EVALUATING INSECTICIDE EXPOSURE RISK FOR GRASSLAND WILDLIFE ON PUBLIC LANDS

Nicole M. Davros and Katelin Goebel[1]

SUMMARY OF FINDINGS

Increasing evidence suggests that acute toxicity to pesticides may be more important than agricultural intensity in explaining declines in grassland-dependent wildlife. Although neonicotinoids (systemic insecticides routinely used on corn and soybeans) are currently under scrutiny for their effects on birds and pollinators, other insecticides are commonly used in Minnesota's farmland regions that may also have negative effects on non-target organisms. Minnesota Department of Natural Resource (MNDNR) wildlife managers and members of the public have reported concerns about 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's farmland regions. Concerns have previously been raised about the impacts of chlorpyrifos, a broad-spectrum organophosphate, and other foliar-application insecticides on water quality and human health, prompting the Minnesota Department of Agriculture (MDA) to release guidelines for voluntary best management practices for their use. Although lab studies have shown chlorpyrifos and other insecticides used to target aphids are highly toxic to non-target organisms, including economically important game species and pollinators, fewer studies have investigated the environmentally-relevant exposure risk of free-ranging wildlife to these chemicals. Our research project will assess the direct and indirect exposure risk of grassland wildlife to common soybean aphid insecticides along a gradient from soybean field edge to grassland interior. The data we obtain on the environmentally-relevant exposure risk of wildlife to these insecticides will be used to help natural resource managers and private landowners better design habitats set aside for grassland wildlife in Minnesota's farmland region.

INTRODUCTION

Grassland habitat 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 increasingly being raised 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).

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[1]Department of Fisheries, Wildlife, and Conservation Biology, University of Minnesota – Twin Cities; United States Geological Survey (USGS) Cooperative Fish and Wildlife Research Unit

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). In response to concerns over yield loss, the University of Minnesota Extension Office (hereafter, UM Extension) released guidelines on how to scout for aphids and when to consider treatment for infested fields (UM Extension 2014). Foliar applications of insecticides using boom sprayers or planes are common treatment methods when chemical control of aphids is considered necessary. The 2 most common insecticides used are chlorpyrifos and lambda-cyhalothrin (MDA 2005, MDA 2007, MDA 2009, MDA 2012, MDA 2014a) but bifenthrin use has also been reported (R. Riley, personal communication; E. Runquist, unpublished data). Withholding times vary by chemical (lambda-cyhalothrin: 45 d; chlorpyrifos: 28 d; bifenthrin: up to 14 d); thus, the timing of product use within the growing season should be considered. If retreatment is necessary due to a continued infestation, landowners are encouraged to use an insecticide with a different mode of action to prevent the development of resistance (UM Extension 2014). Therefore, multiple chemicals may be used on the same field at different times of the year in some situations. Alternatively, landowners 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.

Lambda-cyhalothrin (common trade names include Charge, Demand, Excaliber, Grenade, Hallmark, Icon, Karate, Kung-fu, Matador, Samurai, and 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). Further, 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 names include Bifenture, Brigade, Discipline, Empower, Tundra, and Xpedient) 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 bobwhite quail (*Colinus virginianus*) and mallards (*Anas platyrhynchos*)(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 terrestrial insects are also susceptible to bifenthrin (Siegfried 1993). Bifenthrin is very highly toxic to bumblebees, with one study showing 100% mortality by contact (Besard et al. 2010).

Chlorpyrifos (common trade names include Dursban, Govern, Lorsban, Pilot, Warhawk, and Yuma) is a broad-spectrum organophosphate insecticide that also 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). Further, MDA recently 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 very highly toxic to gallinaceous bird species such as the ring-necked pheasant (*Phasianus colchicus*) and domesticated chickens (*Gallus gallus domesticus*), with a lethal dose causing death in 50% of treated animals (LD_{50}) of 8.41 mg/kg and 32-102 mg/kg, respectively (Tucker and Haegele 1971, Christensen et al. 2009). Several other bird species are also particularly susceptible to chlorpyrifos, including American robins (*Turdus migratorius*), common grackles (*Quiscalus quiscula*), and mallards (Tucker and Haegele 1971, 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) has been documented in both lab and field studies (McEwen et al. 1986, Richards et al. 2000, Al-Badrany and Mohammad 2007, Moye 2008). Thus, sub-lethal effects leading to indirect mortality (e.g., via increased predation rates) may be a concern for wildlife exposed to chlorpyrifos.

Minnesota DNR wildlife managers and members of the public have reported concerns about the effects of these soybean aphid insecticides on non-target wildlife, including economically important bird and pollinator species. The common public perception is that indiscriminate spraying without first scouting for aphid outbreaks has become the norm and fewer birds and insects are observed after spraying has occurred. Yet little is known about the actual exposure risk of birds and terrestrial invertebrates to these insecticides in Minnesota's grasslands. Distances reported for drift from application of foliar insecticides vary widely in the literature (5-75 m; Davis and Williams 1990, Holland et al. 1997, Vischetti et al. 2008, Harris and Thompson 2012), and a recent butterfly study in Minnesota found insecticide drift on plants located up to 1600 m away from potential sources (E. Runquist, personal communication). 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 is to assess the environmentally-relevant exposure risk of grassland wildlife to commonly-used soybean aphid insecticides, especially chlorpyrifos, in Minnesota's farmland region. In particular, we will:

1) Quantify the concentration of insecticides 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 chemicals directly via contact with spray drift and indirectly through insect prey items exposed to the insecticides.

2) Quantify and compare 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 insecticides on food availability for grassland nesting birds and other wildlife.

STUDY AREA

Our study is being conducted within the south-central and southwest regions of Minnesota's farmland zone (Figure 1). These regions are intensively farmed, and corn and soybeans combined account for approximately 75% of the landscape [U.S. Department of Agriculture

(USDA) 2013a, USDA 2013b]. Acres set aside as grassland habitat on public and private land account for 5.8% and 4.6% of the landscape, respectively, in these regions (Davros 2015). 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

A treatment study site will consist of a MNDNR Wildlife Management Area (WMA) immediately adjacent to and downwind from a soybean field that will be sprayed to control for aphids. We are working in close consultation with wildlife managers and private landowner cooperators to choose 6-8 treatment sites. We will choose 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. We will also chose 2-4 control study sites with similar site characteristics except that control sites will not be sprayed with any chemical to control aphids.

Field sampling will occur during summer 2017 and 2018, and approximately half of the study sites will be sampled each year. Within each treatment site prior to spraying, we will establish 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 is large enough, we will also establish a station at a distance of 400 m along each transect. This design will give us a total of 18-21 stations per site. We will establish transects and stations the same way within control sites. At all sites, transects will run perpendicular to the edge of the soybean field and will be spaced 100 m apart to reduce the likelihood of duplicate insecticide exposure from the spraying event.

Data Collection

To assess the potential for direct exposure of birds and other wildlife to soybean aphid insecticides (hereafter, target chemicals), we will deploy passive sampling devices (PSDs) to absorb any chemical drift that occurs. The PSDs will be placed in treatment fields on the morning of but prior to spraying of soybeans. They will be made of Whatman™ Qualitative Filter Paper (grade 2) that is attached to 0.5 in^2 hardwire cloth formed to a cylinder shape to approximate the size and shape of a large songbird or a gamebird chick. We will place the PSDs at two heights (ground and mid-canopy) at each of the sampling stations. Ground-level sampling will help represent 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 will help represent above-ground nesting birds and many species of spiders and insects. We will retrieve the PSDs from the field ≤1 h after spraying and properly store them for later chemical analysis. All ground-level and mid-canopy samples will be analyzed independent of one another. At control sites, we will place PSDs at both ground and mid-canopy levels at each of the stations. We will leave the PSDs on site for the same amount of time as PSDs at treatment sites before we collect and store them for later analysis.

To assess the potential for birds and other insectivorous wildlife to be exposed to the target chemicals indirectly via consumption of prey items, we will sample invertebrates ≤2 h post-spraying at each of the sampling stations. We will sample ground-dwelling invertebrates using a vacuum trap and canopy dwelling invertebrates using a sweepnet. Vacuum trap and sweepnet samples will both be taken along 60 m transects to the left side of the sampling stations and parallel to the soybean field. We will combine vacuum trap and sweepnet samples taken from the same station during the same time period into one sample and properly store them for later chemical analysis. We will sample control sites using the same methods and timing, with the timing based on when we deploy the PSDs at these sites.

To quantify and compare the abundance, richness, diversity, and biomass of invertebrate prey items, we will collect vacuum trap and sweepnet samples from the <1-5 m, 25 m, and 100 m distances along the 3 transects at each site (total = 9 stations/site). The <1 m and 5 m distances will be combined into 1 transect parallel to the soybean field for this effort. We will collect these samples 1-3 d prior to spraying and between 3-5 d and 19-21 d post-spraying at treatment sites. Samples will be taken along 40 m transects but on the right side of the sampling stations and parallel to the soybean field. We will combine vacuum trap and sweepnet samples into 1 sample per station per sampling period and store them in ethanol for later sorting and identification. We will place emphasis on 3 invertebrate orders important in the diets of grassland nesting birds: Araneae (spiders), Orthoptera (grasshoppers, crickets, and katydids) and Coleoptera (beetles). All individuals from these orders will be sorted and identified to at least the family level for analysis. Quantifying the spider community will allow us to examine potential impacts on an additional trophic level since spiders are an important predator of insects.

We will use portable weather meters (Kestrel 5500AG Agricultural Weather Meters) 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 during insect sampling periods at each site.

At each site, we will also collect vegetation data 1-3 days prior to spraying at all sampling stations and again at 3-5 d and 19-21 d post-spraying at the reduced subset of sampling stations coinciding with invertebrate sampling efforts. Data collected will include percent canopy cover, maximum height of live and dead vegetation, litter depth, and vertical density. We are still developing our methods for vegetation data collection but we will likely use the program SamplePoint (Booth et al. 2006) to estimate percent canopy cover as it provides a more objective measure than visual estimation techniques.

We will send the PSD samples and invertebrate samples to an external lab to be analyzed using a solvent-based extraction method. Extracts will be concentrated by evaporation and then analyzed using a gas chromatography/mass spectrometry-negative chemical ionization (GC/MS-NCI) method. Although our experimental design will focus on soybean fields sprayed with foliar insecticides to control aphids, the chemical analyses will allow us to quantify additional pesticides (e.g., neonicotinoids, fungicides) at minimal extra cost. Obtaining information about other pesticide exposure will be valuable supplementary information in support of other Section of Wildlife research and management goals.

Data Analyses

We will use mixed regression models to examine factors related to risk of direct and indirect exposure of wildlife to target chemicals. Chemical concentration will be the dependent variable. We will specify distance from soybean field edge and canopy height (when relevant) as a fixed effect. We may also include other covariates such as site, ordinal date, vegetation data, and weather condition variables where appropriate. We will use similar models to examine differences in the abundance, richness, diversity, and biomass of Aranaeans, Orthopterans and Coleopterans. We will use the sampling period (i.e., 1-3 d prior to spraying, and 7-9 d or 18-20 d post-spraying) as a repeated measure in these analyses, specifying a covariance structure [e.g., autoregressive 1 (AR1)] when appropriate.

RESULTS AND DISCUSSION

To date, we have surveyed 12 farmer cooperatives in 12 counties to gather more specific information about chemical spraying (e.g., type of insecticide, application method) in southern

Minnesota. Congruent with MDA's pesticide usage reports (MDA 2007, MDA 2009, MDA 2012, MDA 2014), the coops reported that chlorpyrifos, lambda-cyhalothrin, and bifenthrin have been the most commonly-used foliar soybean insecticides in recent years. Additionally, we learned that neonicotinoids have also been used in the chemical mixes used as foliar treatment of crop pests. This information is contrary to the widespread belief that neonicotinoids are only used as a prophylactic seed treatment to treat plants systemically.

We also surveyed landowners adjacent to potential WMA study sites to learn more about their soybean aphid spraying practices and to ask for their cooperation with our study (see Appendix 1) since cooperation will be key to timing our field sampling. We mailed 221 letters during the first week of March 2017; 24 letters were returned as undeliverable. The overall response rate for the first mailing was 24.4%. In early April, we sent a second round of 164 letters and had a response rate of 6.1%. Some landowners opted to call us instead and provide their renter's contact information; however, not all landowners provided renter information when they returned the survey by mail. Overall, we were able to identify 11 landowners adjacent to and upwind from a WMA during 2017 who are willing to be cooperators with our study. We are currently contacting these landowners again to determine if they have planted soybeans this year and whether they will be spraying their soybeans for aphids this growing season. Several landowner cooperators have indicated that they do not plan on scouting for aphids. Rather, they plan to spray regardless of infestation levels. This approach to soybean management may be a primary reason why reports of aphid resistance to pyrethroid insecticides are increasing in Minnesota and parts of North Dakota this year (UM Extension 2017).

Further results are forthcoming as no field sampling has occurred yet. Our first year of field sampling will occur during late summer 2017 once soybean aphid spraying begins. A second season of field sampling is also planned for summer 2018.

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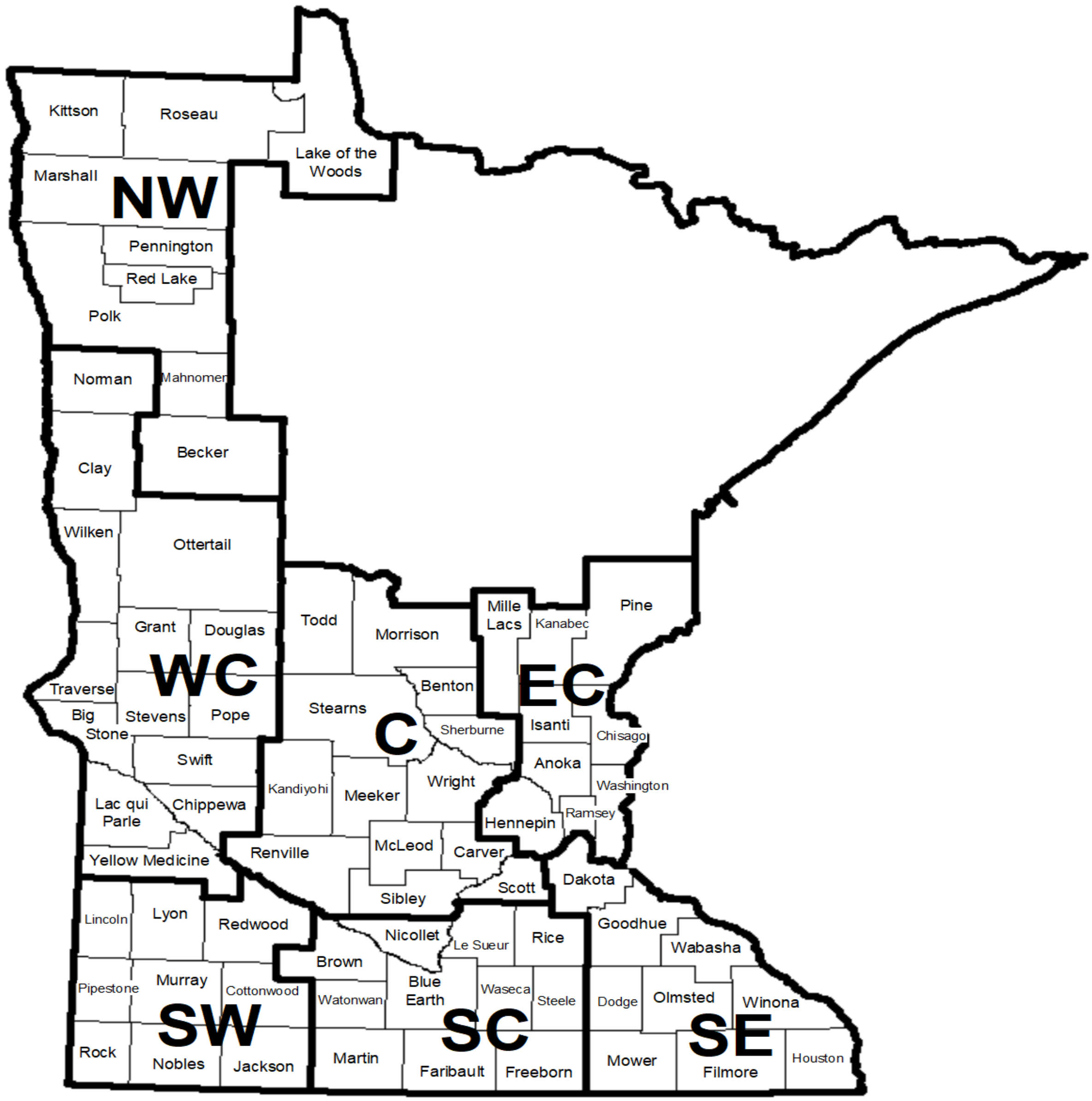

Figure 1. Minnesota's agricultural regions as outlined in MNDNR's annual August Roadside Surveys.

Appendix 1. Survey sent to neighboring landowners (i.e., private landowners with property immediately adjacent to potential Wildlife Management Area study sites) in March and April 2017 to assess soybean aphid spraying practices and to solicit cooperation for summer 2017 sampling efforts.

Print your name here ___________________

Spraying Practices Survey

PART I

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

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

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

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

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

6. **Did you hire an applicator (e.g. agricultural consultant company) to treat your soybeans with foliar insecticides in the past 3-5 years?**
   ○ Yes (please specify company or individual):
   ○ No, I applied insecticides myself

PART II

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

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

3. **How will foliar insecticides likely be sprayed on these soybeans in 2017?**
   ○ Ground boom
   ○ Aerial
   ○ Other (please specify):
   ○ I'm not sure

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

5. **Will you hire an applicator (e.g. agricultural consultant company) to treat these soybeans with foliar insecticides in 2017 if chemical treatment is needed?**
   ○ Yes (please specify company or individual):
   ○ No, I will apply insecticides myself
   ○ I'm not sure

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

Print your name here ____________________

Contact Information Form

1. **May we contact you to identify foliar insecticide spraying date(s) in the summer of 2017?**
   ○ Yes
   ○ No

2. **What is the best way to reach you?**
   ○ Home phone
   ☐☐☐ – ☐☐☐ – ☐☐☐☐

   ○ Cell phone
   ☐☐☐ – ☐☐☐ – ☐☐☐☐

   ○ Both home & cell phones

3. **In order to identify the exact date(s) of spraying, how often are you comfortable with us contacting you during the late summer of 2017?**
   ○ Weekly
   ○ Semi-weekly
   ○ As often as necessary as the spraying date approaches (no more than once daily)

4. **Would you like to receive a paper copy of the LCCMR work plan for our project?**
   This can also be found at: http://www.lccmr.leg.mn/projects/2016/work_plans_may/_2016_03n.pdf
   ○ Yes
   ○ No

5. **Would you like to receive a paper copy of your responses to the Spraying Practices Survey and Contact Information Form?**
   ○ Yes
   ○ No

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

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