EVALUATING GRASSLAND WILDLIFE EXPOSURE TO SOYBEAN APHID INSECTICIDES ON PUBLIC LANDS IN MINNESOTA

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SUMMARY OF FINDINGS

Increasing evidence suggests that pesticides may be an important factor explaining declines in grassland-dependent wildlife in agricultural landscapes. Minnesota Department of Natural Resource (MNDNR) wildlife managers and members of the public have reported concerns about drift from foliar-application insecticides in particular. Such insecticides are used on a variety of crops but their use has been especially important for controlling soybean aphid outbreaks in Minnesota. Lab studies have shown chlorpyrifos (a broad spectrum organophosphate) and other insecticides used to target aphids are highly toxic to non-target organisms, including economically important game species and pollinators, but few studies have investigated the environmentally-relevant exposure of free-ranging wildlife to these chemicals. Our objectives were to assess the direct and indirect exposure of grassland wildlife to the 3 most common soybean aphid insecticides (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) along a gradient from soybean field edge to grassland interior. During summer 2017 and 2018, we sampled 5 treatment and 4 control sites across western and southern Minnesota. We detected chlorpyrifos at all distances examined (0-400 m) at both treatment and control sites, suggesting that some background level of chlorpyrifos exposure is occurring in the environment regardless of landowner activities in the adjacent row crop field. Deposition was higher near field edges than the grassland interior. We also found higher residue amounts on mid-canopy samples than ground-level samples. We detected chlorpyrifos residue amounts on arthropods that were below the acute oral LD_{50} values for birds; however, residue amounts were above the contact LD_{50} for honey bees up to 50 m from the row crop edge. We quantified arthropod abundance, consumable dry biomass, and family richness of insects and spiders pre- and post-spraying to evaluate the indirect effects of spraying on bird food resources. We found short-term reductions in overall arthropod abundance, bird prey abundance (specifically, individuals in the orders Araneae, Coleoptera, Lepidoptera larvae, and Orthoptera), and Coleopteran family richness in treatment sites but our other arthropod measures (i.e., overall consumable dry biomass, bird prey biomass, and richness of other families important in the diets of birds) did not differ between treatment and control sites post-spraying. Overall, our results indicate that wildlife within 25 m of a grassland/row crop edge are more likely to be exposed to drift from foliar-application insecticides, and bees within 50 m of an edge may be exposed to dosages that can cause mortality by contact. Furthermore, reductions in arthropod food abundance for grassland birds may occur up to 21 days post-spraying. Natural resource managers should try to minimize the perimeter-area ratio of grasslands to reduce wildlife exposure to drift from insecticide applications in nearby row crop fields. Additionally, management regimes that increase the percent canopy cover in grasslands also have the potential to decrease exposure of grassland wildlife to these insecticides.

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INTRODUCTION

Grassland loss and fragmentation is a major concern for grassland-dependent wildlife throughout the Midwestern United States (U.S.). In particular, habitat loss due to agricultural intensification has been implicated as a primary reason for the declines of many grassland nesting birds (Sampson and Knopf 1994, Vickery et al. 1999). However, concerns are mounting about the impacts of pesticides on birds and other wildlife in agriculturally-dominated landscapes (e.g., Hopwood et al. 2013, Hallmann et al. 2014, Main et al. 2014, Gibbons et al. 2015), and some evidence exists that acute toxicity to pesticides may be more important than agricultural intensity in explaining grassland bird declines in the U.S. (Mineau and Whiteside 2013).

Soybean aphids were first discovered in southeastern Minnesota during 2000 and subsequently spread throughout the farmland zone by 2001 (Venette and Ragsdale 2004). Although these aphids pose significant risks to agriculture, their presence does not automatically translate to reduced yield or income (Vennette and Ragsdale 2004). Foliar applications of insecticides using ground sprayers or airplanes are common treatment methods when chemical control of aphids is necessary. The 2 most common insecticides used are chlorpyrifos and lambda-cyhalothrin (Minnesota Department of Agriculture [MDA] 2005, MDA 2007, MDA 2009, MDA 2012, MDA 2014a) but bifenthrin is also frequently used (N. Davros, unpublished data; E. Runquist, unpublished data). Withholding times vary by chemical (chlorpyrifos: 28 d; lambda-cyhalothrin: 45 d; bifenthrin: up to 14 d); thus, the timing of product use within the growing season needs to be considered. If retreatment is necessary due to a continued infestation, landowners/operators are encouraged to use an insecticide with a different mode of action to prevent resistance (UM Extension 2014) or reduce the impact of insecticide-resistant aphids (UM Extension 2017, UM Extension 2018). Therefore, multiple chemicals may be used on the same field at different times of the year in some situations. Alternatively, landowners/operators may choose to use a product that combines 2 or more chemicals together (e.g., chlorpyrifos + lambda-cyhalothrin), and such products are readily available on the market.

Chlorpyrifos (common trade names: Dursban, Lorsban) is a broad-spectrum organophosphate insecticide that disrupts the normal nervous system functioning of target- and non-target organisms through direct contact, ingestion, and inhalation (Christensen et al. 2009). Although first registered for use in the U.S. in 1965, its use as an ingredient in residential, pet, and indoor insecticides was removed in 1997 (except for containerized baits) due to human health concerns (Christensen et al. 2009, Alvarez et al. 2013 and references therein, MDA 2014b). Furthermore, MDA released guidelines for best management practices for the use of chlorpyrifos due to water quality concerns (MDA 2014b). Lab studies have shown chlorpyrifos to be toxic to a variety of aquatic and terrestrial organisms (reviewed in Barron and Woodburn 1995), and some bird and beneficial insect species are especially susceptible to acute toxicity from chlorpyrifos exposure (Christensen et al. 2009, MDA 2014a). Chlorpyrifos is toxic to honey bees (*Apis mellifera*) with a contact LD_{50} of 100 ppb (Ostiguy et al. 2019), and exposure to chlorpyrifos in contaminated pollen and nectar of adult honey bees is representative of non-*Apis* bee species as well (e.g., bumblebees; Cutler et al. 2014, U.S. Environmental Protection Agency 2014). Chlorpyrifos is very highly toxic to gallinaceous bird species such as the ring-necked pheasant (*Phasianus colchicus*) with a lethal oral dose causing death in 50% of treated animals (LD_{50}) of 12.2 (Solomon et al. 2001). Several other bird species are also particularly susceptible to chlorpyrifos, including American robins (*Turdus migratorius*), common grackles (*Quiscalus quiscula*), and mallards (*Anas platyrhynchos*; Tucker and Haegele 1971, Solomon et al. 2001, Christensen et al. 2009). Yet few field studies have been able to document direct mortality of birds from chlorpyrifos exposure (e.g., Buck et al. 1996, Martin et al. 1996, Booth et al. 2005), and an ecotoxological risk assessment conducted by Solomon et al. (2001) concluded that the available evidence did not support the presumption that chlorpyrifos use in agroecosystems will result in extensive mortality of wildlife. However, chlorpyrifos exposure leading to morbidity (e.g., altered brain cholinesterase activity, altered behaviors, reduced weight gain, impaired migratory orientation) has been documented in both lab and field studies of several avian species (McEwen et al. 1986, Richards et al. 2000, Al-Badrany and Mohammad 2007, Moye 2008, Eng et al. 2017). Thus, sub-lethal effects leading to indirect mortality (e.g., via increased predation rates) or lost breeding opportunities may be a concern for wildlife, especially birds, exposed to chlorpyrifos.

Lambda-cyhalothrin (common trade name: Warrior) is a broad-spectrum pyrethroid insecticide that affects the nervous systems of target- and non-target organisms through direct contact, ingestion, and inhalation [National Pesticide Information Center (NPIC) 2001]. Although lambda-cyhalothrin is considered low in toxicity to birds, it is highly toxic to pollinators such as bees (NPIC 2001). Furthermore, field studies have shown lower insect diversity and abundance in fields exposed to lambda-cyhalothrin (Galvan et al. 2005, Langhof et al. 2005, Devotto et al. 2006). Because insects are an especially important source of protein for birds during the breeding season, fewer insects could mean reduced food availability for fast-growing chicks.

Bifenthrin (common trade name: Tundra) is a broad-spectrum pyrethroid insecticide that affects the central and peripheral nervous systems of organisms by contact or ingestion (Johnson et al. 2010). Bifenthrin is low in toxicity to birds, including game species such as northern bobwhite (*Colinus virginianus*) and mallards (oral LD_{50} values of 1800 mg/kg and <2150 mg/kg, respectively; Johnson et al. 2010). However, there are exposure risks for birds that feed on fish and aquatic insects because bifenthrin is very highly toxic to aquatic organisms (Siegfried 1993, Johnson et al. 2010). Some non-target terrestrial insects are also susceptible to bifenthrin (Siegfried 1993). For example, bifenthrin is very highly toxic to bumblebees, with one study showing 100% mortality by contact (Besard et al. 2010).

Natural resource managers and members of the public have reported concerns about the effects of soybean aphid insecticides on non-target wildlife, including economically important game bird and pollinator species. Yet little is known about the true exposure of birds and terrestrial invertebrates to drift from these insecticides in Minnesota's grasslands. Distances reported for drift from application of foliar insecticides vary widely in the literature (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). The distance of travel for spray drift is dependent on several factors including droplet size, boom height or width, and weather conditions (e.g., humidity, wind speed, dew point) at the time of application. Guidelines for pesticide application are readily available to landowners and licensed applicators (MDA 2014b, MDA 2014c) so that the likelihood of spray drift can be minimized but there is likely large variation in typical application practices.

OBJECTIVES

Our goal was to assess the environmentally-relevant exposure of grassland wildlife to the 3 most commonly-used soybean aphid insecticides (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin; hereafter, target chemicals) in Minnesota's farmland region. Specific objectives included:

1. *Direct and Indirect Exposure:* We quantified the concentration of target chemicals along a gradient from soybean field edge to grassland interior to assess the potential for grassland wildlife (particularly nesting birds and their young, and beneficial insects) to be exposed to these chemicals: 1a) directly via contact with spray drift, and 1b) indirectly through consumption of insect prey items exposed to the insecticides.

2. *Indirect Effects:* We quantified and compared the relative abundance, richness, diversity, and biomass of invertebrate prey items along a gradient from soybean field edge to grassland interior prior to and post-application to assess the indirect impact of the target chemicals on food availability for grassland nesting birds and other wildlife.

STUDY AREA

We conducted our study within the southwest (SW), west central (WC), and central (C) regions of Minnesota's farmland zone (Figure 1). Corn and soybeans combined account for approximately 90%, 67%, and 71% of the landscape across these three regions, respectively [U.S. Department of Agriculture (USDA) 2019a, USDA 2019b]. Area set aside as grassland cover on public and private land accounted for 6.9%, 10.0%, and 5.6% of the landscape in these regions, respectively (Messinger and Davros 2018). Since 2003, these regions have also experienced some of the highest estimated use of chlorpyrifos and lambda-cyhalothrin (MDA 2005, MDA 2007, MDA 2009, MDA 2012, MDA 2014a).

METHODS

Experimental Design

Our treatment study sites consisted of Minnesota Department of Natural Resources (MNDNR) Wildlife Management Areas (WMA) immediately adjacent to soybean fields that were sprayed to control for aphids. We worked closely with wildlife managers and private landowner cooperators to select treatment sites. We used sites dominated by a diverse mesic prairie mix containing warm-season grasses and forbs because this mix is commonly used by MNDNR managers and agency partners in the farmland zone to restore habitats for the benefit of grassland birds and beneficial insect species. Predominant grass species in our sites included big bluestem (*Andropogon gerardii*), smooth brome (*Bromus inermis*), Canada wild rye (*Elymus canadensis*), and Kentucky bluegrass (*Poa pratensis*), and predominant forb species included wild bergamot (*Monarda fistulosa*), smooth oxeye (*Heliopsis helianthoides*), and Canada goldenrod (*Solidago canadensis*). We also selected control study sites with similar site characteristics except that control sites had corn as the adjacent crop and, to our knowledge, they were not sprayed with foliar chemicals to control aphids. We chose sites that were predicted to be downwind (typically east or north) from cooperators' agricultural fields based on typical wind direction patterns determined from archived daily summaries of National Weather Service data.

We sampled 5 treatment sites and 4 control sites across 2 field seasons (summer 2017 and summer 2018; Table 1). Within each treatment site prior to spraying, we established sampling stations at distances of <1 m, 5 m, 25 m, 50 m, 100 m, and 200 m along each of 3 transects. If the site was large enough, we also established a station at a distance of 400 m along each transect. This design gave us a total of 18-21 stations per site. We established transects and stations the same way within control sites. At all sites, transects ran perpendicular to the edge of the cooperator's field and were spaced 90-100 m apart to reduce the likelihood of duplicate insecticide exposure from the spraying event.

Data Collection

Our methodology required precisely timing our field data collection. We worked closely with cooperators to find out the exact date and time that spraying would occur and we adjusted our pre- and post-spraying field schedules accordingly.

Direct exposure

To assess the potential for direct exposure of birds and other wildlife to our target chemicals, we deployed passive sampling devices (PSDs) to absorb any chemical drift that occurred. We placed PSDs in treatment fields on the day of but prior to spraying of soybeans. The PSDs were

14 cm tall by 7 cm in diameter and consisted of Whatman™ Qualitative Filter Paper (grade 2; GE Healthcare U.K. Ltd, Little Chalfont, United Kingdom) attached to 1.27-cm hardware cloth formed to a cylinder shape to approximate the size and shape of a large songbird or an approximately 3-4 week old gamebird chick. We placed the PSDs at 2 heights (ground level [0 m] and mid-canopy height [0.5 m]) at each of the 18-21 sampling stations per site for a total of 36-42 PSDs/site. Ground-level sampling represented ground-nesting birds and other wildlife that spend the majority of their time on the ground (e.g., gamebirds, small mammals, many species of invertebrates). Mid-canopy sampling represented above-ground nesting birds, songbirds, and many species of spiders and insects. We retrieved the PSDs from the field ≤2.25 h after spraying and properly stored them for later chemical analysis. At control sites, we placed PSDs at both ground and mid-canopy levels at each of the stations. We left the PSDs on site for the same amount of time as PSDs at treatment sites before we collected and stored them for later analysis.

During 2018 only, we deployed PSDs during the pre-spraying period (i.e., 1-3 d prior to spraying) at each <1 m sampling station at 3 treatment and 2 control sites. These samples provided us with a secondary field-based control to determine if our target chemicals could be detected within a treatment site prior to known spraying events.

Indirect exposure

To assess the potential for birds and other insectivorous wildlife to be exposed to the target chemicals indirectly via consumption of prey items, we sampled invertebrates ≤4 h post-spraying at the 0 m, 5 m, and 25 m stations along each transect (total = 9 stations/site). We sampled ground-dwelling invertebrates using a hand-held suction vacuum (BioQuip Products Inc., Rancho Dominguez, CA, U.S.A.) and canopy-dwelling invertebrates using a standard 38-cm diameter sweepnet. We collected vacuum and sweepnet samples along a 30-m doubled transect (30 m x 2 = 60 m total length sampled) to the right side of the sampling stations and parallel to the soybean field. We combined vacuum and sweepnet samples taken from the same station into 1 sample and properly stored them for later chemical analysis. We sampled control sites using the same methods and timing, with the timing based on when we deployed the PSDs at these sites.

Indirect effects

To quantify and compare the effects of target chemicals on the abundance, richness, diversity, and biomass of invertebrate prey items, we collected vacuum and sweepnet samples from the 0 m, 25 m, and 100 m distances along the 3 transects at each site (total = 9 stations/site). We collected these samples 1-3 d prior to spraying and between 3-5 d and 19-21 d post-spraying at treatment sites. We collected samples along a 20-m doubled transect (20 m x 2 = 40 m total length sampled) but on the left side of the sampling stations and parallel to the soybean field. We combined vacuum and sweepnet samples into 1 sample per station per sampling period and stored them in ethanol for later sorting, identification, counting, and measuring. Each time we returned to the site, we started sampling from the endpoint of the previous 20-m sampling transect. We used the same methods and timing to collect our indirect effect samples at each of our control sites after designating an arbitrary "spray day" which occurred within the same range of dates that treatment sites received insecticide applications. During identification in the lab, we placed emphasis on 4 invertebrate orders important in the diets of grassland nesting birds (Wiens and Rotenberry 1979, Rotenberry 1980, Kobal et al. 1998, Linn 2004): Araneae (spiders), Coleoptera (beetles), Lepidoptera (butterflies and moths) larvae, and Orthoptera (grasshoppers, crickets, and katydids). We also placed emphasis on Hemiptera (true bugs) because of their diversity of body forms and trophic levels. We sorted all individuals from these orders and identified them to at least the family level for analysis. All other insects were identified to order only. We counted and measured the body lengths of arthropods to the nearest 0.01 mm and calculated the consumable dry biomass of insects and spiders with formulas reported by Straus and Avilés (2018). We chose to use consumable dry biomass because arthropods' chitinous exoskeletons are not easily digested by birds (e.g., American robin and northern bobwhite chitin digestibility has been estimated at only 7-14%; Weiser et al. 1997). Quantifying the spider community also allowed us to examine potential impacts on an additional trophic level because spiders are an important predator of insects.

Weather

We used portable weather meters (Kestrel 5500AG Agricultural Weather Meters) mounted on tripods and equipped with weather vanes to measure relevant weather data (e.g., temperature, wind speed, wind direction, humidity, dew point) along the center transect at the <1 m, 100 m, and 200 m stations during the deployment of PSDs and at the <1 m, 25 m, and 100 m stations during pre- and post-spraying insect sampling at each site.

Vegetation

At each site, we collected vegetation data 1-3 d prior to spraying at all stations and again at 3-5 d and 19-21 d post-spraying at the reduced subset of stations (i.e., those that coincided with the indirect effects sampling efforts for invertebrates). We sampled multiple vegetation plots at each station: 1 plot at each PSD station and 1 plot at each end of the 20-m and 30-m insect sampling transects. Data collected at each plot included percent ground cover, percent canopy cover, maximum height of live and dead vegetation, litter depth, vertical density, and species richness. Using a modified point-intercept method, we categorized ground cover into bare ground, litter, or other [i.e., woody debris, rock, or gopher mound; Bureau of Land Management (BLM) 1996]. To determine canopy cover, we took a nadir digital photograph of a 30 cm x 55 cm quadrat at a height of 1.5 m above the ground and used the program SamplePoint to estimate percent canopy cover (Booth et al. 2006). Canopy cover categories included grass, forb, standing dead vegetation, woody vegetation, and other. We recorded the maximum height of live and dead vegetation within each plot to the nearest 0.5 dm. We measured litter depth to the nearest 0.1 cm at 1 point within the plot that represented the average condition of the plot. We measured vertical 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 in each of the 4 cardinal directions (Robel et al. 1970). We counted the unique number of grass and forb species in each plot to estimate species richness. Finally, we recorded the dominant grass and forb species (up to 3 species in each category) at each PSD station to obtain a qualitative assessment of the vegetation present at each site.

Chemical analyses in the lab

We sent PSD samples and invertebrate samples (indirect exposure) to the USDA Agricultural Marketing Service's National Science Lab (USDA/AMS-NSL) in Gastonia, NC for chemical residue analysis. Samples were analyzed using a solvent-based extraction method. Extracts were concentrated by evaporation and then analyzed using a gas chromatography/mass spectrometry-negative chemical ionization (GC/MS-NCI) technique or other appropriate method. The USDA/AMS-NSL equipment was capable of an extremely high degree of sensitivity in the limit of detection (LOD) and reported all results to us in parts per billion (ppb).

Data Analyses

Direct and indirect exposure

We used linear mixed-effects models to assess the potential effects of distance from field edge, application spray method (i.e., ground or airplane), and PSD height on direct exposure. The summed value (ppb) of all three target chemicals was the response variable in all models. Using data from treatment sites only, we used a hierarchical model selection approach (see Daly et al. 2015). Using this approach allowed us to examine how our primary factors influenced target chemical deposition after accounting for abiotic and biotic factors expected to affect drift. Our first set of models assessed weather covariates during the spraying event as these are known to affect drift (specifically, continuous covariates for mean air temperature [TEMP; °C] and mean wind speed [WSP; m/s] and a binary covariate for whether transects were downwind of the sprayed field [WDIR]). We retained the covariates from the model with the lowest Akaike's Information Criterion corrected for sample size (AIC_c; Burnham and Anderson 2002) and included them in the models in the second step of analysis. Our second set added vegetation covariates to account for additional variation in the data. We used continuous covariates for maximum height of live vegetation (MHL; dm), vertical density (visual obstruction reading, VOR; dm) from the direction of the sprayed field; and percent canopy cover of live vegetation (CCLIVE; sum of percent cover of grasses and forbs). We used vegetation measurements recorded at each PSD sampling station for models explaining direct exposure, and we used the averaged vegetation measurements from the start and end of insect collection transects for models explaining indirect exposure. We retained weather and vegetation covariates from the model with the lowest AIC_c for inclusion in the final modeling step. In this final step, we incorporated distance from field edge (DIST; m), application spray method (SPRAY), and PSD height (HT). We used similar models to assess indirect exposure except that PSD height was not included as a covariate.

Indirect effects

We used linear mixed-effects models to assess the potential indirect effects of target chemical drift on the abundance, consumable biomass, and family richness of arthropods. Our 8 specific response variables were: total abundance, abundance of bird prey (i.e., individuals from the orders Araneae, Coleoptera, Lepidoptera larvae, and Orthoptera), total consumable dry biomass, consumable dry biomass of bird prey, and family richness of each of the four bird prey taxa. We again used a hierarchical model selection approach. In our first model-building step, we assessed whether vegetation and year influenced arthropod abundance, biomass, and family richness at treatment and control sites at all distances from the field edge prior to spraying. In the second step, we constructed models that included the significant covariates from step 1 and fixed effects of site type (i.e., treatment or control), sample collection timing (i.e., pre-spraying, 3-5 d post-spraying, or 19-21 d post-spraying), and the interaction between site type and sample timing. We excluded data from the 100 m distance because we measured a small difference in target chemical residues at this distance; thus, any differences in arthropod measures detected at 100 m from the field edge would not be due to the effects of target chemical application. If the site type or interaction term was significant in step 2 (i.e., 95% confidence intervals around parameter estimates did not include zero), we then tested whether distance from the field edge influenced our response variables of interest. We subset the data to include only samples that were relevant to the significant predictor in step 2. For example, if we found that samples from treatment sites collected between 3-5 d post-spraying were significantly different than measures at control sites in step 2, then we subset the data to include samples from treatment sites collected between 3-5 d post-spraying at 0 and 25 m from the field edge. Using these data, we constructed a model including distance from field edge as a continuous fixed effect in addition to any covariates that were significant in step 1.

RESULTS

We sampled sites between 28 July – 14 September 2017 and 18 July – 5 September 2018, coinciding with peak activity for aphid spraying in the farmland zone (Table 2). We collected a total of 368 direct exposure PSD samples, 81 indirect exposure invertebrate samples, and 243 indirect effects invertebrate samples across both years. Additionally, we collected 30 pre-spraying PSD samples as our secondary field-based controls in 2018. Cooperators used chlorpyrifos (*n* = 4) and cyhalothrin (*n* = 3) on soybean fields; no cooperators used bifenthrin (Table 1). Airplanes (*n* = 3) and ground sprayers (*n* = 2) were both used to apply insecticides at treatment sites (Table 1). Two of 5 treatment sites were downwind at the time of spraying (i.e., the average wind direction during the spraying event was within 62° of the primary transect orientation).

Direct exposure

We detected target chemicals on PSDs at all distances examined (0-400 m) at both treatment and control sites (Table 3, Figure 2); however, means of target chemical deposition were lower at control sites compared to treatment sites at every distance examined except for 200 m (Table 3). Our PSDs deployed at a subset of sampling stations in 2018 as a secondary, field-based control also contained very low levels of the target chemicals within treatment sites ($\bar{x}$ = 7 ppb, CV = 0.58) and control sites ($\bar{x}$ = 6 ppb, CV = 0.38). Chlorpyrifos was the predominant insecticide detected in these secondary control samples.

Our 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 4). We found a significant inverse association between distance from grassland/soybean field edge and deposition (β = -63.67, $p<0.1$) with target chemical deposition on PSDs being greater within 25 m of the soybean field edge (Table 3, Figure 2). Deposition was higher on PSDs placed at mid-canopy (β = 15,232.28) than ground level (Table 3). Application spray method was not included in the best-supported model. Direction of the wind relative to the WMA during soybean spraying events, mean air temperature, and percent canopy cover of live vegetation were included in the best-supported weather and vegetation models. Sites that were downwind of sprayed soybean fields had a positive association with target chemical deposition (β = 12,735.63). Air temperature and canopy cover of live vegetation had significant and inverse associations with deposition (β = -5,566.73 and β = -608.95, respectively, $p<0.1$).

Our cooperators used chlorpyrifos more often than lambda-cyhalothrin or bifenthrin (Table 1). Additionally, chlorpyrifos had the highest mean deposition values of all chemical residues we measured at both treatment and control sites. Therefore, we analyzed chlorpyrifos residues separately to compare chlorpyrifos deposition to levels shown to be toxic to birds and pollinators (Appendix A). We found overall mean chlorpyrifos residues exceeded 100 ppb on PSDs at 0, 5, 25, 50 and 400 m from the field edge at treatment sites ($\bar{x}$ = 34,875, 16,049, 26,489, and 699 ppb, respectively; Table 5). Mean chlorpyrifos residue values at treatment sites exceeded 100 ppb at both mid-canopy and ground heights and at sites bordered by fields sprayed by airplane at 0-50 m from the field edge (Table 5). Mean chlorpyrifos residues also exceeded 100 ppb at 0 m and 400 m from the field edge at treatment sites where bordering fields were treated with ground sprayers ($\bar{x}$ = 2,509 and 2,254 ppb, respectively; Table 5). Mean chlorpyrifos residues on PSDs did not exceed 100 ppb at any distance from the field edge at control sites ($\bar{x}$ = 38, 20, 19, 21, 21, 18, 24 ppb; Table 5).

We found consistent but small differences in chlorpyrifos deposition associated with PSD height at treatment sites, with slightly higher deposition on mid-canopy PSDs (Table 5, Figure 3). These differences were not significant (Welch's two-sample *t*-test, $p\geq0.213$) and did not show a trend as distance from the field edge increased. We did not detect significant differences between chlorpyrifos residues at mid-canopy and ground height at any distance from the field edge at our control sites ($p\geq0.345$).

Indirect exposure

We detected target chemicals on arthropod samples at all distances (0-25 m) at both treatment and control sites (Figure 4). We detected chlorpyrifos and cyhalothrin but not bifenthrin at treatment sites whereas we detected all 3 target chemicals at control sites.

Our 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 application spray method (Table 6). Mean air temperature and the maximum height of live vegetation were in the best-supported model. Air temperature had a significant inverse association with chemical deposition ($\beta = -764.09$, $p<0.05$) whereas maximum height of vegetation had a significant positive association with deposition ($\beta = 384.05$, $p<0.1$).

We found significantly higher amounts of chlorpyrifos residue on arthropods collected at 0 and 5 m from field edges at control sites than treatment sites ($p = 0.026$, 0.023, respectively; Table 7; Figure 5). Conversely, we found mean chlorpyrifos residue on arthropod samples collected 25 m from the field edge were higher at treatment sites ($\bar{x} = 3{,}050$ ppb) than control sites ($\bar{x} = 810$ ppb)(Table 7); however, these value were not significantly different ($p = 0.303$) and were driven by outliers at the 25 m distance at treatment sites (Figure 5). The median value of chlorpyrifos residues was higher at control sites than treatment sites at the 25 m distance (494 ppb vs. 55 ppb; Figure 5).

Indirect effects

We collected a total of 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%), Araneaens (7.2%), Orthopterans (3.5%), and Lepidopterans (2.12%). All other orders constituted 3.9% of the total number of individuals. Coleopterans and Orthopterans made up the highest percentage of total dry consumable biomass (26% each), followed by Hemipterans (16%), Dipterans (8.1%), Lepidopterans (7.6%), Hymenopterans (5.8%), and Araneaens (4.8%). All other orders constituted 6.4% of the total biomass.

We found significantly lower total arthropod abundance 19-21 d post-spraying at treatment and control sites compared to pre-spraying abundance ($\beta = -46.04$, 95% CI = -76.81, -15.26; Table 8). Samples collected at treatment sites 3-5 d post-spraying had significantly lower measures of total abundance than samples collected in this same timeframe at control sites ($\beta = -49.06$, 95% CI = -89.84, -8.28; Table 8). The abundance of arthropods 3-5 d post-spraying at treatment sites was not related to distance from the field edge ($\beta = -0.38$, 95% CI = -1.12, 0.36).

We also found lower abundance of bird prey at treatment sites 19-21 d post-spraying than control sites in this same timeframe ($\beta = -23.94$, 95% CI = -44.99, -2.88; Table 8). Bird prey abundance at treatment sites 19-21 d post-spraying was not related to distance from the field edge ($\beta = -0.02$, 95% CI = -0.44, 0.39).

For total consumable dry biomass of arthropods, our estimates on days 3-5 and 19-21 post-spraying at treatment and control sites were significantly lower than pre-spraying biomass ($\beta = -99.78$, 95% CI = -169.09, -30.48 and $\beta = -75.91$, 95% CI = -145.28, respectively -6.55; Table 8). Total biomass was not significantly different between treatment and control sites (Table 8).

Our estimates of consumable dry biomass of bird prey collected 3-5 d post-spraying at treatment and control sites were significantly lower than samples collected before spraying ($\beta = -62.36$, 95% CI = -123.91, -0.81; Table 8). Consumable biomass of bird prey arthropods did not differ between treatment and control sites (Table 8).

Our estimates of family richness of Araneans did not differ between pre- and post-spraying periods or between treatment and control sites (Table 8). Coleopteran family richness was lower at treatment than control sites during the 3-5 d post-spraying timeframe (β = -0.94, 95% CI = -1.82, -0.06; Table 8). However, family richness of Coleopterans was not influenced by distance from field edge (β = -0.003, 95% CI = -0.04, 0.03; Table 8). Hemipteran family richness on days 3-5 and 19-21 post-spraying at treatment and control sites was significantly lower than pre-spraying (β = -1.04, 95% CI = -2.03, -0.06 and β = -1.33, 95% CI = -2.32, -0.33, respectively) but did not differ between treatment and control sites (Table 8). Finally, Orthopteran family richness on days 3-5 and 19-21 post-spraying at treatment and control sites was significantly lower than pre-spraying (β = -0.46, 95% CI = -0.83, -0.1 and β = -0.56, 95% CI = -0.95, -0.17, respectively; Table 8) but did not differ between treatment and control sites (Table 8).

DISCUSSION

Our finding that detectable levels of target chemicals, particularly chlorpyrifos, were present within both treatment and control sites suggests that some background level of deposition was occurring in the environment prior to and/or during the time of our sampling regardless of spraying status of our cooperator's field. Although our control sites did not have target chemicals sprayed during our sampling timeframe, our experimental design did not control for nearby fields, including other row crop fields that were adjacent to our WMA sites but not included in our landowner coordination efforts. If insecticide application was occurring in these other fields, drift could have occurred onto our study sites. Chlorpyrifos can persist in the environment after its initial application; its half-life is 4.2 h in the atmosphere and 7-120 d in soils, and residues can remain on plant surfaces up to 14 d post-application (Solomon et al. 2001, Christensen et al. 2009). Although the target chemicals are sometimes used to treat corn pests, over 84% of corn acres in Minnesota in 2018 contained seeds genetically modified to protect again insect pests (Potter et al. 2018). With this technology, the need for foliar insecticides on corn has decreased considerably in recent years (L. Stahl, University of Minnesota Extension, personal communication). Thus, the residue amounts we measured at control sites and the extremely low residue values detected on pre-spraying PSDs suggest that target chemical deposition occurred from sprayed soybean fields beyond our cooperator's fields. Although shorter distances of 5-75 m for drift from application of foliar insecticides are reported in the literature (e.g., Davis and Williams 1990, Holland et al. 1997, Vischetti et al. 2008, Harris and Thompson 2012), a recent butterfly study in western Minnesota found insecticide drift on plants located up to 1,600 m away from potential sources (E. Runquist, personal communication). Runquist et al (2018) also found chlorpyrifos residues in all vegetation samples they collected, with the highest residue amounts measuring 2,290 ppb from a grassland edge. Their samples were collected when nearby spraying was not observed, further supporting our conclusion that measurable chlorpyrifos residues are present in grasslands even in the absence of concurrent spraying in adjacent row crop fields.

We found that distance to the soybean field edge was an important factor explaining direct exposure to insecticide drift within our grasslands. Deposition on PSDs was highest within 25 m of the field edge and decreased with increasing distance from the soybean field edge. Other studies have also found an edge effect of drift (e.g., Threadgill and Smith 1975, Langhof et al. 2005, Carlsen et al. 2006, Nsibande et al. 2015, Holterman et al. 2017, Baio et al. 2019). Furthermore, we analyzed chlorpyrifos separately from our other target chemicals because our cooperators used it more frequently and because of its reported toxicities to wildlife. The deposition amounts we measured exceeded the contact LD_{50} for bees (100 ppb, Appendix A) on PSDs up to 50 m from the soybean field edge at both mid-canopy and ground heights. Chlorpyrifos is highly toxic to honey bees (*Apis mellifera*) and can poison non-target insects for up to 24 h after spraying (Christensen et al. 2009). We did not measure chlorpyrifos at known contact LD_{50} values for common farmland bird species (e.g., ring-necked pheasants, American robins, and common grackles). Notably, the Minnesota Buffer Law requiring perennial vegetative buffers calls for a minimum width of approximately 5 m along public ditches. We suggest these minimal-width buffers have the potential to be ecological traps or population sinks for wildlife vulnerable to our target chemicals, particularly pollinators such as honey bees or bumblebees.

Conversely, we did not find distance to edge to be important in our models explaining indirect exposure to target chemicals. We only collected arthropod samples at 0, 5, and 25 m from the field edge because we predicted the heaviest deposition would occur closer to the field edge. However, an edge distance up to 25 m was likely insufficient for detecting variation in deposition within this shorter range of distances. Notably, the chlorpyrifos residue amounts we detected on arthropods were below the acute oral LD_{50} values for many of the grassland bird species commonly found in Minnesota's farmland landscape (Appendix A). Solomon et al (2001) concluded that chlorpyrifos residues on arthropods in agricultural systems are below acute oral LD_{50} values for birds. However, comparisons to acute oral LD_{50} values do not account for effects that can occur with chronic exposure and at sublethal doses (e.g., impaired movement, reduced foraging, lethargy, reduced body condition, impaired migratory orientation; McEwan et al. 1986, Richards et al. 2000, Al-Badrany and Mohammad 2007, Moye 2008, Eng et al. 2017). Further research is needed to determine if the widespread use of soybean aphid insecticides, particularly chlorpyrifos, which occurs in the landscape leads to chronic exposure and sublethal effects in birds.

Our PSDs at mid-canopy had higher amounts of deposition than ground-level PSDs. Additionally, canopy cover of live vegetation was inversely related to deposition on PSDs which has also been found in other studies (Pratt et al. 2000, Donkersley and Nuyttens 2011, Holterman et al. 2017). Taken together, our results suggest that wildlife that nest and forage on the ground and in thicker cover (e.g., pheasants, some songbirds, small mammals, some pollinators) may experience less direct exposure to insecticide spray drift than those that use the canopy layer of grasslands or select grassland habitat with sparser cover.

Insecticide application spray method can vary depending on field conditions at the time of spraying (e.g., fields are less accessible by tractor when soil is wet or soybeans are too tall), and members of the public perceive that airplanes are more likely than ground sprayers to produce off-target drift. We expected an interaction between spray method and edge distance would best explain drift because our mean values for target chemical deposition on PSDs were generally higher for fields sprayed by airplanes than those sprayed on the ground. Yet our best-fit models for direct drift and indirect drift did not include spray method or its interaction with edge distance. Other factors related to spraying equipment (e.g., spray droplet size, nozzle type, operating pressure, driving speed, boom height, uncontrolled boom movements) and application rate can impact drift (Threadgill and Smith 1975, Nuyttens et al. 2007, Arvidsson et al. 2011, Donkersley and Nuyttens 2011, Nsibande et at. 2015, Nuyttens et al. 2017). Although we requested information from cooperators regarding the spraying equipment they used, we did not control for these variables in our study design or analyses. These factors likely influenced the amount of target chemical deposition on PSD and arthropod samples.

The indirect effects of insecticides, including the declines of arthropods important in bird diets, have become an increasing concern for conservation in agricultural landscapes (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 bird 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). Our results indicated measurable impacts on arthropods that serve as prey items for grassland birds after insecticide spraying events. In particular, we documented short-term reductions in total arthropod abundance (3-5 d post-spraying) and bird prey abundance (19-21 d post-spraying) in grasslands bordered by soybean fields sprayed to control for aphids. We also found lower Coleopteran family richness at treatment sites 3-5 d post-spraying. Similar short-term reductions in arthropod abundance and diversity following insecticide application have been documented in other studies (Barrett 1968, Vickerman and Sunderland 1977, Longley et al. 1997, Galvan et al. 2005, Langhof et al. 2005, Devotto et al. 2006). Although overall abundance of arthropods and Coleoptera family richness were lower within a few days after insecticide application, these measures were similar to pre-spraying levels by week 3 post-spraying, indicating that arthropod populations rebounded during this period. Arthropod communities' recovery rates can vary widely between areas and with arthropods' differing dispersal capabilities, reproductive potentials, and life stages (Campbell et al. 1997, Longley et al. 1997), and arthropods could have recolonized these grasslands from nearby areas that were not treated with or impacted by insecticides during this period (Longley et al. 1997).

Interestingly, we did not detect concomitant decreases in consumable dry biomass between treatment and control sites during either sampling period post-spraying. The effects of insecticides on non-target arthropods have been shown to 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 rate (Stark et al. 2004). Larger-bodied insects which contributed more to our biomass estimates (e.g., Orthopterans) may have not have been affected as much as smaller-bodied insects. Alternatively, arthropod biomass could have been impacted regardless of spray application on the nearest field. The widespread use of soybean aphid insecticides during July-September in Minnesota combined with ubiquitous drift over longer distances that has been documented (at least 400 m, our study; up to 2,000 m, Baio et al. 2019) may have contributed to lower arthropod biomass overall across the landscape, making any differences between our treatment and control sites difficult to detect.

As part of our final LCCMR reporting, we indicated a plan to invite our landowners, cooperators, and other constituent groups (e.g., University of Minnesota's Southwest Agricultural Experiment Station personnel, Soybean Growers' Association, various natural resource professions) to a landowner appreciation seminar during early spring 2020 to engage them, update them on how their participation benefited our research efforts, and show them how the aggregated data is being shared with other groups and in reports and publications. However, the COVID-19 pandemic forced us to delay this seminar for the foreseeable future, and we may have to consider a virtual event instead. Regardless of forum, our proximate goal with this outreach event is multifold: 1) bring awareness to the issue of soybean aphid insecticide drift onto grasslands, 2) engage agricultural partners in a conversation about potential solutions and mitigation efforts to reduce the potential for drift to occur on these grasslands, and 3) promote good will and communication that could be beneficial if MNDNR conducts further pesticide research in the future. However, our ultimate goal is to provide land managers with information on patterns of soybean aphid insecticide drift onto grassland cover in the agricultural matrix of Minnesota to improve management of public and private lands set-aside for grassland wildlife conservation.

MANAGEMENT IMPLICATIONS

Our findings indicate that drift from insecticide applications to control for soybean aphids is ubiquitous in small amounts even in grasslands without immediately adjacent soybean fields. Wildlife associated with row crop edges and sparser grassland vegetation are more likely to be exposed to drift directly. Additionally, less food may be available to breeding birds, their young, and other insectivorous wildlife for up to 3 weeks after spraying operations in the area. Managers should acquire and maintain larger grassland tracts to reduce the edge effects from insecticide drift and provide refugia for arthropods to be able to recolonize affected areas. They should also use seeding mixes that create a thicker, more diverse canopy cover to prevent drift from reaching ground-dwelling wildlife and to support a more diverse arthropod community for insectivores. Further research is needed to the full impacts of insecticide drift on populations of wildlife and pollinators inhabiting narrow buffers (e.g., filter strips, grassed waterways) commonly used alongside row crop fields. Additionally, future research should also address the potential for chronic exposure and sublethal effects of these insecticides on free-ranging wildlife in Minnesota's farmland landscape.

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Table 1. Spray method and application data for soybean aphid spraying events by cooperators adjacent to Wildlife Management Areas (WMA) that were sampled for insecticide spray drift during July-September 2017 and 2018 in Minnesota's farmland zone.

Site ID[a]	Spray method	Insecticide trade name	Insecticide active ingredients	Insecticide application rate (L/ha)	Sprayer application rate (L/ha)	Application speed (m/s)	Boom height (m)	Tank pressure (kPa)
tA	Ground	Endigo	lambda-cyhalothrin + thiamethoxam	0.26	140.3	4.0	0.2-0.3	275.8
tB	Airplane	Bolton	chlorpyrifos + gamma-cyhalothrin	0.88	18.7	67.9	1.5	275.8
tC	Ground	Lorsban 4E	chlorpyrifos	NA[b]	93.5	NA	NA	137.9-206.8
tD	Airplane	Lorsban Advanced	chlorpyrifos	1.17	18.7	55.9	2.7-4.0	275.8
tE[c]	Airplane	Lorsban Advanced; Warrior II	chlorpyrifos; lambda-cyhalothrin	0.44; 0.22	NA	NA	NA	NA

[a]WMA names are not provided to protect private landowner cooperators.
[b]Data is not available because cooperator declined to provide this information.
[c]This cooperator combined two different trade name insecticides during the spraying event.

Table 2. Location, site type, year sampled, and timing of sampling for Wildlife Management Areas (WMA) sampled for insecticide drift from adjacent row crop fields sprayed for soybean aphids during July-September 2017 and 2018 in Minnesota's farmland zone.

Site ID[a]	Region[b]	County	Site type[c]	Year sampled	Range of dates when field sampling occurred[d]
tA	SW	Jackson	Treatment	2017	28 July - 18 Aug
tB	SW	Murray	Treatment	2017	9 Aug - 30 Aug
cA	SW	Jackson	Control	2017	21 Aug - 14 Sept
cB	SW	Lyon	Control	2017	7 Aug - 31 Aug
tC	WC	Lac qui Parle	Treatment	2018	10 Aug - 29 Aug
tD	C	Stearns	Treatment	2018	28 July - 16 Aug
tE	WC	Yellow Medicine	Treatment	2018	7 Aug - 28 Aug
cC	C	Kandiyohi	Control	2018	17 Aug - 5 Sept
cD	WC	Lac qui Parle	Control	2018	18 Jul - 8 Aug

[a]WMA names are not provided to protect private landowner cooperators.
[b]Regions sampled in this study include the southwest (SW), west central (WC), and central (C) regions. The boundaries for these regions follow the same boundaries as outlined in the Minnesota Department of Natural Resources' annual August Roadside Survey.
[c]Treatment sites had adjacent soybean fields that were sprayed for aphids; control sites had adjacent corn fields that were not sprayed for aphids.
[d]Includes first day of pre-spray sampling through last day of post-spray sampling for data collection activities.

Table 3. Means and coefficients of variation ($\bar{x}$ [CV]) of target chemicals (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) detected on passive sampling devices (*n* = 368) by distance from soybean field edge to grassland interior. Mean values are reported in parts per billion (ppb). Treatment sites consisted of grasslands adjacent to soybean fields that were treated with target chemicals by airplanes or ground sprayers; control sites were grasslands adjacent to unsprayed corn fields. PSDs were deployed at mid-canopy height (0.5 m above ground) and ground level. Samples were collected during July-September 2017 and 2018 in Minnesota's farmland zone.

	Distance from soybean field edge (m)						
Site type	0 m	5 m	25 m	50 m	100 m	200 m	400 m
Treatment	35,322 (4.11)	16,260 (3.95)	26,712 (3.48)	385 (2.35)	40 (1.72)	14 (1.44)	699 (5.02)
PSD height							
Mid-canopy	61,981 (3.28)	29,310 (3.08)	38,504 (3.28)	569 (2.11)	52 (1.65)	18 (1.35)	1,387 (3.58)
Ground	8,664 (2.8)	3,210 (2.32)	14,920 (2.68)	201 (2.15)	27 (1.66)	9 (1.35)	11 (0.94)
Spray application method							
Airplane	57,198 (3.25)	27,080 (3.03)	44,504 (2.65)	629 (1.77)	50 (1.69)	7 (1.31)	8 (0.99)
Ground	2,510 (2.21)	30 (1.02)	25 (1.05)	19 (1.07)	24 (1.23)	23 (1.14)	2,254 (2.8)
Control	41 (1.84)	21 (0.94)	21 (0.92)	21 (0.96)	22 (1.03)	19 (0.97)	30 (1.02)

Table 4. Number of parameters (K), Akaike's Information Criterion corrected for small sample size (AIC_c; n = 206), conditional R^2 value (variation explained by the entire model including random effects), deviance (d), and model weight (ω) for models of target chemical deposition (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) onto passive sampling devices (PSDs) at treatment study sites in the farmland region of Minnesota during July-September 2017 and 2018. PSDs were used to assess direct exposure of wildlife to drift from insecticides 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 (specifically, whether the 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 (specifically, percent canopy cover of live vegetation [CCLIVE], maximum height of live vegetation [MHL], and vertical density of vegetation from the direction of the sprayed field [visual obstruction reading; VOR]. The best-supported weather + vegetation model was then used to assess primary factors of interest: distance of the PSD from the grassland/soybean field edge (DIST), height of the PSD (i.e., mid-canopy or ground level; HT), and application spray method (i.e., airplane or ground; SPRAY). The column ΔAICc compares models within each step of model development. Models were linear mixed-effect models, included site as a random effect, and were fit using the maximum likelihood method.

Model	K	AIC_c	ΔAIC_c	R^2	d	ω
Weather						
WDIR + TEMP	5	5172.26	0.00	0.10	5161.96	0.44
TEMP	4	5173.11	0.85	0.08	5164.91	0.29
WSP + WDIR + TEMP	6	5174.37	2.12	0.10	5161.95	0.15
WSP + TEMP	5	5175.12	2.86	0.08	5164.82	0.11
WDIR	4	5179.93	7.67	0.07	5171.73	0.01
WSP + WDIR	5	5182.03	9.77	0.07	5171.73	0.00
WSP	4	5182.34	10.08	0.07	5174.14	0.00
Weather + Vegetation						
WEATHER[a] + CCLIVE	6	5170.36	0.00	0.11	5157.94	0.31
WEATHER + MHL + CCLIVE	7	5171.67	1.30	0.12	5157.10	0.16
WEATHER + MHL	6	5172.09	1.73	0.11	5159.67	0.13
WEATHER	5	5172.26	1.90	0.10	5161.96	0.12
WEATHER + VOR + CCLIVE	7	5172.49	2.13	0.12	5157.92	0.11
WEATHER + MHL + VOR + CCLIVE	8	5173.39	3.02	0.12	5156.65	0.07
WEATHER + VOR	6	5174.05	3.69	0.10	5161.63	0.05
WEATHER + MHL + VOR	7	5174.14	3.78	0.11	5159.57	0.05
Weather + Vegetation + Primary Factors						
VEG[b] + DIST + HT	8	5168.69	0.00	0.14	5151.96	0.22
VEG + DIST	7	5169.31	0.62	0.13	5154.74	0.16
VEG + HT	7	5169.76	1.07	0.13	5155.19	0.13
VEG	6	5170.36	1.68	0.11	5157.94	0.10
VEG + HT + SPRAY * DIST	10	5170.44	1.76	0.15	5149.32	0.09
VEG + DIST + SPRAY + HT	9	5170.58	1.89	0.14	5151.66	0.09
VEG + SPRAY * DIST	9	5171.06	2.37	0.14	5152.14	0.07
VEG + DIST + SPRAY	8	5171.19	2.50	0.13	5154.45	0.06
VEG + SPRAY + HT	8	5171.67	2.99	0.13	5154.94	0.05
VEG + SPRAY	7	5172.26	3.57	0.12	5157.69	0.04

[a]WEATHER = covariates in the top-ranked Weather model (WDIR + TEMP).
[b]VEG = covariates in top-ranked Weather + Vegetation model (WDIR + TEMP + CCLIVE).

Table 5. Means and coefficients of variation (x̄ [CV]) of chlorpyrifos detected on passive sampling devices (*n* = 368) by distance from soybean field edge to grassland interior. Mean values are reported in parts per billion (ppb). Treatment sites consisted of grasslands adjacent to soybean fields that were treated with target chemicals by airplanes or ground sprayers; control sites were grasslands adjacent to unsprayed corn fields. PSDs were deployed at mid-canopy height (0.5 m above ground) and ground level. Samples were collected during July-September 2017 and 2018 in Minnesota's farmland zone.

	Distance from soybean field edge (m)						
Site type	0 m	5 m	25 m	50 m	100 m	200 m	400 m
Treatment	34,875 (4.15)	16,049 (3.98)	26,489 (3.5)	372 (2.36)	38 (1.7)	14 (1.44)	699 (5.02)
PSD height							
Mid-canopy	61,452 (3.31)	28,970 (3.1)	38,272 (3.29)	551 (2.12)	50 (1.62)	18 (1.35)	1,387 (3.58)
Ground	8,297 (2.91)	3,129 (2.34)	14,706 (2.7)	194 (2.16)	27 (1.63)	9 (1.35)	11 (0.94)
Spray application method							
Airplane	56,451 (3.29)	26,729 (3.06)	44,132 (2.66)	608 (1.78)	48 (1.68)	7 (1.31)	8 (0.99)
Ground	2,509 (2.21)	30 (1.02)	25 (1.05)	19 (1.07)	24 (1.23)	23 (1.14)	2,254 (2.8)
Control	38 (1.92)	20 (1)	19 (1.01)	21 (0.98)	21 (1.09)	18 (1.03)	24 (0.94)

Table 6. Number of parameters (K), Akaike's Information Criterion corrected for small sample size (AIC_c; n = 45), conditional R^2 value (variation explained by the entire model including random effects), deviance (d), and model weight (ω) for models of target chemical deposition (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) on arthropod samples collected at treatment study sites in the farmland region of Minnesota during July-September 2017 and 2018. The arthropods were used to assess the potential for wildlife to be exposed to soybean aphid insecticides indirectly via consumption of invertebrate prey exposed to these chemicals via spray drift. A hierarchical model selection approach was used in which the first set of models assessed weather conditions during the spraying event (specifically, whether the 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 (specifically, percent canopy cover of live vegetation [CCLIVE], maximum height of live vegetation [MHL], and vertical density of vegetation from the direction of the sprayed field [visual obstruction reading; VOR]. The best-supported weather + vegetation model was then used to assess primary factors of interest: distance of the PSD from the grassland/soybean field edge (DIST) and application spray method (i.e., airplane or ground; SPRAY). The column ∆AICc compares models within each step of model development. Models were linear mixed-effect models, included site as a random effect, and were fit using the maximum likelihood method.

Model	K	AIC_c	ΔAIC_c	R^2	d	ω
Weather						
TEMP	4	886.60	0.00	0.25	877.60	0.40
WDIR + TEMP	5	886.84	0.25	0.28	875.31	0.36
WSP + TEMP	5	889.06	2.47	0.25	877.53	0.12
WSP + WDIR + TEMP	6	889.51	2.91	0.28	875.30	0.09
WDIR	4	892.82	6.22	0.19	883.82	0.02
WSP	4	895.17	8.57	0.19	886.17	0.01
WSP + WDIR	5	895.36	8.76	0.19	883.82	0.01
Weather + Vegetation						
WEATHER[a] + MHL	5	885.22	0.00	0.31	873.68	0.30
WEATHER + MHL + VOR	6	885.40	0.18	0.35	871.19	0.28
WEATHER	4	886.60	1.38	0.25	877.60	0.15
WEATHER + MHL + CCLIVE	6	887.55	2.32	0.32	873.33	0.10
WEATHER + MHL + VOR + CCLIVE	7	888.19	2.97	0.35	871.16	0.07
WEATHER + CCLIVE	5	889.10	3.88	0.25	877.56	0.04
WEATHER + VOR	5	889.12	3.90	0.25	877.59	0.04
WEATHER + VOR + CCLIVE	6	891.74	6.52	0.25	877.53	0.01
Weather + Vegetation + Primary Factors						
VEG[b]	5	885.22	0.00	0.31	873.68	0.46
VEG + DIST	6	886.47	1.25	0.33	872.26	0.25
VEG + SPRAY	6	887.54	2.32	0.32	873.33	0.14
VEG + DIST + SPRAY	7	888.80	3.58	0.34	871.77	0.08
VEG + SPRAY * DIST	8	888.88	3.65	0.38	868.88	0.07

[a]WEATHER = covariates in the top-ranked Weather model (TEMP).
[b]VEG = covariates in the top-ranked Weather + Vegeation model (TEMP + MHL).

Table 7. Means and coefficients of variation ($\bar{x}$ [CV]) of chlorpyrifos detected on arthropod samples (*n* = 81) by distance from soybean field edge to grassland interior. Mean values are reported in parts per billion (ppb). Treatment sites consisted of grasslands adjacent to soybean fields that were treated with target chemicals by airplanes or ground sprayers; control sites were grasslands adjacent to unsprayed corn fields. Samples were collected during July-September 2017 and 2018 in Minnesota's farmland zone.

	Distance from soybean field edge (m)		
Site type	0 m	5 m	25 m
Treatment	115 (1.89)	54 (0.73)	3,050 (2.64)
Control	628 (1.08)	711 (1.22)	810 (1.25)

Table 8. Coefficient estimates and 95% confidence intervals (CIs) for covariates included in step 2 of a hierarchical model selection approach used to examine the indirect effects of drift from target chemicals (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) used to control soybean aphids on the total abundance, bird prey[a] abundance, total consumable dry biomass, consumable dry biomass of bird prey, and bird prey family richness of arthropods. Arthropod samples were collected from Wildlife Management Areas (WMAs) grasslands in the farmland region of Minnesota during July-September 2017 and 2018. Bold estimates indicate significance based on non-overlapping 95% CIs.

		Site type	Sample timing[d]		Treatment sites[e]	
Response variable	Cond R^{2b}	Treatment[c]	3-5 d	19-21 d	3-5 d	19-21 d
Abundance						
Total	0.43	-17.57	-25.64	**-46.04**	**-49.06**	-18.71
		(-75.6, 40.46)	(-56.35, 5.06)	**(-76.81, -15.26)**	**(-89.84, -8.28)**	(-59.64, 22.22)
Bird prey	0.29	7.53	-11.20	-13.08	-19.38	**-23.94**
		(-13.42, 28.49)	(-26.9, 4.49)	(-28.97, 2.81)	(-40.22, 1.47)	**(-44.99, -2.88)**
Biomass						
Total	0.20	-44.43	**-99.78**	**-75.91**	14.62	-28.42
		(-127.27, 38.42)	**(-169.09, -30.48)**	**(-145.28, -6.55)**	(-77.44, 106.69)	(-120.85, 64.01)
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)
Coleoptera	0.21	-0.21	-0.33	-0.34	**-0.94**	0.03
		(-1.12, 0.70)	(-0.99, 0.33)	(-1.01, 0.33)	**(-1.82, -0.06)**	(-0.85, 0.92)
Hemiptera	0.47	-1.12	**-1.04**	**-1.33**	-0.55	0.16
		(-3.55, 1.32)	**(-2.03, -0.06)**	**(-2.32, -0.33)**	(-1.87, 0.77)	(-1.16, 1.48)
Orthoptera	0.34	-0.37	**-0.46**	**-0.56**	0.02	-0.17
		(-0.97, 0.23)	**(-0.83, -0.10)**	**(-0.95, -0.17)**	(-0.48, 0.52)	(-0.72, 0.38)

[a]Bird prey includes individuals from the orders Araneae, Coleoptera, Lepidoptera (larvae only), and Orthoptera.
[b]Variation explained by the entire model including random effects.
[c]Difference from the estimate of samples collected at control sites.
[d]Difference from the estimate of samples collected prior to spraying event compared to either 3-5 d or 19-21 d post-spraying.
[e]Difference from the estimate of samples collected at control sites either at 3-5 d or 19-21 d post-spraying.

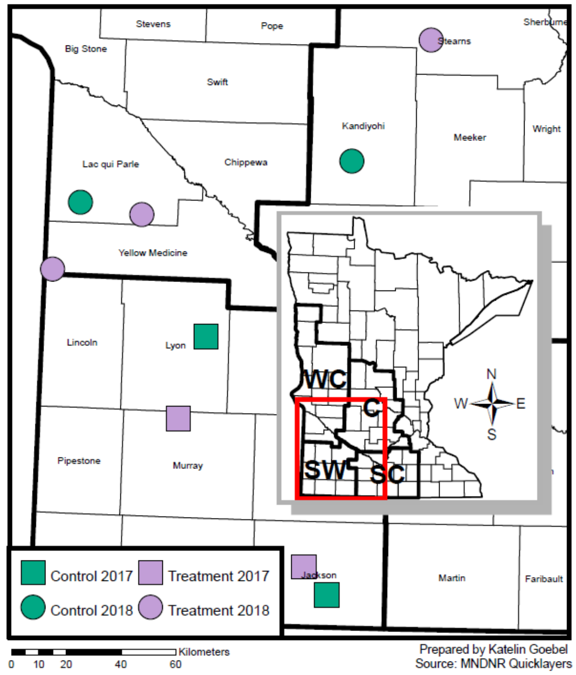

Figure 1. Location of treatment (purple symbols) and control (green symbols) sites during 2017 (square symbols) and 2018 (round symbols) sampling efforts in Minnesota's farmland regions. Treatment sites were Wildlife Management Areas (WMA) grasslands adjacent to soybean fields sprayed for aphids; control sites were WMA grasslands adjacent to corn fields that were not sprayed with insecticides to control soybean aphids.

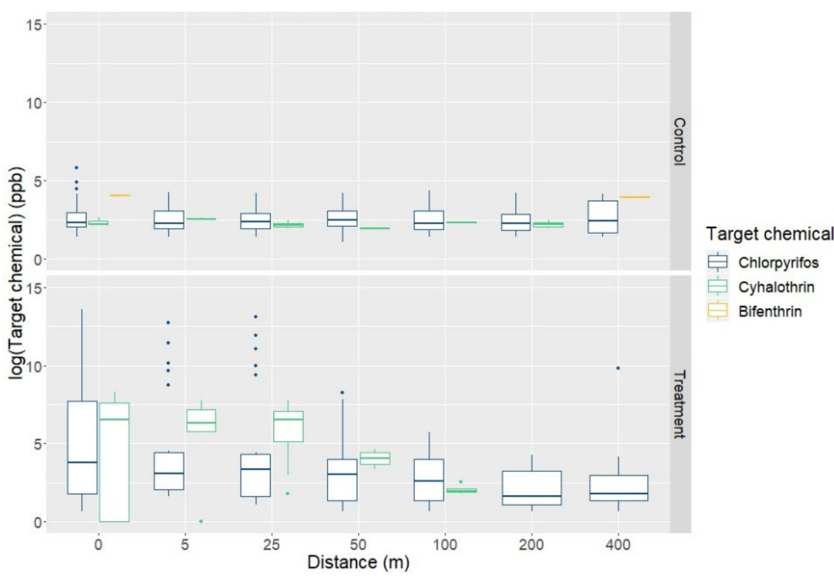

Figure 2. Target chemical deposition (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) on passive sampling devices (PSDs; *n* = 368) by distance from row crop field edge (0 m) to grassland interior for control (top panel) and treatment sites (bottom panel) during July-September 2017 and 2018 in Minnesota's farmland regions. The PSDs were used to quantify the potential for grassland wildlife to be exposed to target chemicals directly through spray drift. Spraying at treatment sites occurred on soybean fields adjacent to grasslands; control sites were grasslands adjacent to unsprayed corn fields.

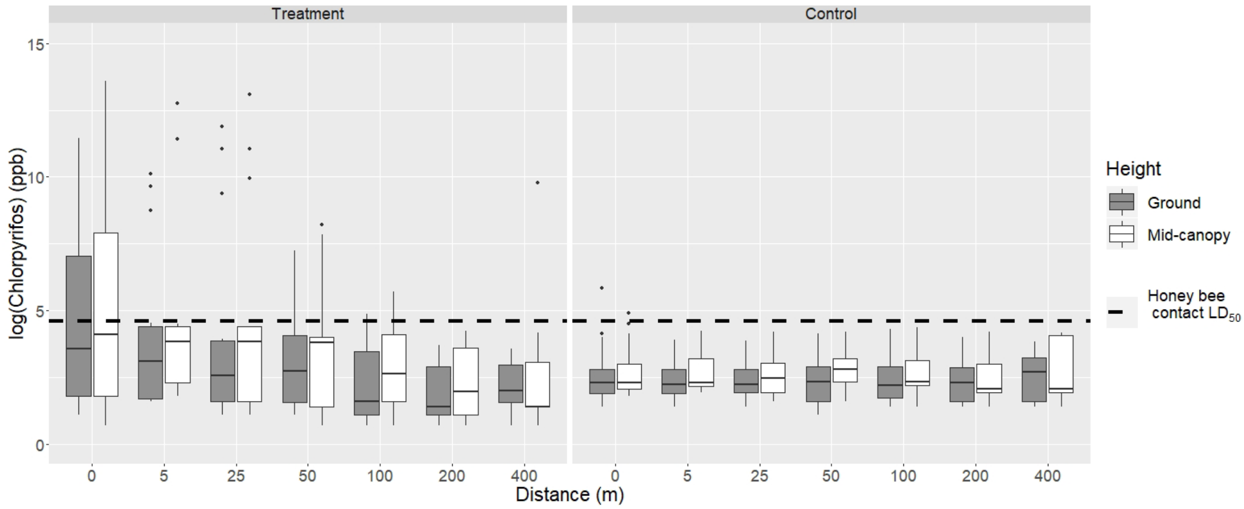

Figure 3. Chlorpyrifos deposition on passive sampling devices (PSDs; *n* = 368) by distance from row crop field edge (0 m) to grassland interior for treatment (left panel) and control sites (right panel) during July-September 2017 and 2018 in Minnesota's farmland regions. The PSDs were used to quantify the potential for grassland wildlife to be exposed to target chemicals directly through spray drift. Spraying at treatment sites occurred on soybean fields adjacent to grasslands; control sites were grasslands adjacent to unsprayed corn fields. The dashed horizontal line shows the acute contact LD_{50} for honey bees (*Apis mellifera*).

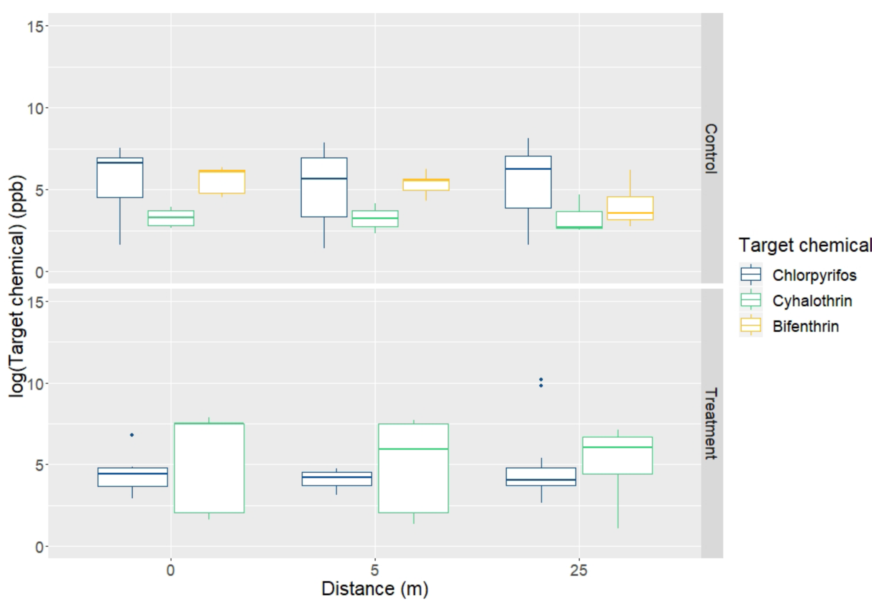

Figure 4. Target chemical deposition (i.e., chlorpyrifos, lambda-cyhalothrin, and bifenthrin) on arthropod samples (*n* = 81) by distance from row crop field edge (0 m) to grassland interior for control (top panel) and treatment sites (bottom panel) during July-September 2017 and 2018 in Minnesota's farmland regions. Samples were collected to quantify the potential for grassland wildlife to be exposed to target chemicals indirectly through consumption of invertebrate prey exposed to drift from spraying. Spraying at treatment sites occurred on soybean fields adjacent to grasslands; control sites were grasslands adjacent to unsprayed corn fields.

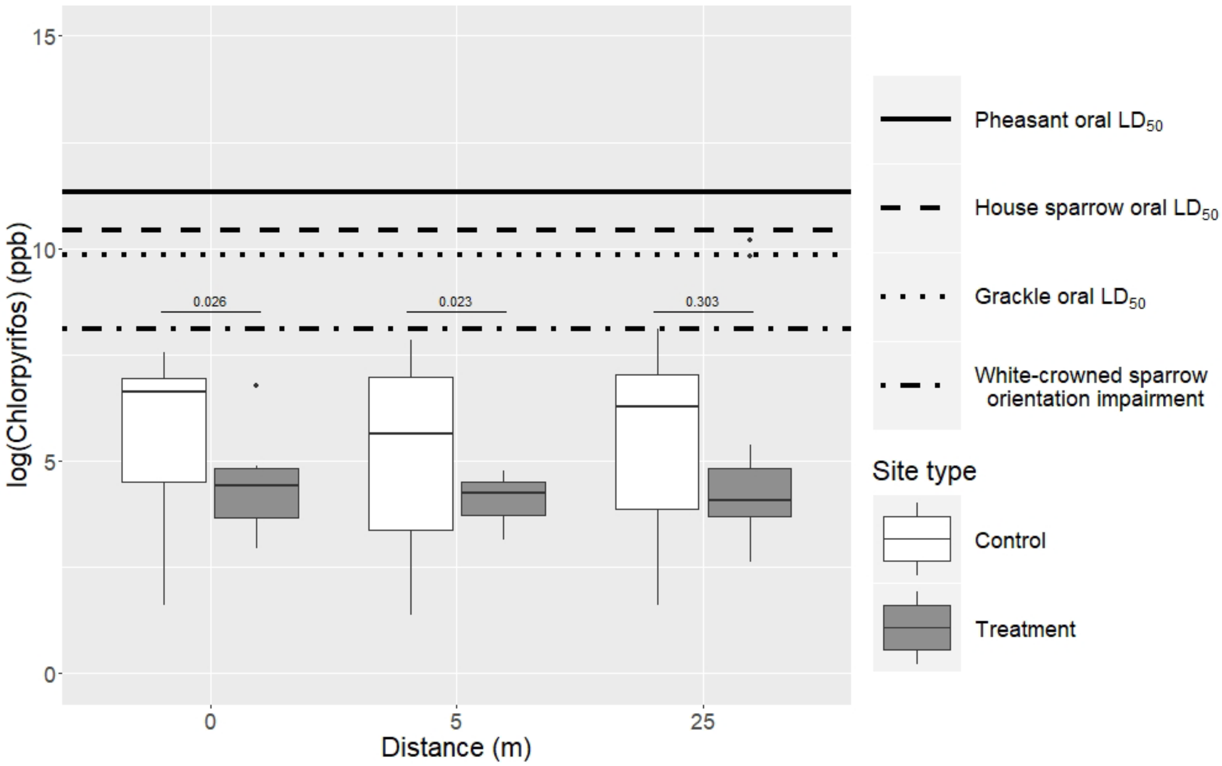

Figure 5. Chlorpyrifos deposition on arthropod samples (*n* = 81) by distance from row crop field edge (0 m) to grassland interior during July-September 2017 and 2018 in Minnesota's farmland regions. Samples were collected to quantify the potential for grassland wildlife to be exposed to target chemicals indirectly through consumption of invertebrate prey exposed to drift from spraying. Spraying at treatment sites occurred on soybean fields adjacent to grasslands; control sites were grasslands adjacent to unsprayed corn fields. The thicker solid horizontal line and the dashed horizontal lines represent the acute oral LD_{50} values for ring-necked pheasants (*Phasianus colchicus*), house sparrows (*Passer domesticus*), and common grackles (*Quiscalus quiscula*) and the acute oral dose causing migratory orientation impairment in white-crowned sparrows (*Zonotrichia leucophrys*), respectively. Values above the shorter horizontal bars are p-values resulting from Welch's two-sample *t*-tests comparing samples from control and treatment sites at each distance.

APPENDIX A.

Acute contact toxicity (lethal does [LD_{50}] values) of chlorpyrifos for honey bees (*Apis mellifera)* and acute oral LD_{50} of chlorpyrifos for bird species commonly found in Minnesota's farmland landscape. The acute contact or oral LD_{50} value is a common measure of acute toxicity and represents the lethal dose that causes death in 50% of treated animals from a single or limited exposure. The acute contact LD_{50} for honey bees is reported in Ostiguy et al. 2019. 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. Environmental Protection Agency et al. 2014). Acute oral LD_{50} (mg/kg) values and acute oral toxicity classifications are reported in Solomon et al. 2001. Acute oral LD_{50} (ppb) values were calculated by dividing the acute oral LD_{50} (mg/kg) by the proportion of body mass eaten as food per day (Solomon et al. 2001) and multiplying by 1,000 (Equation 4, Food Animal Residue Avoidance & Depletion Program 2016).

Species	Scientific name	LD_{50} (mg/kg)	LD_{50} (ppb)	Overall toxicity
Pollinator species				
Honey bee	*Apis mellifera*	59-360 ng/bee	100	Highly toxic
Avian species				
Sandhill crane	*Grus canadensis*	60	250,000	Highly toxic
Canada goose	*Branta canadensis*	60	600,000	Highly toxic
Mallard	*Anas platyrhynchos*	68	680,000	Moderately toxic
Ring-necked pheasant	*Phasianus colchicus*	12.2	122,000	Very highly toxic
Northern bobwhite	*Colinus virginianus*	32	106,667	Highly toxic
Common grackle	*Quiscalus quiscula*	8.5	28,333	Very highly toxic
Red-winged blackbird	*Agelaius phoeniceus*	13.2	44,000	Highly toxic
American robin[a]	*Turdus migratorius*	unknown	unknown	unknown
House sparrow	*Passer domesticus*	29.5	98,333	Highly toxic

[a]American robins are the most frequently reported avian species killed in field incidents; however, the LD_{50} values are unknown (Christensen et al. 2009).