27 | 22.89 | 21.45 | 17.31 | 17.02 |
| Lepidoptera adults | 1.5 | 1 | 3.2 | 3.77 | 2.6 | 1.52 | 4.83 | 3.71 |
| Other | 2.47 | 2.24 | 3.36 | 5.79 | 5.88 | 12.9 | 2.59 | 2.87 |

Appendix D. Means (\bar{x}) and standard deviations (SD) for consumable dry biomass estimates (mg) of arthropods collected via sweep-net and vacuum sampling during 3 sampling periods at treatment and control study sites on secondary transects. Arthropod samples were collected in grasslands in Minnesota’s farmland region during the summers of 2017 and 2018. Treatment sites were grasslands bordered by soybean fields sprayed with foliar insecticides to control soybean aphids; control sites were bordered by corn fields that were not treated with insecticides during sampling. Secondary arthropod collection transects were parallel to the field edge and were visited by researchers repeatedly; reference transects were located > 60 m from other transects to minimize sampling disturbance. Pre-spray samples were collected 1–3 days prior to the spraying event. Arthropods in orders important in the diets of grassland nesting birds are listed under “Bird prey groups”; arthropods less important in their diets are listed under “Other groups.” “Other” includes arthropods in any order not listed in addition to arthropods not identified to order.

| | Treatment sites | | | | | | Control sites | | | | | |
|-------------------------|-----------------------|------|---------------------------------|-------|-----------------------------------|------|-----------------------|------|---------------------------------|-------|-----------------------------------|-------|
| | Pre-spray (n = 45) | | 3–5 d post-spray (n = 45) | | 19–21 d post-spray (n = 45) | | Pre-spray (n = 36) | | 3–5 d post-spray (n = 36) | | 19–21 d post-spray (n = 36) | |
| | \bar{x} | SD | \bar{x} | SD | \bar{x} | SD | \bar{x} | SD | \bar{x} | SD | \bar{x} | SD |
| <i>Bird prey groups</i> | | | | | | | | | | | | |
| Araneae | 3.05 | 5.09 | 2.66 | 5.64 | 2.51 | 3.53 | 3.27 | 5.75 | 9.51 | 53.24 | 4.38 | 13.23 |
| Anyphaenidae | - | NA | 0.14 | NA | - | NA | - | NA | - | NA | 0.1 | NA |
| Araneidae | 3.6 | 5.03 | 5.26 | 7.23 | 9.92 | NA | 2.77 | 5.88 | 14.33 | 32.84 | 20.73 | 48 |
| Clubionidae | 0.28 | NA | 0.15 | 0.13 | - | NA | - | NA | 2.62 | NA | - | NA |
| Ctenidae | - | NA | - | NA | - | NA | 0.01 | NA | 0.01 | NA | - | NA |
| Dictynidae | - | NA | - | NA | - | NA | - | NA | - | NA | 0.01 | 0.01 |
| Eutichuridae | - | NA | 0.58 | NA | - | NA | 0.01 | 0 | - | NA | - | NA |
| Linyphiidae | - | NA | 0.55 | NA | 0.11 | NA | 0.83 | 1.13 | 0.23 | 0.31 | - | NA |
| Liocranidae | - | NA | - | NA | - | NA | 0.65 | NA | - | NA | - | NA |
| Lycosidae | 0.04 | NA | 0.43 | 0.32 | 0.08 | NA | 1.65 | 2.28 | - | NA | - | NA |
| Oxyopidae | 1.66 | 2.25 | 0.32 | 0.4 | 0.71 | 0.76 | 0.04 | 0.05 | 1.33 | 2.06 | 3.47 | 11.52 |
| Philodromidae | 3.37 | 4.32 | 6.35 | 10.29 | 2.9 | 5.26 | 4.63 | 8.02 | 2.38 | 1.74 | 1.73 | 1.75 |
| Pholcidae | 6.34 | NA | - | NA | - | NA | - | NA | - | NA | - | NA |
| Pisauridae | 0.29 | NA | - | NA | 0.09 | 0.09 | 3.25 | 0.54 | 3.46 | NA | - | NA |
| Salticidae | 2.34 | 4.68 | 2.32 | 6.1 | 3.68 | 2.92 | 4.73 | 7.36 | 20.92 | 91.33 | 5.36 | 6.31 |
| Tetragnathidae | 9.73 | 7.2 | 5.36 | 3.77 | 5.14 | 5.73 | 6.1 | 5.38 | 6.96 | 5.46 | 5.37 | 2.73 |
| Theridiidae | - | NA | - | NA | - | NA | 0.04 | NA | - | NA | - | NA |

| | | | | | | | | | | | | |
|---------------------|--------|--------|-------|--------|-------|-------|-------|-------|-------|-------|-------|-------|
| Thomisidae | 0.48 | 1.21 | 0.41 | 1.04 | 0.54 | 1.17 | 1.76 | 3.21 | 0.66 | 1.01 | 0.17 | 0.39 |
| Unidentified | 1.3 | 1.8 | 1.92 | 3.59 | 1.67 | 2.63 | 1.53 | 2.09 | 2.12 | 2.93 | 2.08 | 2.15 |
| Coleoptera | 18.31 | 53.09 | 14.54 | 53.33 | 9.66 | 34.83 | 15.78 | 43.24 | 15.19 | 29.7 | 11.65 | 27.19 |
| Anthicidae | 0.52 | NA | - | NA | - | NA | - | NA | - | NA | - | NA |
| Bruchidae | - | NA | - | NA | - | NA | 0.38 | 0.38 | 0.14 | NA | - | NA |
| Cantharidae | 108.11 | 133.18 | 73.33 | 141.07 | 71.39 | 94.46 | 76.46 | 90.8 | 59.2 | 53 | 61.55 | 59.81 |
| Carabidae | 3.49 | 4.31 | 4.17 | NA | 2.7 | 5.35 | 1.99 | 2.62 | 1.85 | 2.55 | 1.76 | 1.89 |
| Chrysomelidae | 18.35 | 18.43 | 14.9 | 16.61 | 7.18 | 8.28 | 17.87 | 19.64 | 19.82 | 23.93 | 14.13 | 13.02 |
| Cleridae | - | NA | - | NA | - | NA | - | NA | 0.78 | NA | 0.55 | NA |
| Coccinellidae | 2.46 | 1.88 | 2.08 | 3.34 | 1.13 | 1.4 | 2.22 | 4.07 | 3.21 | 5.64 | 0.87 | 0.78 |
| Corylophidae | - | NA | - | NA | - | NA | 0.06 | NA | - | NA | - | NA |
| Cryptophagidae | 0.19 | 0.22 | 0.21 | 0.13 | 0.21 | 0.18 | 0.15 | 0.16 | 0.09 | 0.05 | 1.62 | 3.02 |
| Curculionidae | 2.3 | 3.28 | 1.34 | 1.38 | 2.44 | 4.95 | 16.38 | 55.63 | 4.36 | 6.12 | 3.16 | 4.35 |
| Elateridae | 0.68 | NA | - | NA | - | NA | - | NA | 26.7 | NA | - | NA |
| Erotylidae | - | NA | - | NA | - | NA | - | NA | 2.83 | NA | - | NA |
| Lampyridae | 4.07 | 1.38 | - | NA | - | NA | - | NA | 1.66 | 0.16 | - | NA |
| Meloidae | 31.9 | 13.45 | 24.34 | 23.71 | 20.17 | 17.86 | 14.98 | 14.17 | 11.76 | 8.41 | 17.58 | NA |
| Mordellidae | 0.25 | 0.02 | - | NA | - | NA | 0.09 | 0.02 | 0.3 | 0.12 | - | NA |
| Nitidulidae | 1 | 0.91 | 2.53 | 3.34 | 0.75 | 0.97 | - | NA | 0.65 | NA | 0.08 | 0.01 |
| Phalacridae | 2.47 | 4.37 | 0.82 | 1.08 | 0.35 | 0.24 | 0.46 | 0.34 | 0.29 | 0.18 | 0.32 | 0.26 |
| Ptiliidae | - | NA | - | NA | - | NA | - | NA | - | NA | 0.09 | 0.03 |
| Silphidae | - | NA | 21.04 | NA | - | NA | - | NA | - | NA | - | NA |
| Sphaeriidae | - | NA | - | NA | - | NA | - | NA | - | NA | 0.1 | NA |
| Staphylinidae | 0.32 | 0.24 | 0.17 | 0.17 | 0.43 | 0.3 | 0.56 | 0.28 | 0.19 | NA | - | NA |
| Unidentified | 0.3 | 0.18 | 1.77 | 2.09 | 1.02 | 0.43 | 1.83 | 1.3 | 0.83 | 0.47 | 1.08 | 0.72 |
| Lepidoptera larvae | 24.53 | 36.07 | 18.48 | 27.32 | 10.12 | 17.41 | 10.09 | 9.65 | 8.77 | 7.75 | 24.04 | 54 |
| Orthoptera | 37.99 | 39.2 | 31.06 | 37.87 | 35.68 | 44.89 | 44.13 | 50.49 | 23.47 | 23.48 | 32.58 | 28.42 |
| Acrididae | 53.84 | 51.05 | 38.04 | 37.35 | 56.18 | 61.38 | 47.23 | 49.93 | 29.03 | 28.55 | 39.61 | 22.05 |
| Gryllacrididae | - | NA | 0.98 | NA | 3.69 | NA | - | NA | 6.21 | NA | - | NA |
| Gryllidae | 28.83 | 25.16 | 16.11 | 10.76 | 21.09 | 14.94 | 20.7 | 14.53 | 14.91 | 9.54 | 18.73 | 16.88 |
| Tettigoniidae | 29.38 | 30.75 | 44.28 | 58.26 | 19.83 | 9.76 | 68.28 | 66.91 | 27.96 | 26.14 | 44.48 | 47.03 |
| <i>Other groups</i> | | | | | | | | | | | | |
| Diptera | 7.96 | 9.44 | 5.01 | 8.98 | 12.77 | 22.31 | 8.51 | 11.78 | 6.23 | 6.95 | 5.81 | 4.86 |
| Hemiptera | 4.35 | 6.17 | 4.23 | 6.71 | 3.42 | 5.88 | 6.73 | 11.18 | 4.39 | 5.99 | 4.87 | 7.19 |
| Acanaloniidae | 3.74 | 0.46 | 1.64 | 1.47 | 2.63 | 1.33 | 3.73 | 3.46 | 6.48 | 8.42 | 10.07 | 10.18 |

| | | | | | | | | | | | | |
|--------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| Aleyrodidae | - | NA | - | NA | - | NA | - | NA | - | NA | 0.26 | NA |
| Alydidae | 7.37 | 7.52 | 4.21 | 6.21 | 5.39 | 6.39 | 13.83 | 15.47 | 3.17 | 3.81 | 7.09 | 5.51 |
| Anthocoridae | 0.34 | 0.42 | 0.25 | 0.34 | 0.2 | 0.18 | 0.06 | 0.03 | 0.3 | 0.36 | 0.15 | 0.07 |
| Aphididae | 0.56 | 0.63 | 0.61 | 0.96 | 0.64 | 1.8 | 0.85 | 1.38 | 1.28 | 3.62 | 0.32 | 0.44 |
| Berytidae | - | NA | 3.43 | NA | - | NA | 3.43 | NA | - | NA | 1.09 | 1.27 |
| Caliscelidae | 0.08 | 0.06 | 0.12 | 0.1 | 0.41 | 0.19 | 0.24 | 0.14 | 0.5 | 0.08 | 1.28 | 1.25 |
| Cercopidae | 2.13 | 1.86 | 2.34 | 2.06 | 1.57 | 2.06 | 10.47 | 17.15 | 4.62 | 4.22 | 2.54 | 3.88 |
| Cicadellidae | 7.03 | 6.5 | 6.84 | 6.16 | 2.3 | 2.35 | 13.05 | 14.42 | 7 | 5.01 | 3.51 | 3.5 |
| Cixiidae | - | NA | - | NA | - | NA | 0.33 | NA | - | NA | - | NA |
| Delphacidae | 1.07 | 1.1 | 0.5 | 0.35 | 0.56 | 0.3 | 1.22 | 1.6 | 0.59 | 0.57 | 0.61 | 0.41 |
| Derbidae | 0.23 | 0.04 | 0.36 | 0.17 | 0.14 | NA | - | NA | - | NA | - | NA |
| Dictyopharidae | 5.57 | 4.07 | 3.34 | 2 | 4.06 | 1.2 | 9.74 | 18.38 | 3.47 | 2.05 | 4.42 | 2.89 |
| Fulgoridae | - | NA | - | NA | - | NA | - | NA | - | NA | 0.56 | 0.07 |
| Issidae | - | NA | - | NA | - | NA | 1.41 | NA | 0.43 | NA | - | NA |
| Lygaeidae | 3.15 | 3.62 | 7.6 | 13.77 | 3.53 | 5.55 | 3.47 | 3.37 | 3.4 | 2.32 | 2.28 | 1.93 |
| Membracidae | 1.52 | 0.73 | 3.21 | 1.79 | 1.91 | 1.12 | 2.6 | 1.65 | 1.73 | 0.33 | 2.23 | 1.27 |
| Miridae | 4.73 | 5.49 | 4.07 | 5.65 | 3.1 | 4.8 | 2.87 | 3.05 | 3.24 | 3.98 | 4.39 | 5.64 |
| Nabidae | 9.64 | 8.09 | 6.51 | 9.52 | 3.95 | 3.72 | 10.68 | 13.59 | 9.8 | 15.28 | 6.43 | 6.85 |
| Pentatomidae | 8.58 | 10.07 | 5.51 | 6.7 | 7.87 | 9.33 | 11.38 | 12.25 | 6.48 | 5.87 | 9.88 | 9.49 |
| Phymatidae | 6.03 | 2.66 | 7.1 | 4.24 | 4.89 | 0.79 | 7.6 | 4.62 | 5.48 | 2.5 | 9.41 | 7.78 |
| Psyllidae | 1.58 | 2.3 | 1.07 | 1.25 | 2.49 | 4.52 | 1.21 | 1.34 | 0.6 | 1 | 0.49 | 0.59 |
| Reduviidae | 9.96 | 5.88 | 12.7 | 8.36 | 14.45 | 11.2 | 14.68 | 12.47 | 11.44 | 8.36 | 13.36 | 6.33 |
| Rhopalidae | 0.63 | 0.43 | 0.4 | 0.45 | 2.59 | 3.61 | 1.43 | 1.37 | 2.32 | NA | - | NA |
| Scutelleridae | 0.83 | 0.75 | 1.6 | 2.08 | - | NA | 0.4 | 0.23 | - | NA | 3.85 | NA |
| Thyreocoridae | - | NA | 0.41 | 0.39 | 0.68 | NA | - | NA | - | NA | - | NA |
| Tingidae | 0.11 | 0.08 | 0.19 | NA | - | NA | 0.14 | 0.11 | 0.54 | 0.33 | 12.56 | 24.76 |
| Unidentified | 1.53 | 1.35 | 0.36 | 0.37 | 0.13 | NA | 0.06 | 0.06 | 0.39 | 0.61 | 0.23 | NA |
| Hymenoptera | 6.21 | 6.01 | 3.9 | 3.72 | 7.27 | 7.27 | 11.5 | 18.27 | 10.39 | 12.43 | 8.2 | 6.89 |
| Lepidoptera adults | 7.59 | 14.69 | 12.11 | 24.83 | 9.9 | 15.97 | 10.96 | 31.58 | 7.81 | 11.01 | 50.45 | 106.68 |
| Other | 11.93 | 28.89 | 17.03 | 38.5 | 7.32 | 10.98 | 4.79 | 10.13 | 6.35 | 15.8 | 8.84 | 22.55 |

Appendix E. Means (\bar{x}) and standard deviations (SD) for consumable dry biomass estimates (mg) of arthropods collected via sweep-net and vacuum sampling during 2 sampling periods at treatment and control study sites on additional reference transects. Arthropod samples were collected in grasslands in Minnesota’s farmland region during the summers of 2017 and 2018. Treatment sites were grasslands bordered by soybean fields sprayed with foliar insecticides to control soybean aphids; control sites were bordered by corn fields that were not treated with insecticides during sampling. Secondary arthropod collection transects were parallel to the field edge and were visited by researchers repeatedly; reference transects were located > 60 m from other transects to minimize sampling disturbance. Pre-spray samples were collected 1–3 days prior to the spraying event. Arthropods in orders important in the diets of grassland nesting birds are listed under “Bird prey groups”; arthropods less important in their diets are listed under “Other groups.” “Other” includes arthropods in any order not listed in addition to arthropods not identified to order.

| | Treatment sites | | | | Control sites | | | |
|-------------------------|------------------------------|-------|--------------------------------|-------|------------------------------|-------|--------------------------------|-------|
| | 3–5 d post-spray (n = 15) | | 19–21 d post-spray (n = 15) | | 3–5 d post-spray (n = 12) | | 19–21 d post-spray (n = 12) | |
| | \bar{x} | SD | \bar{x} | SD | \bar{x} | SD | \bar{x} | SD |
| <i>Bird prey groups</i> | | | | | | | | |
| Araneae | 3.47 | 5.74 | 0.63 | 0.71 | 3.07 | 4.65 | 1.66 | 2.04 |
| Coleoptera | 24.26 | 55.54 | 8.94 | 20.99 | 14.21 | 22.92 | 13.71 | 25.78 |
| Lepidoptera larvae | 40.73 | 56.87 | 17.57 | 35.42 | 9.04 | 9.48 | 54.66 | 28.24 |
| Orthoptera | 44.05 | 41.93 | 44.40 | 65.23 | 45.73 | 46.31 | 52.38 | 81.17 |
| <i>Other groups</i> | | | | | | | | |
| Diptera | 4.95 | 6.36 | 9.99 | 11.98 | 12.21 | 15.81 | 8.17 | 9.39 |
| Hemiptera | 5.44 | 8.82 | 4.38 | 8.97 | 4.12 | 5.44 | 3.47 | 3.78 |
| Hymenoptera | 5.47 | 5.05 | 5.93 | 5.20 | 12.48 | 8.77 | 8.74 | 7.29 |
| Lepidoptera adults | 5.44 | 3.92 | 10.61 | 23.44 | 6.67 | 7.33 | 37.55 | 46.24 |
| Other | 23.95 | 62.44 | 5.37 | 6.94 | 12.77 | 15.51 | 3.07 | 5.79 |

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