Insecticide Exposure Risk for Grassland Wildlife on Public Lands[1]

2017 Annual Report

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Abstract: There is growing concern about the potential effects of insecticides on grassland wildlife that inhabit landscapes dominated by agriculture. In the agricultural region of southern and western Minnesota, there is particular concern about the risk of exposure of wildlife on public lands to insecticides used to control soybean aphids. Our objectives are to assess the direct and indirect exposure risks of grassland birds and their insect food resources to insecticides encountered via aerial drift. We are quantifying chemical residues in public grasslands, measuring chemical residues on invertebrates, and assessing effects of insecticide exposure to invertebrate abundance near sprayed fields before and after routine applications of insecticides are used to control soybean aphids. We collected data from 2 treatment and 2 control sites during summer 2017 and are preparing for our final field season in summer 2018. Our results will inform land managers and private landowners on how best to manage grasslands to reduce risks of wildlife to insecticide drift.

INTRODUCTION

Loss and fragmentation of grassland cover is a major concern for grassland-dependent wildlife throughout the Midwestern United States. Increasing evidence suggests that acute toxicity to pesticides may be a greater threat to grassland bird populations than habitat loss due to agricultural intensification (Mineau and Whiteside 2013). In Minnesota, many remaining grasslands are highly fragmented and surrounded by row crops, including over 3 million hectares of soybeans (USDA 2016*a*). The insecticides used to combat soybean aphids, namely chlorpyrifos, lambda-cyhalothrin, and bifenthrin, disrupt nervous system functioning of organisms and are highly effective against target insect pests; however, they are highly toxic to non-target organisms such as birds and pollinators (NPIC 2001, Christensen et al. 2009, Johnson et al. 2010). Chlorpyrifos is an organophosphate insecticide, whereas lambda-cyhalothrin and bifenthrin are pyrethroids. Members of the public and Minnesota Department of Natural

Resources (MNDNR) wildlife managers have observed fewer birds and insects after these insecticides are applied in late summer, raising concerns regarding the impacts of these chemicals on populations of grassland wildlife. However, little is known about the deposition of these pesticides in grasslands and the exposure risk to wildlife in an agricultural matrix under typical application conditions.

One important avenue of exposure of grassland wildlife to agricultural insecticides is through aerial drift associated with routine application to prevent and control pest outbreaks. Drift occurs when insecticides are sprayed on crops but environmental factors result in their transport to areas beyond the targeted application area. Distance of travel for insecticide drift is highly dependent on factors such as humidity, wind speed, and application method. Furthermore, the reported drift distances vary widely, ranging from 5 m to 1,600 m (Davis and Williams 1990, E. Runquist, MN Zoo, personal communication). For many standard insecticide application regimes in agricultural landscapes, there is little or no information about drift and exposure risk to wildlife in grassland cover types - information necessary to effectively design grasslands set aside and managed for wildlife.

Restoring grasslands within the agricultural matrix is a priority conservation concern in western Minnesota. Information about risk of exposure of grassland wildlife to insecticides in this landscape is lacking, but this knowledge would help managers with grassland conservation efforts. Agricultural practices and policies that influence cover-type composition [e.g., a 2016 Minnesota law that requires perennial vegetation buffers of an average of 15 m (50 ft) width and 9 m (30 ft) minimum width along public waters and 5 m (16.5 ft) width along public drainage systems] may result in addition of grassland cover to the landscape. However, how and to what extent grassland birds, their insect prey, and beneficial insects such as pollinators using these buffers are exposed to spray drift from adjacent field operations is unknown. Similarly, Minnesota's Pheasant Summit Action Plan (MNDNR 2015), Prairie Conservation Plan (MN Prairie Plan Working Group 2011), and Wildlife Action Plan (MNDNR 2016) aim to offset grassland cover losses by establishing grassland/wetland complexes within the agricultural matrix.

Chlorpyrifos, lambda-cyhalothrin, and bifenthrin have all been shown to have detrimental effects on non-target organisms. Lab studies have shown chlorpyrifos to be very highly toxic to several bird species including ring-necked pheasants (*Phasianus colchicus*), American robins (*Turdus migratorius*), common grackles (*Quiscalus quiscula*), and mallards (*Anas platyrhynchos*; Tucker and Haegele 1971). Furthermore, sub-lethal effects in birds resulting from chlorpyrifos exposure (e.g., altered brain cholinesterase activity, altered behaviors, reduced weight gain) have been documented in both lab and field studies (McEwen et al. 1986, Richards et al. 2000, Al-Badrany and Mohammad 2007, Moye 2008, Eng et al. 2017). Thus, exposure to sub-lethal doses of chlorpyrifos has the potential to cause indirect mortality of wildlife through factors such as increased predation risk or exposure to harsh weather conditions. Lambda-cyhalothrin is highly toxic to pollinators including bees and mildly toxic to birds (NPIC 2001). Insect abundance and diversity has decreased in fields exposed to this insecticide during field studies (Galvan et al. 2005, Langhof et al. 2005, Devotto et al. 2007). Birds relying on insects as a source of protein therefore may face reduced food availability when lambda-cyhalothrin is applied in agricultural landscapes. Bifenthrin is low in toxicity to upland birds; however, it is very highly toxic to aquatic organisms and its use may decrease food availability for birds that feed on fish and aquatic insects (Siegfried 1993, Johnson et al. 2010). Bifenthrin is also very highly toxic to bumblebees (*Bombus* spp.), with one study showing 100% mortality by contact (Besard et al. 2010). Consequently, these insecticides have the potential to detrimentally affect both birds and their insect food resources.

Reduced insect abundance and diversity resulting from insecticide application may pose a threat to grassland wildlife that use insects as a food source. Protein-rich insects are especially important for breeding grassland birds during egg-laying and the nestling and fledgling periods. The majority of breeding grassland birds' diets consist of insects, and insects are the primary food item fed to nestlings (Wiens and Rotenberry 1979, Kaspari and Joern 1993). Furthermore, there is correlative evidence between reduced insect food supplies and reduced nesting success for birds in fragmented habitat surrounded by cultivated fields (Zanette et al. 2000). Thus, the reduction of food availability via mortality of non-target insects from insecticides has the potential to negatively impact grassland bird reproduction and survival.

The objectives of our research are to assess the direct and indirect exposure risks of grassland birds and their insect food resources to soybean aphid insecticides in Minnesota's farmland region. First, we are quantifying the concentration of insecticides along a gradient from soybean field edge to grassland interior to assess the potential for grassland wildlife to be directly exposed to chemicals via contact with insecticides resulting from spray drift. Second, we are quantifying the chemical residue on invertebrates that serve as prey items of grassland birds, predatory insects, and other insectivores. This will allow us to assess the indirect exposure risk of birds and other wildlife to these chemicals through consumption of invertebrates. Finally, we are quantifying and comparing the relative abundance, richness, diversity, and biomass of invertebrates along a gradient from soybean field edge to grassland interior prior to and post-application to assess the indirect impact of insecticides on food availability for grassland-nesting birds and other wildlife. Our research will allow us to inform decision-making by land managers and private landowners so they can better incorporate areas of grassland cover within agricultural landscapes, thus reducing the impacts of spray drift on wildlife in these systems.

STUDY AREA

Our potential study sites are in the west-central (WC), central (C), southwest (SW), and south-central (SC) agricultural regions of Minnesota (Fig. 1). Corn and soybean fields account for approximately 50% of the landscape in these four regions. The SW and SC regions are the most intensively farmed; corn and soybeans are planted on 75% of those landscapes (USDA 2016*a*, *b*). Our 2017 study sites consisted of Wildlife Management Areas (WMAs) owned by the MNDNR and managed with the intent of providing high quality habitat for wildlife. We may consider other public lands (e.g., Scientific and Natural Areas) in addition to WMAs for our 2018 study sites.

We identified 16 potential study sites via GIS prior to the start of the field season but in-person site visits reduced our potential list to 7 treatment sites for various reasons (e.g., adjacent row crop was corn instead of soybeans) and 4 control sites. Four out of 7 landowners for our potential treatment sites agreed to cooperate with our study so that we could precisely time our sampling efforts. However, 1 landowner did not spray for aphids in 2017 and 1 landowner failed to give us advanced notice of his spraying efforts. Ultimately, we sampled 2 treatment and 2 control sites in summer 2017 (Table 1).

Each treatment site consisted of a WMA including upland grassland directly adjacent to and east of a soybean field. We chose this configuration based on the prevailing wind direction in the region to increase the likelihood that our treatment study sites would be downwind of sprayed soybean fields. We prioritized 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 and create grassland bird and beneficial insect habitat. Control sites had similar characteristics except that control sites were east of cornfields. We plan to sample an additional 6-8 treatment sites and 2 control sites during summer 2018.

METHODS

We conducted sampling to assess both direct and indirect exposure risks to grassland wildlife, especially birds and insects, immediately after spraying and at additional periods post-application. Within each treatment site, we conducted sampling at stations placed at 7 distances (0, 5, 25, 50, 100, 200, and 400 m) along each of 3 transects extending from a treated soybean field edge to an adjacent grassland interior (Fig. 2). Thus, there were 21 drift sampling stations at each treatment (i.e., pesticide application) or control (i.e., non-application) site. We aligned transects perpendicular to the soybean field edge and spaced them 100 m apart to reduce the likelihood of duplicate insecticide exposure during the spraying event. As a control, we also conducted sampling in grasslands adjacent to cornfields. We 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 temperature, wind speed, wind direction, humidity, and dew point at the time of spraying and during insect sampling periods (use of trade names does not imply endorsement by the U.S. Government, the University of Minnesota, or the MNDNR). We also measured vegetation characteristics at each station. Weather conditions and vegetation characteristics may influence deposition rates of insecticides and we will include covariates related to these factors in mixed linear models of insecticide deposition. In addition, weather influences the availability of insects to be collected (Southwood and Henderson 2000).

Direct Exposure Risk

To assess the potential for direct exposure of birds and other wildlife to soybean aphid insecticides, we measured the amount of chemicals deposited in grasslands within hours of soybean fields being sprayed. We placed passive sampling devices (PSDs) and water-sensitive cards at ground level and mid-canopy level at each station described in the 2 hrs prior to spraying. Ground level sampling measures potential insecticide drift exposure of ground-nesting birds and other ground-dwelling wildlife. Mid-canopy sampling measures potential exposure of above-ground-nesting birds and many species of spiders and insects to insecticide drift. Ground-level and mid-canopy samples will be analyzed independent of one another. We collected 42 filter paper and 42 water-sensitive card samples at each site. We retrieved these samples within 2.5 hrs of treatment sites being sprayed, wrapped them in aluminum foil, enclosed them in airtight plastic storage bags, and placed them in a cooler with dry ice. At control sites, we sampled at the same 21 stations within the same timeframe as at treatment sites. We are storing filter paper from the PSDs in a -80° C freezer until we send them to the laboratory for chemical analysis.

PSDs were composed of Whatman grade 2 filter paper (GE Healthcare UK Ltd., Little Chalfont, UK) covering 1.27 cm (0.5 in) mesh steel hardware cloth in the shape of a cylinder. Organic molecules adhere to the surface of filter paper, and a 3-dimensional PSD mimics an animal being exposed to insecticides. We attached PSDs to upright plastic fence posts with zip ties. Similarly, we attached 4 water-sensitive cards (Syngenta, Basel, CH) to steel mesh hardware cloth (2 on the vertical plane and 2 on the horizontal plane) to collect spray droplets. These cards change color from yellow to purple when they encounter liquid.

Indirect Exposure Risk

To assess the potential for birds and other insectivorous wildlife to be exposed to insecticides indirectly, we will examine the chemical residues on invertebrates collected on the day of spraying at each treatment site. We sampled ground-dwelling invertebrates using vacuum sampling and canopy dwelling invertebrates via sweep netting (Southwood and Henderson 2000). These sampling methods collected invertebrates of differing size classes and taxa (Doxon et al. 2011). We collected these samples on paired 30-m transects extending perpendicular to the field edge from the 0-, 5-, and 25-m stations for a total of 9 stations (Fig. 3). We placed vacuum sampling and sweep netting transects 1-2 m apart at each station to minimize disturbance of sampling and maximize the likelihood that the invertebrate communities being sampled were similar (Doxon et al. 2011). We combined vacuum samples and sweep net samples taken from the same station into 1 sample in sterilized plastic bags and placed them on dry ice immediately after collection in the field. We are storing them in a -80◦ C freezer until later chemical analysis.

We will send samples that require chemical analysis to the U. S. Department of Agriculture - Agricultural Marketing Service (USDA-AMS) National Science Laboratory (Gastonia, NC, U.S.A.) to test for chemical levels of our three primary chemicals of interest (chlorpyrifos, lambda-cyhalothrin, and bifenthrin) and several additional pesticides (particularly those classified as neonicotinoids and fungicides) commonly used in Minnesota's agricultural region. Chemical analyses will use a solvent extraction method followed by concentration of the extracts by evaporation. Concentrated extracts will then be subjected to Gas Chromatography/Mass Spectrometry (MS)-Negative Chemical Ionization to test for organophosphates and pyrethroids, and Liquid Chromatography/MS/MS for neonicotinoids and fungicides. Chemical residues will be reported in parts per billion.

Indirect Effects of Exposure

To quantify and compare the abundance, richness, diversity, and biomass of invertebrate prey items before and after spraying, we collected additional vacuum and sweep net samples 1-3 d prior to spraying and 3-5 d and 19-21 d post-spraying. We collected these samples between the 0- and 5-m stations and at the 25- and 100-m stations on paired 20-m transects. Additionally, we collected insect samples at the same 3 distances along an added transect 3-5 d and 19-21 d post-spraying. This transect was not adjacent to our 3 original transects to ensure that these samples were not affected by our previous disturbance of the area due to sampling activities. We combined vacuum and sweep net samples from each station into 1 Whirl-Pak plastic bag and preserved insects in ethanol. We are sorting and identifying these insects to the family level. We are placing emphasis on invertebrate orders important in the diets of grassland nesting birds, including: Araneae (spiders), Orthoptera (grasshoppers, crickets, and katydids), and Coleoptera (beetles). After identification, we will dry and weigh invertebrates to measure biomass and measure them to sort into size classes preferred by grassland birds and nestlings.

Vegetation Measurements

We measured ground cover, canopy cover, litter depth, maximum height of live and dead vegetation, vertical vegetation density, and species richness at 3 locations parallel to the field edge at each station and at both ends of insect sampling transects (Fig. 3). We recorded these vegetation characteristics at the 21 drift sampling stations 1-3 d prior to spraying and at each of the 9 insect sampling stations at 1-3 d prior to spraying and 3-5 d and 19-21 d post-spraying. Using a modified point-intercept method, we categorized ground cover into bare ground, litter, and other (i.e., woody debris, rock, or gopher mound; BLM 1996). We determined canopy cover from nadir digital photographs taken of each plot from 1.5 m above the ground using the program SamplePoint (Booth et al. 2006). Canopy cover categories included grass, forb, standing dead, woody vegetation, and other. 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 recorded the maximum height of live and dead vegetation within each plot to the nearest 0.5 dm. We measured vertical vegetation density by placing a Robel pole in the center of each plot and estimating the visual obstruction reading (VOR) in each of the 4 cardinal directions (Robel et al. 1970). We recorded the lowest 0.5-dm mark visible on the pole from 4 m away and 1 m above the ground. Finally, we listed the dominant grass and forb species in each plot along the center transect only. This list was composed of up to 3 species of grasses and 3 species of forbs that constituted significant portions of the canopy cover within the sampling frame and provided a qualitative assessment of the vegetation present at each site. We will include covariates derived from these measurements in mixed linear models of chemical deposition and abundance, richness, and diversity of invertebrates.

Researcher Safety

Long-term exposure to organophosphate and pyrethroid insecticides have been linked to increased human health risks in pesticide 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). The specimen labels of insecticide mixes including chlorpyrifos, lambda-cyhalothrin, and bifenthrin contain warnings of short-term side effects of exposure including eye, skin, nose, and throat irritation; headaches; nausea; and dizziness (Dow AgroSciences LLC 2014, Syngenta Crop Protection LLC 2014).

To reduce our exposure to these chemicals, we followed the Personal Protective Equipment (PPE) recommendations listed on the specimen labels of mixes containing chlorpyrifos. These mixes had more PPE recommendations than those containing lambda-cyhalothrin or bifenthrin, because chlorpyrifos has more severe health risks. We were equipped with more PPE than necessary, because the PPE recommendations on specimen labels are aimed at pesticide applicators who spend several days per year working in close proximity to these chemicals (D.

Herzfeld, University of MN, personal communication). Our overall exposure levels were very low, as we spent 4-5 h in grasslands adjacent to sprayed fields on only 1 d per each treatment site. We wore Tychem QC 127 series hooded Tyvek 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 on the day of spraying immediately after chemical application. We had chemical-resistant goggles and half-mask air-purifying respirators on-hand should we have experienced eye, skin, nose, or throat irritation while in the field, but did not need to use them during our fieldwork in summer 2017.

Contacting Farmer Cooperatives
We contacted 12 farmer cooperatives (with the assistance of T. Klinkner, MNDNR) during fall 2016 to request the trade names of the soybean insecticides they most commonly applied during summer 2016 to decide the active ingredients upon which to focus our sampling efforts. We also requested information regarding the application method of these chemicals (i.e., ground boom or aerial). These cooperatives were located in Cottonwood, Kandiyohi, Redwood, Stearns, Swift, Meeker, and Watonwan counties in Minnesota. Several representatives reported using multiple active ingredients to combat soybean aphids (e.g., chlorpyrifos + lambda-cyhalothrin). This is a common practice, as active ingredients have differing withholding times and modes of action, and such products are readily available commercially (Koch et al. 2016).

Landowner Contact
Landowner cooperation is vital to timing our field sampling efforts. To request the cooperation of landowners and learn about their soybean-aphid-spraying practices, we mailed surveys to 206 landowners who owned land bordering 29 potential study sites in March and April 2016. We ultimately solicited landowner cooperation for our treatment sites by directly calling landowners and visiting their residences. This approach was more effective than mailing surveys and we will contact landowners in this manner to request their cooperation in early summer 2018.

RESULTS

Insecticides Used in Our Study Area
Lambda-cyhalothrin was the most common active ingredient reported to us by farmer cooperative representatives in our study area, followed by chlorpyrifos and bifenthrin. This reflects statewide insecticide usage trends from 2013: the Minnesota Department of Agriculture found that lambda-cyhalothrin was the most widely used chemical on 16% of surveyed soybean acres, followed by 13% being treated with chlorpyrifos and 5% with bifenthrin (MDA 2016). Overall, cooperative representatives estimated 56% of insecticide applications to control soybean aphids were by air and 44% were via ground boom in recent years. Our 2 treatment sites in summer 2017 were sprayed with insecticides with the trade names Bolton (chlorpyrifos + gamma-cyhalothrin; Cheminova, Inc., Research Triangle Park, NC, U.S.A.) and Endigo (lambda-cyhalothrin + thiamethoxam; Syngenta Canada Inc., Guelph, ON, CA). One treatment site was sprayed by air and the other was sprayed via ground boom.

Landowner Contact

Of the 206 surveys we sent to landowners who owned land adjacent to potential study areas, 28.1% were returned. However, not all landowners filled out the survey completely. Many landowners did not complete the survey because they rent their land and did not have information on aphid-spraying practices; this was the case for the landowners with soybean fields adjacent to our 2 treatment sites in 2017. We then called these landowners to request their renters' information. Approximately 13.6% of landowners completed the survey in its entirety and 7 landowners indicated that they would be planting soybeans adjacent to a WMA in 2017 and were willing to be contacted during the growing season. However, we did not select these WMAs at treatment sites for summer 2017.

Field Sampling

During our first field season in summer 2017, we collected 166 direct-exposure samples, 36 indirect-exposure invertebrate samples, and 132 indirect-effect invertebrate samples in 2 treatment and 2 control sites (Table 2). We will send samples requiring chemical analysis to the USDA-AMS National Science Laboratory upon approval of a multi-year master contract. This process has taken longer than expected, but the lab typically processes samples in 10 business days once they receive them; thus, analyses will commence quickly upon contract approval.

The aim of using water-sensitive cards was to provide an immediate visual assessment of whether drift occurred at our treatment sites in low humidity. However, at high humidity levels these cards demonstrated a color change in the absence of chemical drift. We were unable to attain quantifiable measures of chemical drift from these cards and thus, we will be discontinuing their use in 2018.

ACCOMPLISHMENTS

Through January 2017

- Contacted representatives at 12 farmer cooperatives across 7 counties to gather information about current spraying methods used in our study area (T. Klinkner, MNDNR)
- Identified the insecticides that will constitute the focus of our sampling efforts
- Drafted a research summary letter and survey to be sent to potential cooperating landowners

January 2017-present

- Sent a research summary letter and survey to 206 landowners who own property adjacent to 29 potential study site WMAs
- Contacted landowners via phone and in-person to request their cooperation with the project
- Refined the sampling design
- Purchased project supplies and equipment
- Hired 2 technicians through the MN DNR to assist with field sampling efforts
- Identified 16 potential study sites using GIS and in-person site visits

- Collected 166 direct exposure samples, 36 indirect exposure invertebrate samples, and 132 indirect effect invertebrate samples in 2 treatment and 2 control sites during our first field season
- Recruited 3 undergraduate students to process indirect effect invertebrate samples

Work in Progress

- Setting up a multi-year master contract with the USDA-AMS National Science Laboratory in Gastonia, NC, U.S.A. following MNDNR purchasing policies
- Sorting insect samples collected in summer 2017
- Analyzing preliminary vegetation data
- Identifying additional study sites for 2018 field season
- Advertising a job posting to hire 1 technician for our 2018 field season

PUBLICATIONS

Davros, N. M., and Goebel, K. M. *In press*. Evaluating insecticide exposure risk for grassland wildlife on public lands. 2016 Summary of Wildlife Research Findings, Division of Fish & Wildlife: Minnesota Department of Natural Resources. St. Paul, Minnesota, U.S.A.

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PRESENTATIONS

Davros, N. M. 2016. Overview of grassland wildlife/insecticide exposure study. MN DNR Region 4 Wildlife Meeting, New Ulm, Minnesota, U.S.A. (July 2016)

Davros, N. M. 2016. Introduction of grassland wildlife/insecticide exposure study. LCCMR Pollinator & Partner Projects Meeting, St. Paul, Minnesota, U.S.A. (December 2016)

Davros, N. M. 2016. Update of grassland wildlife/insecticide exposure study. MN DNR Region 4 Wildlife Meeting, Lamberton, Minnesota, U.S.A. (December 2016)

Goebel, K. M., Davros, N. M., and D. E. Andersen. 2017. Insecticide exposure risk for grassland wildlife on public lands. Poster. Midwest Fish and Wildlife Conference, Lincoln, Nebraska, U.S.A. (February 2017)

Goebel, K. M., Davros, N. M., and D. E. Andersen. 2017. Insecticide exposure risk for grassland wildlife on public lands. Poster. Annual Meeting of the MN Chapter of The Wildlife Society, Callaway, Minnesota, U.S.A. (February 2017)

Davros, N. M. 2017. Does diversity matter? Ring-necked pheasant nest site selection and nest survival in grassland reconstructions. Little Lunch on the Prairie Webinar, WebEx Meeting. <https://www.youtube.com/watch?v=kidTWvK0a30&index=9&list=PLeh-ajY3F3JK8MgVek1eeWwtKibPLgzdc&t=2647s> (December 2017)

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Goebel, K. M., Davros, N. M., Andersen, D. E., and P. J. Rice. 2018. Evaluating insecticide exposure risk for grassland wildlife on public lands. LCCMR Pollinator and Partner Projects Meeting, St. Paul, Minnesota, U.S.A. (March 2018)

MEDIA OUTLET

Kennedy, T. October 2017. DNR wildlife researcher Nicole Davros working to help upland birds thrive. Star Tribune, Minneapolis, Minnesota, U.S.A. <http://www.startribune.com/dnr-wildlife-researcher-nicole-davros-working-to-help-upland-birds-thrive/450349283/>

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Figure 1. Minnesota's agricultural regions as outlined in the Minnesota Department of Natural Resources (MNDNR) annual August Roadside Surveys. The study sites for this project include Wildlife Management Areas owned and managed by the MNDNR and potentially other publically-owned grasslands in the west-central (WC), central (C), southwest (SW), and south-central (SC) regions of the state.

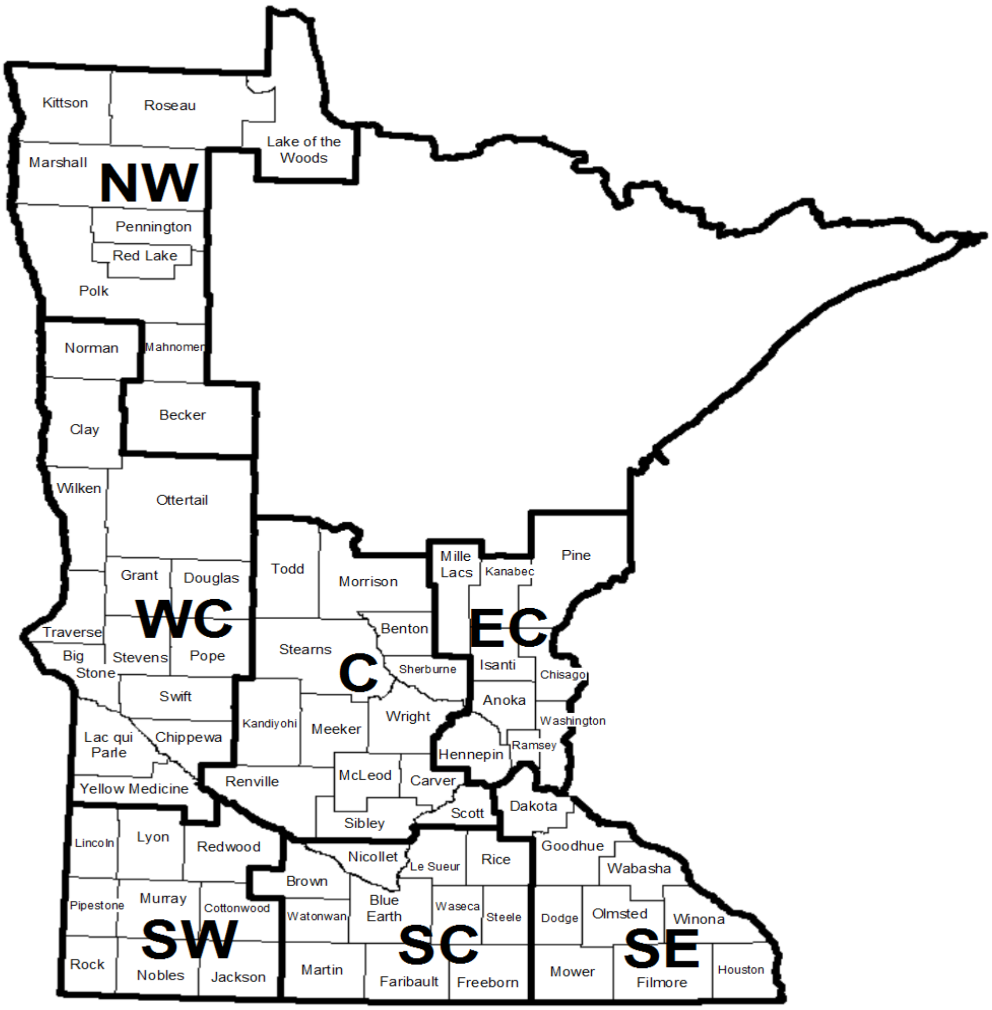

Figure 2. Field sampling design used to assess the exposure risk of grassland wildlife to soybean aphid insecticides. We conducted sampling on 2 WMAs east of privately owned soybean fields treated with insecticides to combat aphids in 2017. Our control sites are WMAs adjacent to cornfields. White lines indicate sampling transects established 100 m apart, perpendicular to the soybean field edge, and extending 400 m into the grassland. Yellow circles represent sampling stations at 0, 5, 25, 50, 100, 200, and 400 m from the field edge. This transect and station layout is used in both our treatment and control sites.

Figure 3. Field sampling design showing the layout of transects, chemical drift sampling, insect sampling, and vegetation sampling in each sample site. Features are color-coded to represent the timing of sampling in relation to the day of spraying. Numbers indicate distances in meters (not to scale).

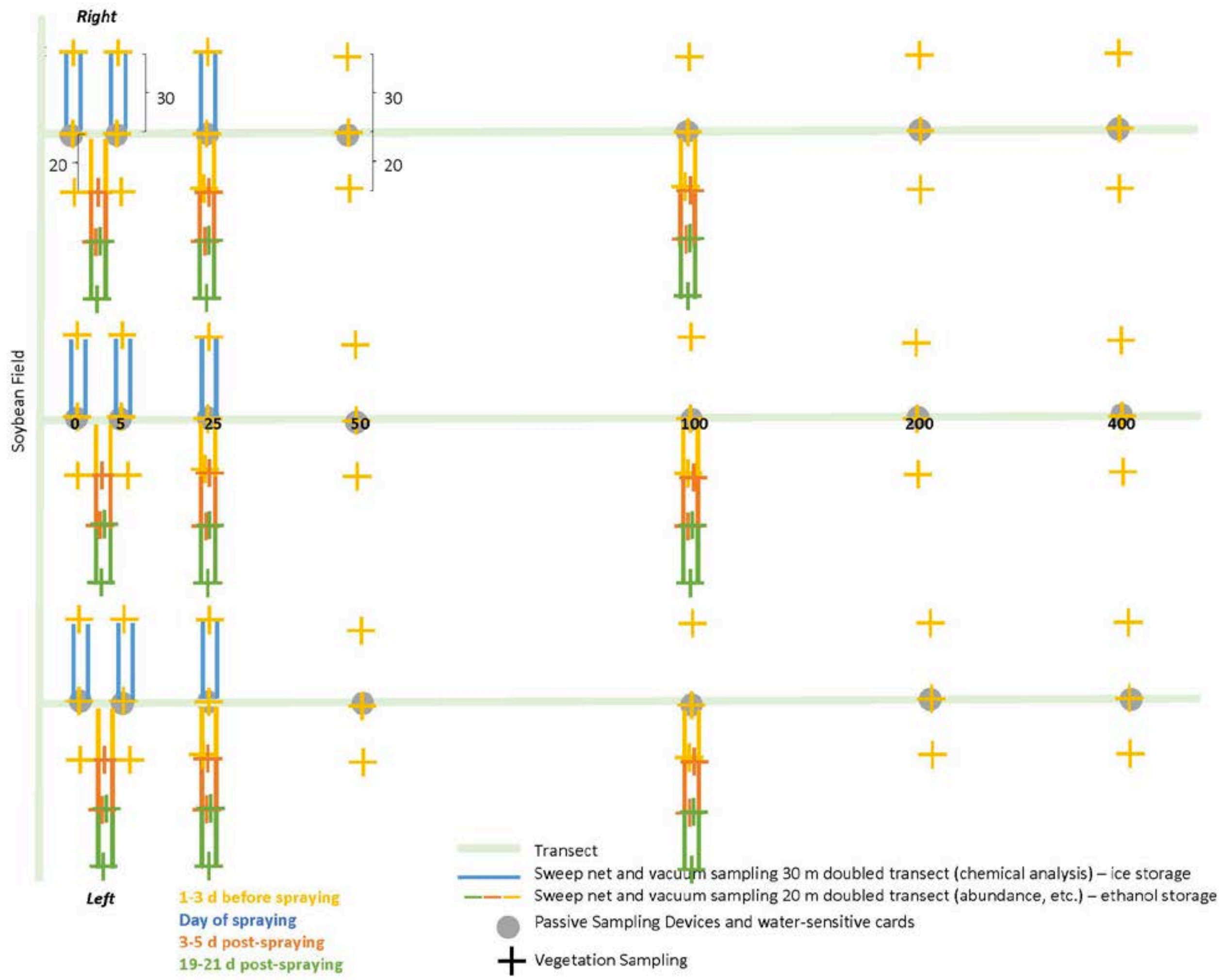

Table 1. Wildlife Management Areas (WMAs) selected as study sites for our summer 2017 field season. Agricultural region refers to the regions outlined in the Minnesota Department of Natural Resources annual August Roadside Surveys.

Site name	County	Site type	Agricultural region
Dead Horse WMA	Jackson	Control	Southwest
Heron Lake WMA: South Heron Unit	Jackson	Treatment	Southwest
Lake Maria WMA	Murray	Treatment	Southwest
Rolling Hills WMA	Lyon	Control	Southwest

Table 2. Timing and number of samples collected during our summer 2017 field season at 2 treatment and 2 control sites. (EF) denotes indirect effect invertebrate samples stored in ethanol and (EX) denotes indirect exposure invertebrate samples to be submitted for analysis of chemical residues.

Timeframe	Sample type	Number of samples/site	Total number of samples collected during July-Sept 2017
1-3 d before spraying	Insects (EF)	9	36
Day of spraying	PSDs & water-sensitive cards	*42	166
Day of spraying	Insects (EX)	9	36
3-5 d after spraying	Insects (EF)	12	48
19-21 d after spraying	Insects (EF)	12	48

*We omitted 1 400-m station at 1 treatment site (Heron Lake WMA: South Heron Unit) due to transect length constraints. We collected 40 PSD and water-sensitive card samples at that site.