

**INTEGRATING CHEMICAL AND BIOLOGICAL CONTROLS FOR  
MANAGEMENT OF SOYBEAN APHID**

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## **Dedication**

*To my mom, Suzie  
Thanks for teaching me to not only talk about my aspirations,  
but to achieve them as well.*

*To my father, Nhuan  
Thank you for everything. This thesis is for you.*

## Abstract

Integrated pest management (IPM) is an approach to integrating different control techniques to minimize control costs and reduce pest damages. In soybean, *Glycine max* (L.) Merrill, further IPM research is needed for management of the soybean aphid, *Aphis glycines* Matsumura. Management of *A. glycines* relies on application of broad-spectrum insecticides, which can negatively impact the natural enemies associated with *A. glycines*. My research examined two approaches to improve integration of natural enemies in *A. glycines* management.

First, I compared the effects of sulfoxaflor (i.e., a new selective insecticide) and broad-spectrum insecticides on *A. glycines* and predators in a two-year field experiment. In addition to field studies, laboratory bioassays were performed to characterize the lethal effects of sulfoxaflor by exposing aphid predators to insecticide residues. Field results showed that sulfoxaflor was as effective as the broad-spectrum insecticide in suppressing *A. glycines* populations and was less impactful on predator populations. The laboratory bioassays showed that sulfoxaflor varied in toxicity to different predator species. However, application of insecticides is currently based only on *A. glycines* density and removal of natural enemies can cause negative consequences. As a step toward incorporating natural enemy densities into *A. glycines* management, I developed sequential fixed-precision sampling plans for the most abundant aphid predators in soybean fields. The development of sequential sampling plans should provide guidance for improved estimation of these predator densities for *A. glycines* management and for research purposes.

The results of my thesis will provide information on how to better integrate biological control into current *A. glycines* management programs. Improved integration of natural enemies should reduce reliance on broad-spectrum insecticides, which will result in more sustainable soybean production.

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## Introduction

Soybean, *Glycine max* (L.) Merrill, was first cultivated in China; however, the United States has become one of the largest soybean-producing countries (Qui and Chang 2010). In 2016, approximately 34 million hectares of soybean were planted in the United States (NASS 2016) with 80% of the soybean production occurring in the North-Central Region (USDA ERS 2016). Among all of the insect pests that occur in soybean, *Aphis glycines* (Hemiptera: Aphididae) Matsumura, is the most economically important in the United States (Ragsdale et al. 2011). *Aphis glycines*, commonly known as the soybean aphid, was first described in Japan in 1917 (GBIF 2016) and belongs to the largest genus of Aphididae with more than 400 species (Blackman and Eastop 2000). *Aphis glycines* is widely distributed in soybean growing regions throughout east and southeast Asia (Ragsdale et al. 2004) and was first detected in the United States in 2000 in Wisconsin (Wedberg et al. 2001). By 2009, *A. glycines* was found in 30 states and three Canadian provinces (Ragsdale et al. 2011).

*Aphis glycines* is a heteroecious and holocyclic species, meaning the aphid alternates between two different host plants, with buckthorn, *Rhamnus* spp. (L.), as the primary host and soybean as the secondary host, and has both sexual and asexual morphs (Dixon 1973, Ragsdale et al. 2004). Due to the ability of *A. glycines* to reproduce asexually on soybean, under optimal environmental conditions the population can increase rapidly (McCornack et al. 2004) and can cause damage to soybean (Ragsdale et al. 2007, Beckendorf et al. 2008). *Aphis glycines* has piercing-sucking mouthparts, which are used to extract nutrients from soybean plants. Extensive feeding from *A. glycines* can

cause economic damage by reducing soybean plant height, pod number, seed quality and size (Beckendorf et al. 2008). In addition to direct feeding injury, *A. glycines* is capable of transmitting plant pathogens (Clark and Perry 2002) and promoting the growth of sooty mold, which may reduce the soybean plant ability to photosynthesize (Hirano et al. 1996, Wedberg et al. 2001). The direct and indirect damages exhibited by *A. glycines* can cause yield decreases as high as 40% (Ragsdale et al. 2011).

Since the arrival of *A. glycines*, the soybean pest management regime has changed by increased scouting efforts and pesticide applications (Ragsdale et al. 2011). These changes in soybean practices consequently increased soybean production cost (Song et al. 2006). Therefore, there has been an extensive amount of research to improve *A. glycines* management through integrated pest management (IPM). Integrated pest management can be loosely defined as combining different techniques that will minimize control cost and reduce pest damage (Gray et al. 2009). These different control techniques include but are not limited to, resistant crop varieties, cultural practices, biological control, and species-specific chemical control (Gray et al. 2009).

The predominant method for managing *A. glycines* has been application of foliar insecticides (Hodgson et al. 2012). Ragsdale et al. (2007) found that the economic threshold for *A. glycines* is 250 aphids per plant with 80% of the soybean field infested and the population increasing. At the economic threshold, an insecticide application should be made to prevent densities from reaching the economic injury level of 674 aphids per plant (Higley and Pedigo 1996, Ragsdale et al. 2007). When aphid populations reach the economic injury level, the cost of treatment will be equivalent to the value of

yield loss (Higley and Pedigo 1996). The development of an economic threshold to protect yield loss and reduced prophylactic sprays has had net benefit of USD \$1.34 billion between 2003 and 2017 (Song and Swinton 2009).

In conjunction with the economic threshold, further research to reduce foliar applications of insecticides has focused on host plant resistance. Host plant resistance is a heritable ability of plants to resist arthropod pests (Smith 2005). Host plant resistance in soybean is currently being researched as part of a management program for *A. glycines* and many soybean lines have been identified as having resistance to *A. glycines* (Hanson et al. 2016). However, soybean varieties with known aphid-resistance are currently of limited availability for commercial use (Hesler et al. 2013).

The use of biological control is another important component of IPM (Naranjo et al. 2015). Classical biological control, also known as importation biological control, is management of an invasive pest by introducing natural enemies from the native range of the pest (DeBach 1964). Different classical biological control agents, particularly parasitoids, have been researched extensively for release against *A. glycines* (Heimpel et al. 2004, Wyckhuys et al. 2009, APHIS 2016). Though the exotic natural enemies are able to suppress *A. glycines* populations below economic levels in the native range (Heimpel et al. 2004, Wu et al. 2004), their ability to establish in North America can be difficult due to environmental interference (Hall and Ehler 1979, Grevstad 1999, Ragsdale et al. 2011).

In addition to foreign exploration for natural enemies, researchers have identified many different natural enemies attacking *A. glycines* in North America (Rutledge et al.

2004, Nielsen and Hajek 2005, Kaiser et al. 2007, Ragsdale et al. 2011). Exclusion cage studies have shown that natural enemies are important for suppressing *A. glycines* populations (Fox et al. 2004, Desneux et al. 2006, Costamagna et al. 2007, Miao et al. 2007) and that soybean fields with diverse landscapes typically have lower *A. glycines* and higher natural enemy densities (Gardiner et al. 2009, Noma et al. 2010). Although research has shown that natural enemies are important for suppressing *A. glycines* population, *A. glycines* densities can reach economically damaging levels and require insecticide application (Johnson et al. 2009, Tran et al. 2016).

Therefore, the focus of this thesis is to investigate IPM options to better integrate chemical and biological controls for *A. glycines* management. In Chapter 1, I investigate a new selective insecticide, sulfoxaflor, for its ability to control *A. glycines* population and reduce non-targeted impacts on natural enemies of *A. glycines*. In Chapter 2, I develop sampling plans for key predators of *A. glycines*, because this is an important step toward incorporating natural enemies into pest management decision making. The results from my thesis will provide information to improve IPM by integrating natural enemies into *A. glycines* pest management to create a more sustainable soybean production.

**Chapter I: Potential for Sulfoxaflor to Improve Conservation Biological Control of  
*Aphis glycines* (Hemiptera: Aphididae) in Soybean**

## Summary

Soybean aphid, *Aphis glycines* Matsumura, is one of the most important insect pests of soybean in the north central United States. Management of *A. glycines* currently relies on applications of broad-spectrum insecticides. However, broad-spectrum insecticides can negatively impact the natural enemies associated with aphids. Selective insecticides, on the other hand, are promising control tactics for reducing the negative impact of insecticide applications. Here, we compared the effects of sulfoxaflor (a new selective insecticide) and broad-spectrum insecticides on *A. glycines* and predators in a two-year field experiment. We sampled *A. glycines* and aphid predator populations using visual whole-plant inspection. In addition, sweep-net sampling was performed to monitor predator populations. To evaluate the toxicity of the insecticides on predator populations, laboratory bioassays were performed on *Hippodamia convergens* Guérin-Ménéville, *Orius insidiosus* (Say), and *Chrysoperla rufilabris* (Burmeister). Field results showed that sulfoxaflor was as effective as the broad-spectrum insecticide in suppressing soybean aphid populations and was less impactful on predator populations. The laboratory bioassays showed that sulfoxaflor was moderately harmful to *O. insidiosus*, harmless to slightly harmful to *H. convergens*, and harmless to *C. rufilabris*. These studies suggest that sulfoxaflor holds promise for improving integration of chemical and biological controls for *A. glycines* management.



## Introduction

Soybean, *Glycine max* (L.) Merrill, is an important field crop in the United States with approximately 33,600,000 ha harvested in 2016 (NASS 2016). Among the most important soybean pests in the north central United States is the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) (Ragsdale et al. 2011). *Aphis glycines* causes up to 40% yield loss (Ragsdale et al. 2007) and has increased production costs by US \$25 - \$50 per ha since its invasion in 2000 (Hodgson et al. 2012). Current management practices for *A. glycines* rely on scouting and threshold-based applications of broad-spectrum insecticides, such as pyrethroids and organophosphates (Tilmon et al. 2011, Hodgson et al. 2012) However, broad-spectrum insecticides can negatively impact natural enemies (Stuebaker and Kring 2003, Desneux et al. 2007, Roubos et al. 2014). Natural enemies are important for preventing and suppressing outbreaks of *A. glycines* (Rutledge et al. 2004, Fox et al. 2005, Costamagna et al. 2008). In North America, over 40 species of predators and parasitoids that attack *A. glycines* have been identified (Ragsdale et al. 2011).

Removal of natural enemy populations can lead to resurgence or replacement of pest populations (Hardin et al. 1995, Dutcher 2007, Pedigo and Rice 2009). To reduce the likelihood of such adverse impacts of insecticide use, selective insecticides are needed for management of *A. glycines*. Selective insecticides are toxic to the targeted pest, but relatively less toxic to natural enemies (Ripper et al. 1951, Weinzierl 2009). Use of selective insecticides is one method of conservation biological control (Bartlett 1964, Pedigo and Rice 2009). Biological control and selective insecticides have been shown to be compatible tactics in integrated pest management programs (Giles and Obrycki 1997,

Naranjo et al. 2002, Weinzierl 2009) and can minimize the likelihood of pest resurgence (Johnson and Tabashnik 1999), prevent pest replacement (Grafton-Cardwell et al. 2008), and possibly reduce the number of insecticide applications (Naranjo 2001, Hutchison et al. 2004). In field studies, Ohnesorg et al. (2009) and Varenhorst and O'Neal (2012) found that some selective insecticides suppressed *A. glycines* populations, while having intermediate effects on the natural enemy populations. Despite this potential, selective insecticides have not been widely adopted for *A. glycines* management.

Sulfoxaflor is a systemic insecticide within the sulfoxamine insecticide class (Babcock et al. 2010, Sparks et al. 2013) and is under consideration as an alternative to broad-spectrum insecticides. The mode of action of sulfoxaflor is similar to that of the neonicotinoid insecticides; however, it is structurally different (Sparks et al. 2013), has novel activity on the nicotinic acetylcholine receptor (Babcock et al. 2010, Watson et al. 2011, Zhu et al. 2010, Cutler et al. 2013) and does not show cross resistance with neonicotinoid insecticides (Babcock et al. 2010, Zhu et al. 2010, Longhurst et al. 2013, Herron et al. 2014). Sulfoxaflor is effective against a broad range of piercing-sucking insect pests such as whitefly, *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) (Longhurst et al. 2013), tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Siebert et al. 2012), the San Jose scale, *Diaspidiotus perniciosus* Comstock (Sazo et al. 2015) and brown planthopper, *Nilaparvata lugens* (Stål) (Ghosh et al. 2013).

Sulfoxaflor can reduce *A. glycines* densities to levels similar to those of broad-spectrum insecticides (e.g., chlorpyrifos and  $\lambda$ -cyhalothrin) (Dana et al. 2012). However, Hodgson and VanNostrand (2012) observed reduced effectiveness when lower rates of sulfoxaflor were used against *A. glycines*. Further work is needed to evaluate the

effectiveness and utilization of sulfoxaflor for *A. glycines* management. In addition, impacts of sulfoxaflor on natural enemies of *A. glycines* remain to be studied. Sulfoxaflor has shown potential for conserving natural enemies in rice (Ghosh et al. 2013) and was found to be compatible with *Chrysoperla carnea* (Stephens) larvae and adults, but toxic to *Adalia bipunctata* (L.) larvae (Garzón et al. 2015). Therefore, we evaluated the impact of sulfoxaflor on *A. glycines* and key predators of this pest through field experiments. In addition, as an initial step toward characterizing lethal effects of sulfoxaflor, laboratory experiments were performed with representative species of three common taxa of aphid predators exposed to insecticide residues (e.g., US EPA 2016). Results from this study will provide insight on the selectivity of sulfoxaflor and could lead to improved conservation biological control for *A. glycines*.

## **Materials and Methods**

### **Field experiment**

Two field experiments were conducted at the University of Minnesota Outreach, Research and Education (UMore) Park, University of Minnesota, near Rosemount, MN, in 2013 and 2014. On 31 May 2013, soybean seeds (variety: NK S19-A6) were sown 3.20 cm deep at a seeding rate of 370,000 seeds per ha. On 27 May 2014, soybean seeds (variety: S17-B3) were sown 3.80 cm deep at a seeding rate of 409,000 seeds per ha. In both years, spacing between rows was 76.20 cm. In 2013, plots were 6.10 m wide by 9.10- m long. In 2014, plots were 3.10 m wide by 9.10 m long. Plots within replications were separated by 1.50 m of bare ground and replications were separated by 3.10 m of bare ground. Plots were maintained using standard production practices (Bennet et al.

1999), but without insecticide input other than the treatments described below. In both years, plots were arranged in a randomized complete block design with five treatments and four replications. In 2013, the five treatments were: 1) sulfoxaflor at the low labeled rate (25.78 g a.i./ha, Transform, Dow AgroSciences, LLC, Indianapolis, IN), 2) sulfoxaflor at the high labeled rate (34.75 g a.i./ha), 3)  $\lambda$ -cyhalothrin at the low labeled rate (17.46 g a.i./ha, Warrior II, Syngenta Crop Protection, Inc., Basel, Switzerland), 4)  $\lambda$ -cyhalothrin at the high labeled rate (29.10 g a.i./ha), and 5) untreated check. In 2014, the five treatments were: 1) sulfoxaflor at the high labeled rate (34.75 g a.i./ha), 2)  $\lambda$ -cyhalothrin + sulfoxaflor at the low labeled rate (22.95 g a.i./ha and 15.30 g a.i./ha, respectively, Seeker, Dow AgroSciences, LLC, Indianapolis, IN), 3)  $\lambda$ -cyhalothrin + sulfoxaflor at the high labeled rate (28.41 g a.i./ha and 18.94 g a.i./ha, respectively), 4)  $\lambda$ -cyhalothrin at the high labeled rate (29.10 g a.i./ha), and 5) untreated check. Insecticides were applied on 13 August 2013 and 12 August 2014 at R4 and R2 soybean growth stages, respectively (Fehr and Cavines 1977). Insecticide applications were made with a CO<sub>2</sub>-pressurized backpack sprayer using a 3.10-m boom with eight nozzles (XR-Teejet 8002 flat fan, with no screen). The sprayer was calibrated to deliver 187 liters/ha at 275.80 kPa.

*Aphis glycines* and aphid predators were sampled by nondestructive, visual whole-plant inspection of randomly-selected plants. Counts of *A. glycines* were recorded on five plants per plot on 12, 16 and 20 August 2013 and three plants per plot on 27 August and 3 September 2013. Counts of aphid predators were recorded on ten plants per plot on 12, 16, 20 and 27 August and from eight plants per plot on 3 September 2013. Counts of *A. glycines* were recorded on five plants per plot on 8, 15, 18, and 26 August

and 5 September 2014. Counts of aphid predators were recorded on ten plants per plot on 8, 15, 18 and 26 August and 5 September 2014. In addition to visual whole-plant inspection, one set of 20 pendulum-style sweeps with a 39-cm-diameter sweep net were taken from each plot on 12, 16, 21 August, and 3 September 2013 and 8, 15, 18, 26 August and 5 September 2014. In 2013, sweeps samples were taken from two interior rows not sampled for visual counts. In 2014, sweeps samples were taken from the same rows sampled for visual counts, but were taken 3 to 4 h after visual counts. Contents of the sweep nets were transferred in the field to plastic zipper-locking bags, which were stored in the freezer for later identification in the laboratory.

Whole-plant samples were averaged per plant for each plot on each sample date. Insect abundance over time [i.e., cumulative insect-days (CID)] was calculated per plot across sample dates within each year for *A. glycines* (apterae + alates) and the three most abundant taxa of aphid predators [Coccinellidae (larvae and adults of all species), *Orius* spp. (nymphs and adults of all species), and Chrysopidae (larvae and adults of all species)]. CID was calculated using the following equation adapted from Hanafi et al. (1989):

$$CID = \sum_{i=1}^n \left[ \left( \frac{x_i + x_{i-1}}{2} \right) \right] \times (t_i - t_{i-1})$$

where,  $n$  is the number of sample dates,  $x_i$  is the mean number of insects per plant on sample date  $i$ ,  $x_{i-1}$  is the mean number of insects per plant on the preceding sample date, and  $t_i - t_{i-1}$  is the number of days between two consecutive sample dates. For Coccinellidae, *Orius* spp., and Chrysopidae collected via sweep net sampling,  $x_i$  and  $x_{i-1}$ , was the total number of predators per 20 sweeps on sample date  $i$  and on the

preceding sample date.  $\text{Log}(x+1)$ -transformed CID based on whole-plant counts or sweep-net sampling were analyzed using ANOVA on the last evaluation date (i.e., 13 Aug 2013 and 12 Aug 2014) and means were separated with Tukey's HSD (R Core Team 2015).

### **Laboratory experiments**

Bioassays were performed on adult *Hippodamia convergens* Guérin-Ménéville, adult *Orius insidiosus* (Say), and third-instar *Chrysoperla rufilabris* (Burmeister) at the University of Minnesota, Saint Paul, MN. *Hippodamia convergens* and *C. rufilabris* were purchased through Arbico Organics (Oro Valley, AZ) and *O. insidiosus* was purchased through Beneficial Insectary (Redding, CA). Insects were shipped overnight and used within 6 h of arrival except for *C. rufilabris*, which was allowed to develop from first-instar to the third-instar under conditions described below. Predators were evaluated in separate randomized complete block design experiments. All treatments described below were delivered using a motorized spray chamber with a XR-Teejet 8002 flat-fan nozzle calibrated to deliver approximately 233.85 liters/ha of each product and water on to the interior of the lids and bottoms of plastic petri dishes. The treatments were allowed to dry for 1 h before transferring insects to the treated petri dishes using a clean fine-tipped (size 0) paintbrush. Insects were kept in the treated petri dishes for 24 h before being transferred to untreated petri dishes. After transferred to untreated petri dishes, insects were provisioned with *Ephestia kuehniella* eggs *ad libitum* and water through moistened floral foam. Food and water was replenished every 2 d. Insects were held in a growth chamber at 25°C, 75% RH, and a photoperiod of 16:8 h (L:D). Insects were checked over time and during each observation insects were prodded with a fine-tipped (size 0)

paintbrush and rated as alive (i.e., ability to walk), moribund (i.e., movement, but uncoordinated), or dead (i.e., no movement). Dead insects were removed from the petri dishes.

Two experiments were performed to evaluate insecticide residual toxicity to *H. convergens* adults. For these two experiments, treatments were applied to 100×15 mm plastic petri dishes and each petri dish contained five individuals. The first experiment with *H. convergens* consisted of five treatments and three replications with 20 individuals per replication (i.e., 60 individuals total per treatment). Treatments were: 1) sulfoxaflor at the low labeled rate (25.78 g a.i./ha, Transform, Dow AgroSciences, LLC, Indianapolis, Indiana), 2) sulfoxaflor at the high labeled rate (34.75 g a.i./ha), 3) λ-cyhalothrin at the low labeled rate (17.46 g a.i./ha, Warrior II, Syngenta Crop Protection, Inc., Basel, Switzerland), 4) λ-cyhalothrin at the high labeled rate (29.10 g a.i./ha), and 5) untreated check. The second experiment with *H. convergens* consisted of four treatments and four replications with 10 individuals per replication (i.e., 40 individuals total per treatment). The treatments were 1) sulfoxaflor at the high labeled rate (34.75 g a.i./ha, 2) λ-cyhalothrin + sulfoxaflor at the high labeled rate (28.41 g a.i./ha and 18.94 g a.i./ha, respectively, Seeker, Dow AgroSciences, LLC, Indianapolis, IN), 3) λ-cyhalothrin at the high labeled rate (29.10 g a.i./ha) and 4) untreated check. For the two experiments, insects were checked 1 h post exposure to treatments and every 24 h for 7 d.

To evaluate insecticide residual toxicity to *O. insidiosus* adults, four treatments and three replications were used. Treatments were applied to 100x15-mm petri dishes with approximately 45 small plastic beads (Bead Landing™ Crafting Beads, Irving, TX). The beads were treated in a similar matter and were added to the petri dish to increase

surface area to reduce cannibalism. Each petri dish contained eight individuals with 32 individuals per replication (i.e., 96 individuals total per treatment). The four treatments were: 1) sulfoxaflor at the high labeled rate (34.75 g a.i./ha), 2)  $\lambda$ -cyhalothrin + sulfoxaflor at the high labeled rate (28.41 g a.i./ha and 18.94 g a.i./ha, respectively), 3)  $\lambda$ -cyhalothrin at the high labeled rate (29.10 g a.i./ha), and 4) untreated check. Insects were checked at 1, 15 and 24 h post exposure to treatments.

To evaluate insecticide residual toxicity on *C. rufilabris*, four treatments and three replications were used. Treatments were applied to 60×15-mm petri dishes and each petri dish contained one individual with 10 individuals per replication (i.e., 30 individuals total per treatment). The treatments were: 1) sulfoxaflor at the high labeled rate (34.75 g a.i./ha), 2)  $\lambda$ -cyhalothrin + sulfoxaflor at the high labeled rate (28.41 g a.i./ha and 18.94 g a.i./ha, respectively), 3)  $\lambda$ -cyhalothrin at the high labeled rate (29.10 g a.i./ha), and 4) untreated check. Insects were checked at 1 h post exposure to treatments and every 24 h for 7 d.

The proportion of *H. convergens* and *C. rufilabris* surviving (i.e., those rated as alive) at 168 h (7 d) post exposure to treatments and the proportion of *O. insidiosus* surviving at 24 h post exposure to treatments were arcsine square-root transformed prior to analysis. The response variables were analyzed using ANOVA and means were separated with Tukey's HSD (R Core Team 2015).

## **Results**

### **Field experiment**

In 2013, the mean CID of *A. glycines* in the untreated check from whole-plant sampling was about six times greater than that of the insecticide treatments, but not



different among insecticide treatments ( $F = 70.35$ ;  $df = 4, 12$ ;  $P < 0.0001$ ) (Figure 1.1A). Across sampling methods (i.e., whole-plant sampling and sweep-net sampling), sample dates, and treatments, Coccinellidae (larvae + adults), *Orius* spp. (nymphs + adults), and Chrysopidae (larvae + adults) comprised 56.8%, 18.2%, and 3.2%, respectively, of the total generalist aphid predators (Table 1.1). For whole-plant sampling, mean CID of Coccinellidae were significantly reduced in the insecticide treatments compared to the untreated check; however, mean CID for both labeled rates of sulfoxaflor were significantly higher than both labeled rate of  $\lambda$ -cyhalothrin ( $F = 37.84$ ;  $df = 4, 12$ ;  $P < 0.0001$ ) (Figure 1.2A). For whole-plant sampling, the high labeled rate of  $\lambda$ -cyhalothrin was the only treatment that significantly reduced mean CID of *Orius* spp. compared to the untreated check ( $F = 3.68$ ;  $df = 4, 12$ ;  $P = 0.0353$ ) (Figure 1.2C). Mean CID of Chrysopidae based on whole-plant sampling did not differ among treatments ( $F = 0.76$ ;  $df = 4, 12$ ;  $P = 0.5693$ ) (Figure 1.2E). For sweep-net sampling, the high labeled rate of  $\lambda$ -cyhalothrin was the only treatment that significantly reduced mean CID of Coccinellidae ( $F = 5.42$ ;  $df = 4, 12$ ;  $P = 0.0098$ ) (Figure 1.2B) and *Orius* spp. ( $F = 3.94$ ;  $df = 4, 12$ ;  $P = 0.0287$ ) (Figure 1.2D). Mean CID of Chrysopidae based on sweep-net sampling did not differ significantly among treatments ( $F = 1.64$ ;  $df = 4, 12$ ;  $P = 0.2274$ ) (Figure 1.2F).

In 2014, mean CID of *A. glycines* in the untreated check from whole-plant sampling was about seven times greater than that of the insecticide treatments, but mean CID was not different among insecticide treatments ( $F = 15.14$ ;  $df = 4, 12$ ;  $P = 0.0001$ ) (Figure 1.1B). Across sampling methods (i.e., whole-plant sampling and sweep-net sampling), sample dates, and treatments, Coccinellidae (larvae + adults), *Orius* spp. (nymphs + adults), and Chrysopidae (larvae + adults) comprised 51.1%, 26.3% and 9.5%,

respectively, of generalist aphid predators (Table 1.1). For whole-plant sampling, both labeled rates of the mixture  $\lambda$ -cyhalothrin + sulfoxaflor and the high labeled rate of  $\lambda$ -cyhalothrin significantly reduced mean CID of Coccinellidae compared to the untreated check ( $F = 9.39$ ;  $df = 4, 12$ ;  $P = 0.0011$ ) (Figure 1.3A). For whole-plant sampling, sulfoxaflor treatment did not reduce mean CID of Coccinellidae compared to the untreated check (Figure 1.3A). For whole-plant sampling, insecticide treatments did not reduce mean CID of *Orius* spp. ( $F = 0.97$ ;  $df = 4, 12$ ;  $P = 0.4591$ ) (Figure 1.3C) and Chrysopidae ( $F = 1.36$ ;  $df = 4, 12$ ;  $P = 0.3040$ ) compared to the untreated check (Figure 1.3E). For sweep-net sampling, both labeled rates of the mixture of  $\lambda$ -cyhalothrin + sulfoxaflor and the high labeled rate of  $\lambda$ -cyhalothrin significantly reduced mean CID of Coccinellidae compared to the untreated check ( $F = 7.86$ ;  $df = 4, 12$ ;  $P = 0.0024$ ) (Figure 1.3B). For sweep-net sampling, all insecticide treatments reduced mean CID of *Orius* spp. compared to the untreated check ( $F = 13.47$ ;  $df = 4, 12$ ;  $P = 0.0002$ ) (Figure 1.3D). Among the insecticide treatments, sulfoxaflor was the only treatment with mean CID of *Orius* spp. significantly greater than  $\lambda$ -cyhalothrin (Figure 1.3D). Mean CID of Chrysopidae based on sweep-net sampling did not differ significantly among treatments ( $F = 2.25$ ;  $df = 4, 12$ ;  $P = 0.1237$ ) (Figure 1.3F).

### **Laboratory experiments**

In the first experiment with *H. convergens* adults, survivorship was significantly reduced 168 h after exposure to both rates of  $\lambda$ -cyhalothrin compared to the untreated check and both rates of sulfoxaflor ( $F = 14.13$ ;  $df = 4, 10$ ;  $P = 0.0004$ ) (Figure 1.4A). In the second experiment, survivorship of *H. convergens* was significantly reduced 168 h after exposure to the high labeled rate of  $\lambda$ -cyhalothrin and the high labeled rate of the

mixture of  $\lambda$ -cyhalothrin + sulfoxaflor ( $F = 206.16$ ;  $df = 3, 12$ ;  $P < 0.0001$ ) (Figure 1.4B). In the bioassay with *O. insidiosus* adults, survivorship was significantly reduced 24 h after exposure to all insecticide treatments compared to the untreated check ( $F = 125.04$ ;  $df = 3, 8$ ;  $P < 0.0001$ ) (Figure 1.4C). In the bioassay with *C. rufilabris* larvae, survivorship was significantly reduced 168 h after exposure to the high labeled rate of  $\lambda$ -cyhalothrin and the high labeled rate of the mixture of  $\lambda$ -cyhalothrin + sulfoxaflor ( $F = 20.04$ ;  $df = 3, 8$ ;  $P = 0.0004$ ) (Figure 1.4D).

## Discussion

There is a paucity of literature on the impact of sulfoxaflor on natural enemies. This study provides the first evaluation of the compatibility of this novel selective insecticide with natural enemies for management of *A. glycines* in soybean production. Sulfoxaflor received registration in the United States for use in soybean in 2013. However, registration was canceled in 2015 (Lewis 2015). As sulfoxaflor is being evaluated for use on soybean, further data on the effects of sulfoxaflor on soybean insect pests and their associated natural enemies may be necessary. Under field conditions, sulfoxaflor provided levels of *A. glycines* suppression equivalent to those of a standard broad-spectrum insecticide (i.e.,  $\lambda$ -cyhalothrin) in 2013 and 2014 (Figure 1.1). The effectiveness of sulfoxaflor on *A. glycines* was also observed by Dana et al. (2012), but such efficacy was not observed by Hodgson and VanNostrand (2012). The difference in pest suppression exhibited by sulfoxaflor between these studies is likely due to differences in sulfoxaflor rates. Dana et al. (2012) and our study used a higher rate of sulfoxaflor compared to the rate used by Hodgson and VanNostrand (2012). Sulfoxaflor

effectiveness against *A. glycines* was expected because sulfoxaflor has proven effective against populations of other aphid species, such as the cotton aphid, *Aphis gossypii* Glover (Gore et al. 2013, Koo et al. 2014) the green peach aphid, *Myzus persicae* (Sulzer) (Tang et al. 2015), and the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Rodríguez-del-Bosque and Terán 2015). Additionally, neonicotinoid insecticides, which have a similar mode of action as sulfoxaflor, are commonly used for management of *A. glycines* (Magalhaes et al. 2008, Qu et al. 2015) and other aphid pest species (Nauen and Elbert 2003, Elbert et al. 2008, Joshi and Sharma 2009, Shi et al. 2011).

Other field studies found selective insecticides to have reduced impacts on predator populations compared to broad-spectrum insecticides (Koss et al. 2005, Ohnesorg et al. 2009, Varenhorst and O'Neal 2012). Our study showed that although mean CID for predators were generally lower in the insecticide treatments compared to the untreated check, sulfoxaflor was less impactful than  $\lambda$ -cyhalothrin on Coccinellidae and *Orius* spp. Counts of *A. glycines* predators and numbers of predatory species observed varied between whole-plant and sweep-net sampling (Table 1.1). This variation is likely due to differences between the sampling methods in effectiveness to capture the number and type (e.g., life stages, mobility of insects) of predators that are present in the field (Schmidt et al. 2008, Bannerman et al. 2015). Furthermore, differences in the number of predatory species recorded for each sampling method may have been due to predators being more readily identified to the genus or species levels in the laboratory (sweep-net samples) compared to in the field (whole-plant samples).

Field studies, like those described above, may confound the direct toxic effects of the insecticides on the predators with indirect effects of the insecticides on prey

abundance. Therefore, complementary laboratory studies are required to understand the direct contact toxicity of insecticides to natural enemies (Galvan et al. 2005). To classify the level of contact toxicity the insecticides had on the insects we followed the International Organization for Biological Control (IOBC) ratings for laboratory-based pesticides, which are harmless (i.e., <30% mortality), slightly harmful (i.e., 30-79% mortality), moderately harmful (i.e., 80-99% mortality), and harmful (i.e., >99% mortality) (Hassan et al. 1994). Based on the IOBC rating scale, the contact toxicity of sulfoxaflor residuals varied among *O. insidiosus*, *H. convergens*, and *C. rufilabris* (Figure 1.4).

Sulfoxaflor was moderately harmful to *O. insidiosus*, causing 96% mortality within 24 h (Figure 1.4C). The greater toxicity of sulfoxaflor to *O. insidiosus* compared to the other predators could be due to the closer phylogenetic relatedness of this predator to targeted pest groups. For example, Wanumen et al. (2016) found that sulfoxaflor was harmful to the predatory hemipteran, *Nesidiocoris tenuis* (Reuter). Sulfoxaflor is ineffective against non-hemipteran insects, such as the southern corn rootworm, *Diabrotica undecimpunctata* Barber, fruit fly, *Drosophila melanogaster* Meigan, yellow fever mosquito larvae, *Aedes aegypti* (L.), Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Babcock et al. 2010) and grape colaspis beetle, *Colaspis brunnea* (F.) (Alves et al. 2016). However, as described above, sulfoxaflor is effective against Hemiptera, to which *O. insidiosus* belongs. Although sulfoxaflor caused high mortality on *O. insidiosus*, the rate at which the mortality occurred was less compared to the insecticides that contained  $\lambda$ -cyhalothrin. Within 1 h of exposure, sulfoxaflor caused 25% mortality while insecticides with  $\lambda$ -cyhalothrin caused 86-98% mortality. The greater

impact of sulfoxaflor on *O. insidiosus* in the laboratory compared to the field may be due to the lack of pesticide-free refuges in the petri-dish bioassays performed in the laboratory. In a comparison of selective insecticides and broad-spectrum insecticides in petri-dish, greenhouse and field experiments, Studebaker and Kring (2003) proposed that the field and greenhouse bioassays provided areas to avoid the insecticides, whereas there was constant exposure to the insecticides in the petri dishes.

In the two laboratory bioassays performed on *H. convergens* adults, sulfoxaflor was in the harmless to slightly harmful range causing less than 35% mortality (Figures 1.4A and 1.4B). Similarly, Garzón et al. (2015) found that sulfoxaflor was harmless to adults of a different species of Coccinellidae, *A. bipunctata*, where there was 23% mortality 3 d after being exposed to the insecticide residuals (Garzón et al. 2015). In contrast to sulfoxaflor, the insecticide treatments that contained  $\lambda$ -cyhalothrin were moderately harmful to harmful to *H. convergens* adults, causing 80-100% mortality (Figures 1.4A and 1.4B). Likewise,  $\lambda$ -cyhalothrin caused high mortality of *H. convergens* adults in a laboratory assay performed by Pezzini and Koch (2015) and in other Coccinellidae species (Galvan et al. 2005, Jalali et al. 2009).

Sulfoxaflor was harmless to *C. rufilabris* third-instar larvae, causing 7% mortality (Figure 1.4D). Our results were comparable to the experiment described by Garzon et al. (2015) where sulfoxaflor was harmless to the third-instar larvae of *C. carnea*. Insecticides containing  $\lambda$ -cyhalothrin were slightly harmful to *C. rufilabris* larvae (Figure 1.4D). The higher mortality rates of *C. rufilabris* larvae treated with  $\lambda$ -cyhalothrin observed by Pezzini and Koch (2015) may have been due to their use of first-instar larvae compared to third-instars in the present study. The lower susceptibility of Chrysopidae larvae to

pyrethroid insecticides compared to other taxa of predators observed here is consistent with other studies (Pree et al. 1989, Medina et al. 2004, Pezzini and Koch 2015) and is due to Chrysopidae having a natural tolerance to pyrethroids associated with their high pyrethroid esterase(s) activity (Ishaayn and Casida 1981).

Natural enemies are important for preventing and suppressing *A. glycines* outbreaks (Rutledge et al. 2004, Fox et al. 2005, Costamagna et al. 2008, Ragsdale et al. 2011) and likely contribute to prevention of pest resurgence and replacement (e.g., Dutcher 2007). However, complete reliance on natural enemies is not sufficient to keep *A. glycines* below the economic injury level and application of foliar insecticides may be required to protect soybean yield (Hodgson et al. 2012). Sulfoxaflor was indeed effective against soybean aphid and had less impact on some predators than a broad-spectrum insecticide. However, when sulfoxaflor was combined with  $\lambda$ -cyhalothrin as a formulated mixture (i.e., Seeker) the selectivity was diminished and the impacts on predators were similar to  $\lambda$ -cyhalothrin.

Sulfoxaflor holds promise for better integration of chemical and biological controls for pests like *A. glycines*. Though sulfoxaflor was less impactful than broad-spectrum insecticides to predators of *A. glycines*, further work is needed to examine toxicity via other routes of exposure (e.g., oral exposure) and potential sublethal effects of this insecticide on predators. For example, Garzon et al. (2015) observed sublethal effects of sulfoxaflor on the ability of *A. bipunctata* larvae to pupate and emerge into adults, and the fertility decreased in *C. carnea*. Further characterization of the selectivity of sulfoxaflor will guide recommendations on how to better integrate chemical and biological controls for more sustainable soybean production. In addition, because

management of *A. glycines* in the north central United States currently relies primarily on foliar applications of only two modes of action (i.e., pyrethroid and organophosphate insecticides) (Hodgson et al. 2012), there is risk of *A. glycines* developing insecticide resistance. The availability of an additional effective insecticide of a different mode of action would improve insecticide rotations for *A. glycines* management and help postpone the development of insecticide resistance.



**Table 1.1:** Adjusted experiment-wide totals of mobile stages of aphid predators collected using visual whole-plant and sweep-net sampling in Rosemount, MN in 2013 and 2014.

Order/Family	Species	2013		2014	
		Whole-plant <sup>a</sup>	Sweep-net <sup>b</sup>	Whole-plant <sup>a</sup>	Sweep-net <sup>b</sup>
<b>Araneae</b>	Unidentified <sup>c</sup>	0.1	0.0	0.3	2.0
<b>Opiliones</b>	Unidentified <sup>c</sup>	0.0	0.8	1.7	1.3
<b>Coleoptera</b>					
Coccinellidae	<i>Coccinella septempunctata</i> <sup>c</sup>	0.0	0.0	1.4	2.1
	<i>Cycloneda munda</i> <sup>c</sup>	0.0	0.1	0.1	0.1
	<i>Harmonia axyridis</i> <sup>c</sup>	5.4	9.5	5.0	8.9
	<i>Hippodamia convergens</i> <sup>c</sup>	0.9	3.0	0.1	0.1
	<i>Hippodamia parenthesis</i> <sup>c</sup>	0.1	0.0	0.1	0.1
	<i>Hippodamia tredecimpunctata</i> <sup>c</sup>	0.0	0.1	0.0	0.0
	<i>Hippodamia variegata</i> <sup>c</sup>	0.4	1.3	1.6	6.2
	Larvae	24.9	19.5	15.4	24.8
<b>Diptera</b>					
Cecidomyiidae	<i>Aphidoletes aphidimyza</i> larvae	8.8	0.0	3.1	0.1
Syrphidae	<i>Allograpta obliqua</i> <sup>c</sup>	*	*	*	0.1
	<i>Eupeodes americanus</i> <sup>c</sup>	*	*	*	0.1
	<i>Sphaerophoria</i> sp. <sup>c</sup>	*	*	*	0.1
	<i>Toxomerus marginatus</i> <sup>c</sup>	*	*	*	0.2
	Unidentified <sup>d</sup>	13.1	0.9	1.7	0.2
<b>Hemiptera</b>					
Anthocoridae	<i>Orius</i> spp. <sup>e</sup>	10.1	10.8	7.8	26.2
Berytidae	Unidentified <sup>e</sup>	0.0	0.0	0.0	0.3
Nabidae	<i>Nabis</i> spp. <sup>e</sup>	0.3	1.2	0.3	1.7

Order/Family	Species	2013		2014	
		Whole-plant <sup>a</sup>	Sweep-net <sup>b</sup>	Whole-plant <sup>a</sup>	Sweep-net <sup>b</sup>
<b>Neuroptera</b>					
Chrysopidae	<i>Chrysopa</i> spp. <sup>c</sup>	*	*	*	1.2
	<i>Chrysoperla plorabunda</i> <sup>c</sup>	*	*	*	4.6
	Unidentified <sup>d</sup>	1.9	1.8	2.5	4.0
Hemerobiidae	<i>Hemerobius stigma</i> <sup>c</sup>	*	*	*	0.2
	<i>Micromus</i> spp. <sup>c</sup>	*	*	*	1.2
	Unidentified <sup>d</sup>	0.0	0.0	0.9	1.9
<b>Total</b>		66.0	49.0	42.0	87.0

<sup>a</sup> Total number of individuals adjusted to counts per 100 plants

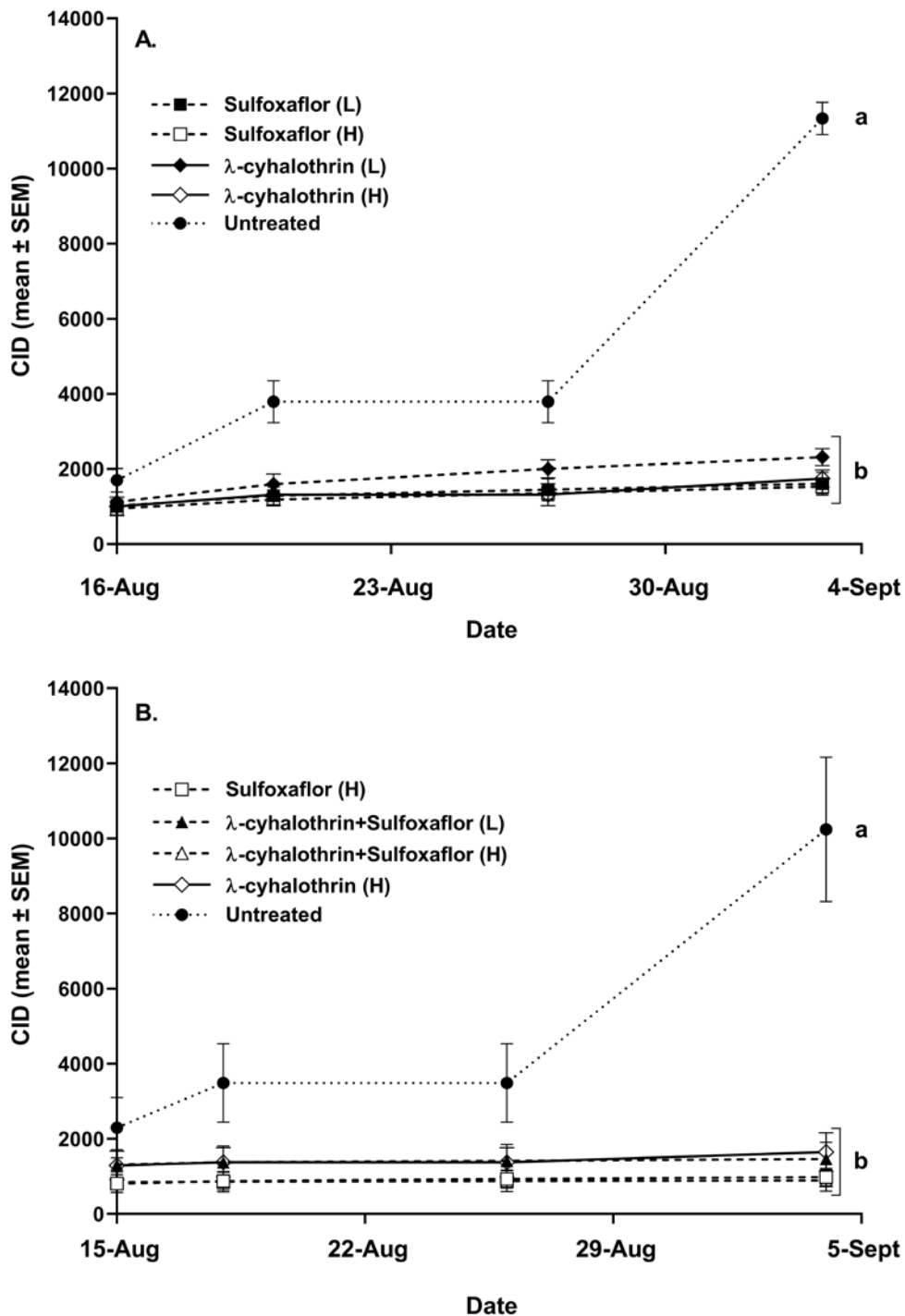
<sup>b</sup> Total number of individuals adjusted to counts per 100 sweeps

<sup>c</sup> Species total include only adults

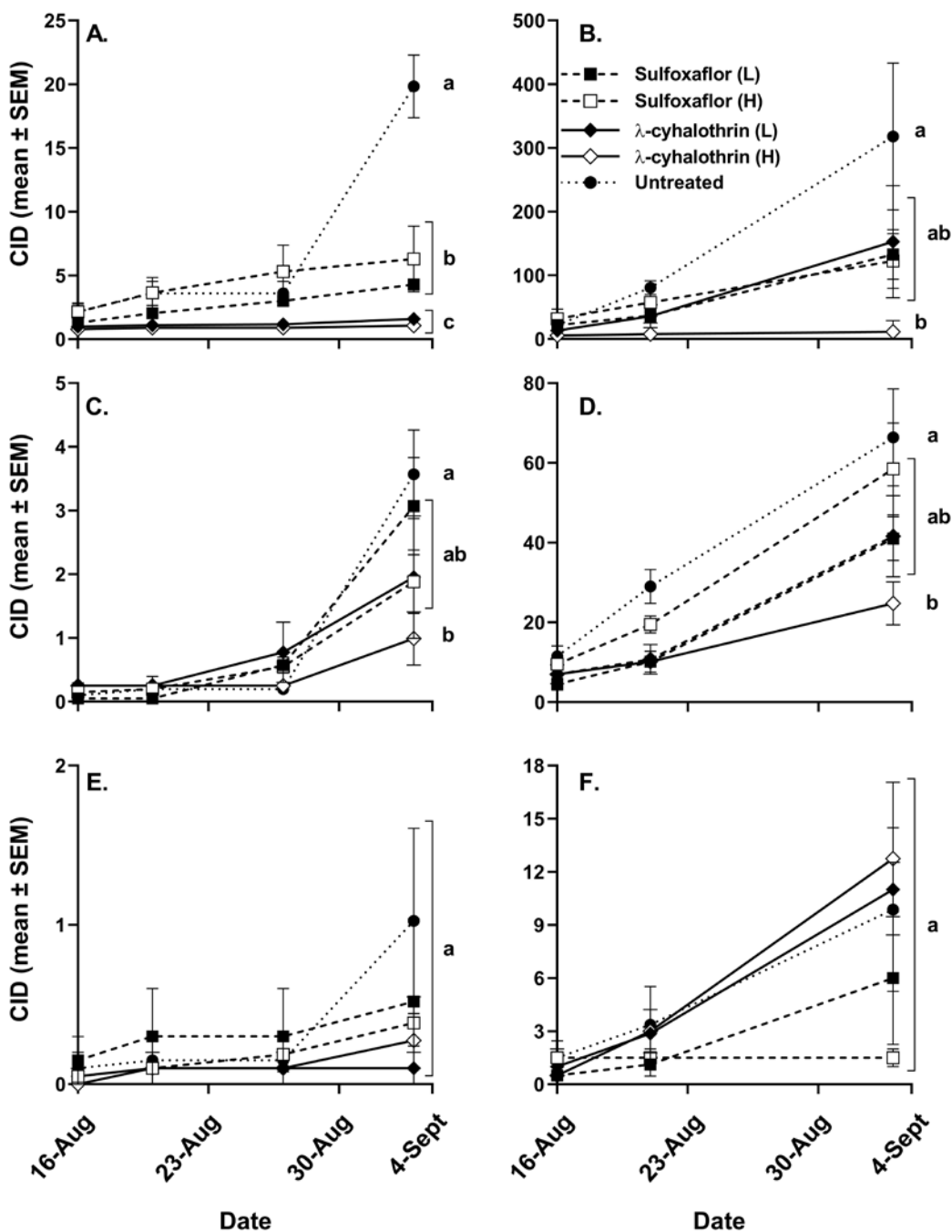
<sup>d</sup> Species total includes larvae and adults

<sup>e</sup> Species total includes nymphs and adults

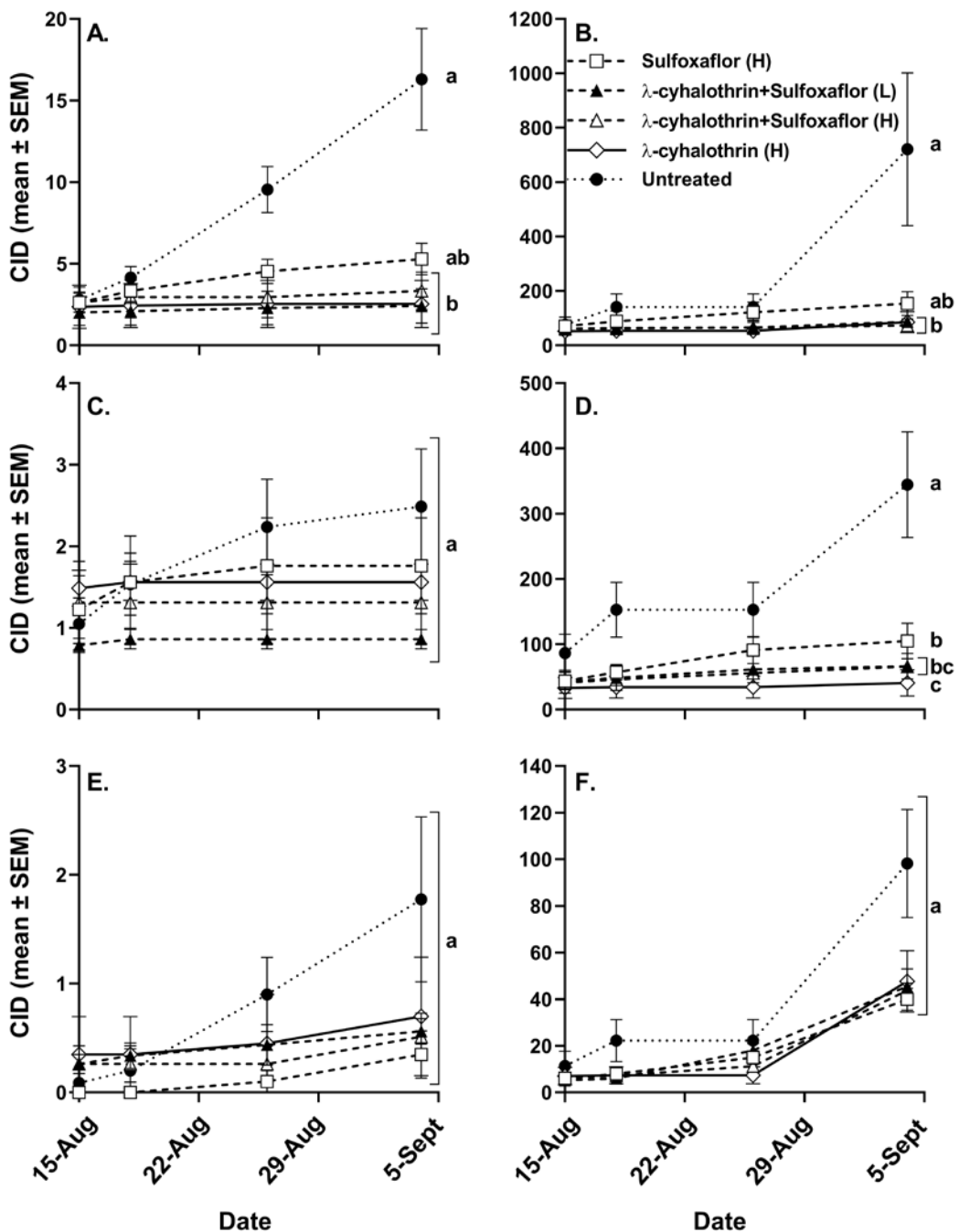
\* The species was not recorded



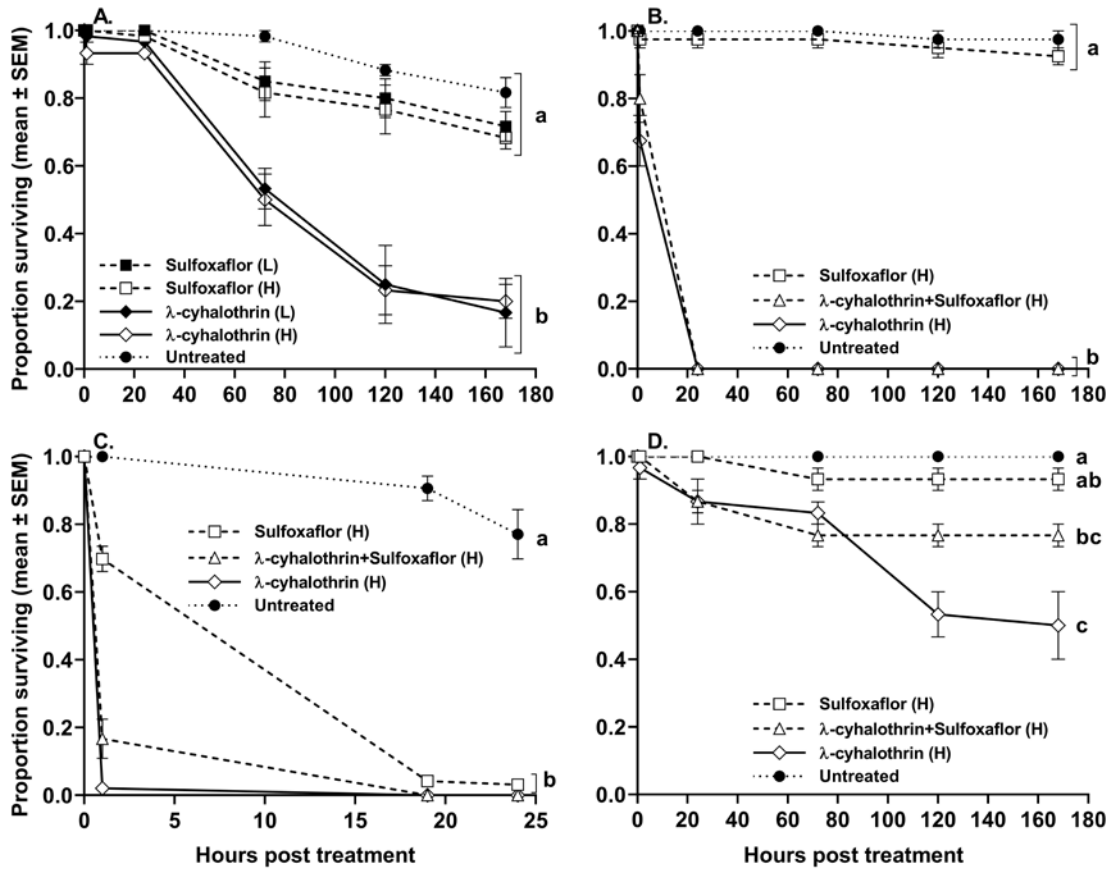
**Figure 1.1:** Cumulative insect days (CID) of *Aphis glycines* under field conditions in Rosemount, MN, in 2013 (A) and 2014 (B). Low and high labeled rates of treatments are represented by L and H, respectively. Insecticides were applied on 13 August 2013 and 12 August 2014. Treatment means with the same letter are not significantly different (Tukey HSD,  $P > 0.05$ ). A bracket represents more than one treatment mean associated with the letter.



**Figure 1.2:** Cumulative insect days (CID) from visual whole-plant samples (A, C, and E) and sweep-net samples (B, D, and F) for larvae + adults of Coccinellidae (A and B), nymphs + adults *Orius* spp. (C and D), and larvae + adults Chrysopidae (E and F) under field conditions in Rosemount, MN in 2013. Low and high labeled rates of treatments are represented by L and H, respectively. Insecticides were applied on 13 August. Treatment means with the same letter are not significantly different (Tukey HSD,  $P > 0.05$ ). A bracket represents a more than one of treatment mean associated with the letter.



**Figure 1.3:** Cumulative insect days (CID) from visual whole-plant samples (A, C, and E) and sweep-net samples (B, D, and F) for larvae + adults Coccinellidae (A and B), nymphs + adults *Orius* spp. (C and D), and larvae + adults of Chrysopidae (E and F) under field conditions in Rosemount, MN in 2014. Low and high labeled rates of treatments are represented by L and H, respectively. Insecticides were applied on 12 August. Treatment means with the same letter are not significantly different (Tukey HSD,  $P > 0.05$ ). A bracket represents a more than one of treatment mean associated with the letter.



**Figure 1.4:** Survival of *Hippodamia convergens* adults (A and B), *Orius insidiosus* adults (C), and *Chrysoperla rufilabris* larvae (D) under laboratory conditions. Low and high labeled rates of treatments are represented by L and H, respectively. Treatments with the same letter are not significantly different (Tukey HSD,  $P > 0.05$ ). A bracket represents more than one of treatment mean associated with the letter.

**Chapter II: Spatial Patterns and Sequential Sampling Plans for Predators of *Aphis*  
*glycines* (Hemiptera: Aphididae) in Minnesota Soybean**

## Summary

The soybean aphid, *Aphis glycines* Matsumura, is an economically important soybean pest. Many studies have demonstrated that predatory insects are important in suppressing *A. glycines* population growth. However, to improve the utilization of predators in *A. glycines* management, sampling plans need to be developed and validated for predators. Aphid predators were sampled in soybean fields near Rosemount, Minnesota from 2006 – 2007 and 2013 – 2015 with sample sizes of 20 – 80 plants per sample date. Sampling plans were developed for the most abundant *A. glycines* predators (i.e., *Orius insidiosus* (Say), *Harmonia axyridis* (Pallas) and all aphidophagous Coccinellidae species). Taylor's power law parameters from the regression of the log variance versus log mean suggested aggregated spatial patterns for immature and adult stages combined for *O. insidiosus*, *H. axyridis* and Coccinellidae in soybean fields. Using the parameters from Taylor's power law and Green's method, sequential fixed-precision sampling plans were developed to estimate the density for each predator taxon at desired precision levels of 0.10 and 0.25. To achieve a desired precision of 0.10 and 0.25, the average sample number (ASN) ranged from 398 – 713 and 64 – 108 soybean plants, respectively, for all species. Resulting ASNs were relatively large and assumed impractical for most purposes; therefore, the desired precision levels were adjusted to determine the level of precision associated with a more practical ASN. Final analysis indicated an ASN of 38 soybean plants provided precision of 0.32 – 0.40 for the predators. Development of sequential sampling plans should provide guidance for improved estimation of predator densities for *A. glycines* integrated pest management programs and for research purposes.



## Introduction

The soybean aphid, *Aphis glycines* Matsumura, is an invasive insect from Asia and is a major pest of soybean, *Glycine max* (L.) Merrill in North America (Ragsdale et al. 2004). Feeding injury from *A. glycines* can cause economic damage with yield loss as high as 40% (Ragsdale et al. 2007, Tilmon et al. 2011). Before *A. glycines* established in North America, less than 2% of soybean fields were scouted for insect pests; however, since the invasion, scouting efforts increased 40-fold (Ragsdale et al. 2011). Along with the increase in scouting, there has been a large increase in insecticide applications to manage *A. glycines* (Ragsdale et al. 2011, Heimpel et al. 2013). Current management of *A. glycines* relies on scouting soybean fields and applying broad-spectrum, foliar insecticides when pest populations reach an economic threshold of 250 aphids per plant, with 80% of the soybean field infested and an increasing population (Ragsdale et al. 2007, Hodgson et al. 2012). However, studies have shown that application of insecticides can remove natural enemy populations (Ohnesorg et al. 2009, Varenhorst and O'Neal 2012, Tran et al. 2016), which can result in negative consequences (Desneux et al. 2007, Dutcher 2007). Furthermore, over-reliance on insecticides may accelerate development of *A. glycines* insecticide resistance.

In North America, approximately 40 species of predators have been confirmed to attack *A. glycines* (Ragsdale et al. 2011). These natural enemies have been documented as important for preventing and suppressing *A. glycines* population growth (Rutledge et al. 2004, Fox et al. 2005, Costamagna and Landis 2007). Of the natural enemies, *Orius insidiosus* (Say) and *Harmonia axyridis* (Pallas) and other Coccinellidae have been identified as key predators in North America (Rutledge and O'Neil 2005, Xue et al. 2009,

Koch and Costamagna 2016). Hallet et al. (2014) developed a model incorporating natural enemy population densities to adjust the *A. glycines* economic threshold. The results from Hallet et al. (2014) could improve *A. glycines* management-decisions, but there is a lack of information for sampling natural enemies. Different methods can be implemented for sampling natural enemies (e.g., sweep-net, sticky cards, shake cloth) (Shepard et al. 1974, Marston et al. 1976, Schmidt et al. 2008, Bannerman et al. 2015). In soybean, Schmidt et al. (2008) and Bannerman et al. (2015) found that sweep-net sampling was efficient for collecting mobile predators such as coccinellids, but underestimated *O. insidiosus* density. In contrast, whole-plant counts were more efficient at detecting *O. insidiosus*, but not coccinellids (Schmidt et al. 2008). However, visual whole-plant counts is the recommended method for *A. glycines* scouting (Hodgson et al. 2012) and Bannerman et al. (2015) suggest this sampling method is effective for sampling both *O. insidiosus* and coccinellids, but recognize that fewer coccinellids will be detected in comparison to sweep-net.

However, sampling plans have not been developed for natural enemies of *A. glycines* in soybean fields. Sampling plans are fundamental components of integrated pest management (IPM), because they provide estimates of insect density without examining an entire field, retain a known level of precision, and the information can be utilized to make management decisions (Irwin and Shepard 1980, Hutchison 1994, Moon and Wilson 2009). Sequential sampling plans have been widely adopted for sampling because of the ability to set a fixed-precision level prior to sampling that allows for reduced sampling efforts (Kendall 1946, Binns 1994, Hutchison 1994). When using such plans, sampling can cease when sample numbers reach the desired precision level compared to

sampling until a fixed-sample size is met (Binns et al. 2000). To develop a sequential sampling plan it is important to consider the spatial pattern of the targeted organism (Shepard 1980). The spatial pattern (i.e., uniform, random, or aggregated) exhibited by the targeted organism can affect the sampling plan (Ruesink 1980) and change depending on life stage (e.g., egg, larva or nymph, and adult), environment and time (Hutchison 1994).

Sampling plans have been developed extensively for insect pests in many crops such as western flower thrips, *Frankliniella occidentalis* (Pergande), and cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter) in cotton (Parajulee et al. 2006), two-spotted spider mite, *Tetranychus urticae* Koch, in peppermint (Tollerup et al. 2013), sugarcane weevil, *Acrotomopus atropunctellus* (Boheman), in sugar cane (Pérez et al. 2015), squash bug, *Anasa tristis* (De Geer) in watermelon (Dogramaci et al. 2006), and *A. glycines* in soybean (Hodgson et al. 2004). Yet, limited work has been done to develop sampling plans for natural enemies. The development of sampling plans for natural enemies of *A. glycines* could improve integration of natural enemies in management of this pest and be used to better estimate natural enemy densities in soybean research. Sampling plans have been developed for *Orius* spp. and coccinellids in several crop systems, such as wheat (Elliot et al. 1997), cotton (Knutson et al. 2008), sweet pepper (Shipp et al. 1992) and in sweet corn (Musser et al. 2004, Koch et al. 2006). A sampling plan exists for *O. insidiosus* in Iowa soybean (Bechinski and Pedigo 1981); however, that sampling plan was developed prior to the invasion by *A. glycines*.

Due to their importance in *A. glycines* population dynamics, sampling plans should be developed for natural enemies of *A. glycines*, such as *O. insidiosus*,

Coccinellidae in general and *H. axyridis* in particular in soybean (Koch and Costamagna 2016). Therefore, the purpose of this study was to characterize the spatial patterns and develop and validate sequential sampling plans for *A. glycines* predators, *O. insidiosus*, *H. axyridis*, and Coccinellidae. The development of sequential sampling plans should provide guidance for improved estimation of these predator densities for management of *A. glycines* and for research purposes.

### **Materials and Methods**

Sampling for *A. glycines* predators was performed from 2006 to 2007 and 2013 to 2015 at the University of Minnesota Outreach, Research and Education Park, Rosemount, Minnesota, USA. Data used for these analyses were from insecticide-free, aphid-susceptible soybean plots that were part of nine larger randomized complete block design experiments that investigated management tactics (e.g., host plant resistance, cover crops) for *A. glycines* and each experiment had four replications of each treatment. Row spacing for all experiments was 76.2 cm and adjacent plots were separated by at least 1.5 m. Additional agronomic details for each experiment were as follows. On 24 May 2006, soybean seeds (variety: IA2053, Iowa State University Research Foundation (ISURF), Ames, IA) were planted at a seeding rate of 370,658 seeds per ha into 5,000 m<sup>2</sup> plots. On 22 May 2007, soybean seeds (variety: IA2053, ISURF, Ames, IA) were planted at a seeding rate of 370,658 seeds per ha into 5,000 m<sup>2</sup> plots. On 31 May 2013, soybean seeds (variety: NK S19-A6, Syngenta AG, Basel, Switzerland) were planted at a seeding rate of 369,029 seeds per ha into 55.66 m<sup>2</sup> plots. On 11 June 2013, soybean seeds (variety: IA2037, ISURF, Ames, IA) were planted at a seeding rate of 516,450 seeds per ha into

10.47 m<sup>2</sup> plots. On 27 May 2014, soybean seeds (variety: S17-B3, Syngenta AG, Basel, Switzerland) were planted at a seeding rate of 409,390 seeds per ha into 10.47 m<sup>2</sup> plots. On 27 May 2014, soybean seeds (variety: IA2037, ISURF, Ames, IA) were planted at a seeding rate of 516,450 seeds per ha into 10.47 m<sup>2</sup> plots. On 28 May 2015, soybean seeds (variety: IA3027, ISURF, Ames, IA) were planted at a seeding rate of 370,658 seeds per ha into 10.47 m<sup>2</sup> plots. On 16 June 2014, soybean seeds (variety: 90M92, DuPont Pioneer, Johnston, IA) were planted at a seeding rate of 409,400 seeds per ha into 27.88 m<sup>2</sup> plots. Plots were maintained using standard production practices (Bennet et al. 1999).

Predator sampling occurred approximately weekly and encompassed the V1 to R6 soybean growth stages (Fehr and Cavines 1977). Predators of *A. glycines* were sampled using non-destructive, visual whole-plant inspection of randomly selected plants. The sample unit for all samples was an individual soybean plant and sample sizes across all experiments ranged from 20-80 plants. Sample units were randomly selected within the plots while avoiding the plot edges. Counts of immatures (i.e., nymphs or larvae) and adults of each predator were recorded. Immatures and adults of the three most abundant predator taxa observed across experiments, *O. insidiosus*, *H. axyridis* and Coccinellidae (across all aphidophagous species), were used for analyses. The spatial patterns of the immatures and adults for each taxon was characterized by Taylor's power law:

$$s^2 = am^b$$

where  $m$  is the sample mean,  $s^2$  is the sample variance,  $b$  is the slope and  $a$  is the anti-log of the intercept. The intercept and slope values were calculated using a linear regression of the log variance and log mean for the immature and adult life stages of *O. insidiosus*, *H. axyridis* and all other Coccinellidae. Slopes of the regressions were compared to 1

using a one-sample t-test. Slopes less than 1, equal to 1, or greater than 1 represent uniform, random, or aggregated spatial patterns, respectively (Taylor 1961). To determine if spatial patterns differed between immatures and adults of each taxon, slopes of the regressions for immatures and adults of each taxon were compared with a two-sample t-test. If the life stages (i.e., immatures and adults) were not significantly different ( $P > 0.05$ ), the data sets were pooled for Taylor's power law calculations for the species. All statistical analyses were performed using R Core Team (2015). Prior to estimating Taylor's power law parameters for each predator taxon, we removed 10 data sets, which were later used for independent validation of the sampling plans. The 10 data sets were selected to represent the range of mean densities observed, but the minimum mean density for selected data sets was maintained above 0.05 insect per plant because the validation software would not allow lower sample means.

The development and validation of sampling plans was performed by using the Resampling Validation of Sample Plans (RVSP), following the protocol of Naranjo and Hutchison (1997). The sampling plan for each focal taxon and life stage was developed using Green's fixed-precision sequential sampling plan. The calculation for the sampling stop line under Green's plan is:

$$T_n \geq (an^{1-b}/D^2)^{1/(2-b)}$$

where  $T_n$  is the cumulative number of individuals sampled,  $n$  is the total number of samples,  $D$  is the precision (SEM/mean), and  $a$  and  $b$  values are from Taylor's power parameter (as described above). Sampling plans were developed for precision levels of 0.10 and 0.25 for ecological and pest management purposes, respectively (Southwood and Henderson 2000). The ten data sets that were excluded from calculation of Taylor's

power law for each focal taxon and life stage, were used for validation of the sampling plans via resampling simulation. The simulations randomly selected samples with replacement from the validation data sets until the corresponding stop line was exceeded. The mean density, sample number and precision was obtained from 500 iterations for each data set. In some instances, the resulting mean precision values from the simulations were lower than the desired precision values (0.10 or 0.25), so the desired precision levels were relaxed to allow the resulting mean precision to approach the desired level (Burkness and Hutchison 1997, Galvan et al. 2007). In addition, further simulations were performed because the resulting average sample numbers (ASN) were relatively large and assumed impractical for most purposes. The desired precision was adjusted iteratively to determine the level of precision that would be required to achieve the ASN of 38 soybean plants, which is the recommended average sample size for *A. glycines* sequential sampling (Hodgson et al. 2004). For each of the precision levels, the resulting mean sample numbers were averaged to obtain the recommended sample numbers for estimating *O. insidiosus*, *H. axyridis* and Coccinellidae.

## **Results and Discussion**

To our knowledge, sampling plans have not been developed for common predators of *A. glycines* since its arrival in North America. Developing and validating a sampling plan can be useful for gaining information on estimating insect population density with a known degree of precision and our research provides this information for three primary predators of *A. glycines*. In our study, *O. insidiosus* and coccinellids made up 39 and 33% of the total *A. glycines* predators sampled, respectively (Table 2.1).

Within Coccinellidae, *H. axyridis* made up 45% of the total coccinellid species sampled (Table 2.1). Furthermore, *O. insidiosus*, *H. axyridis* and coccinellids were the focus for developing sampling plans because of their documented importance for regulating *A. glycines* populations (Rutledge and O'Neil 2005, Desneux et al. 2006, Xue et al. 2009, Koch and Costamagna 2016).

### ***Orius insidiosus***

For *O. insidiosus* nymphs and adults, a total of 71 data sets were collected across experiments with mean densities ranging from 0.01 to 2.98 *O. insidiosus* per plant. A total of 32, 50 and 61 data sets were used to estimate Taylor's power law parameters for nymphs, adults and both stages combined, respectively (Table 2.2). Taylor's power law regressions for *O. insidiosus* showed a positive relationship between log variance and log mean for all life stages (nymphs, adults, and combined) with  $r^2$  values  $\geq 0.91$  (Table 2.2). The  $b$  values from Taylor's power law for nymphs and combined (nymphs + adults), were significantly greater than 1 (nymphs:  $t = 2.11$ ;  $df = 30$ ;  $P = 0.0431$ , combined:  $t = 3.13$ ;  $df = 59$ ;  $P = 0.0027$ ), indicating these stages of *O. insidiosus* had an aggregated spatial pattern (Table 2.2). However, *O. insidiosus* adults had a random spatial pattern with the  $b$  value equal to 1 ( $t = 1.11$ ;  $df = 48$ ;  $P = 0.2705$ ) (Table 2.2). These two different spatial patterns for nymphs and adults of *O. insidiosus* were similar to the spatial patterns described by Shipp et al. (1992). Shipp et al. (1992) observed two species of *Orius*, *Orius tristicolor* (White) and *O. insidiosus*, in sweet pepper grown in greenhouses and found that nymphs and adults had aggregated and random spatial patterns, respectively. However, Bechinski and Pedigo (1981) found that *O. insidiosus* nymphs and adults were both aggregated, but nymphs were more aggregated than adults.



The differences in spatial patterns between nymph and adult *O. insidiosus* could be due to the adults being more mobile than nymphs (Shipp et al. 1992). In the present study, the *b* values for nymphs and adults of *O. insidiosus* did not differ from each other ( $t = -0.38$ ;  $df = 78$ ;  $P = 0.7044$ ); therefore, further sampling analyses utilized the pooled nymph and adult data sets, and the combined stages had an aggregated distribution (Table 2.2). Despite working in different crops (Shipp et al. 1992) and with different sampling methods (Bechinski and Pedigo 1981), *O. insidiosus* nymph and adult stages were able to be combined for sampling plan development.

For nymphs and adults of *O. insidiosus* combined, the ASN decreased when density per plant increased and desired precision was relaxed (Figure 2.1). The original simulation output for Green's sequential sampling plan resulted in a better average precision level than the desired precision levels of 0.10 and 0.25 for combined life stages of *O. insidiosus*. A relaxed desired precision level of 0.11 provided an average precision level of 0.10 with an ASN of 398 (range: 146-517) soybean plants (Table 2.3; Figures 2.1A and 2.1B). A relaxed desired precision level of 0.28 provided an average precision level of 0.25 with an ASN of 64 (range: 30-120) soybean plants (Table 2.3; Figures 2.1C and 2.1D). Targeting a desired ASN of 38 soybean plants for *O. insidiosus* resulted in an average precision level of 0.32 (Table 2.3; Figures 2.1E and 2.1F). We were unable to compare our sampling plans to Shipp et al. (1992) and Bechinski and Pedigo (1981), due to differences in sampling methods among studies.

### ***Harmonia axyridis***

A total of 8 species of Coccinellidae were observed across our data sets (Table 2.1). Within Coccinellidae, *H. axyridis* was the most abundant species and is a well-

known voracious predator that is capable of preventing and suppressing *A. glycines* outbreaks (Xue et al. 2009, Koch and Costamagna 2016). Because of the documented abundance and importance of *H. axyridis* and its distinguishable characteristics for identification (Koch 2003), a separate sampling plan for *H. axyridis* in soybean was developed.

For *H. axyridis* larvae and adults, a total of 81 data sets were collected across experiments with mean densities ranging from 0.01 to 1.45 *H. axyridis* per plant. A total of 43, 41 and 71 (larvae, adults and both stages combined, respectively) data sets were used to estimate Taylor's power law parameters (Table 2.2). Taylor's power law regressions for *H. axyridis* showed a positive relationship between log variance and log mean for all life stages (larvae, adults, and combined) with  $r^2$  values  $\geq 0.94$  (Table 2.2). The  $b$  values from Taylor's power law for larvae, adults and combined (larvae + adults), were greater than 1 (larvae:  $t = 3.60$ ;  $df = 41$ ;  $P = 0.0008$ , adults:  $t = 2.39$ ;  $df = 39$ ;  $P = 0.0220$ ; combined:  $t = 4.54$ ;  $df = 69$ ;  $P < 0.0001$ ), indicating that *H. axyridis* had an aggregated spatial pattern in soybean fields (Table 2.2).

Other studies have described *H. axyridis* larvae and adults having an aggregated spatial pattern in field corn (Park and Obrycki 2004) and in wine grapes (Galvan et al. 2007), even when *H. axyridis* was functioning as a pest. However, Koch et al. (2006) found that *H. axyridis* had a random spatial pattern in sweet corn. Coccinellids may display different spatial patterns dependent on their prey's spatial pattern (Dixon 2000). Hodgson et al. (2004) found that *A. glycines* were highly aggregated, which could explain the aggregated distribution of *H. axyridis* in soybean. In addition, Galvan et al. (2007) and Park and Obrycki (2004) mentioned that there were instances where *H. axyridis* had

random spatial patterns, but the patterns varied over time (i.e., early or late season) and by life stage. Similar to *Orius* spp., adult *H. axyridis* are more mobile compared to the immature stage and this could contribute to differences in spatial patterns (Koch et al. 2006). There was no significant difference between the  $b$  values for larval and adult stages for *H. axyridis* ( $t = -0.94$ ;  $df = 80$ ;  $P = 0.3517$ ); therefore, further sampling analyses utilized the pooled larvae and adults data sets.

For *H. axyridis* larvae and adults combined, the ASN of soybean plants decreased with increasing density per plant and when desired precision was relaxed (Figure 2.2). For combined life stages of *H. axyridis*, the original simulation output for Green's sequential sampling plan resulted in a better average precision level than the desired precision levels of 0.10 and 0.25. A relaxed desired precision level of 0.11 provided an average precision level of 0.10 with an ASN of 449 (range: 183-609) soybean plants (Table 2.3; Figures 2.2A and 2.2B). A relaxed desired precision level of 0.27 provided an average precision level of 0.25 with an ASN of 77 (range: 37-152) soybean plants (Table 2.3; Figures 2.2C and 2.2D). Targeting a desired ASN of 38 soybean plants resulted in an average precision level of 0.35 (Table 2.3; Figures 2.2E and 2.2F). Our sample plan for *H. axyridis* in soybean is comparable to the ASN in wine grapes and sweet corn. In wine grapes, an ASN of 546 and 180 provided precision levels of 0.10 and 0.25, respectively (Galvan et al. 2007). However in sweet corn, the ASN was lower and an ASN of 205 and 77 provided precision levels of 0.10 and 0.25, respectively (Koch et al. 2006).

### **Coccinellidae**

For assessment of spatial pattern and development of sampling plans, we combined different adult coccinellid species because coccinellids are often considered

acting as a guild of predators not necessarily broken out by species (Obrycki et al. 2009, Noma et al. 2010, Woltz et al. 2012). In addition, it can be difficult to distinguish the larvae of different coccinellid species in the field (Michels et al. 1997). Similar sampling plans, combining coccinellid species, have been developed for wheat (Elliot et al. 1997, Michels et al. 1997) and cotton (Knutson et al. 2008).

For coccinellid larvae and adults of all species combined, a total of 103 data sets were collected across experiments with mean densities ranging from 0.01 to 1.60 coccinellid per plant. A total of 76 and 69 (larvae and adults, respectively) data sets were used to estimate Taylor's power law parameters (Table 2.2). Taylor's power law regression for Coccinellidae showed a positive relationship between log variance and log mean for all life stages (larvae and adults) with  $r^2$  values  $\geq 0.85$  (Table 2.2). The  $b$  values from Taylor's power law for larvae and adults, were greater than 1 (larvae:  $t = 4.01$ ;  $df = 74$ ;  $P = 0.0001$ , adults:  $t = 3.90$ ;  $df = 67$ ;  $P = 0.0002$ ), indicating that Coccinellidae had an aggregated spatial pattern (Table 2.2).

Michel et al. (1997) and Knutson et al. (2008) developed sampling plans that combined different adult species of coccinellids, but did so without comparing spatial patterns among species. However, Elliot et al. (1997) compared the spatial patterns of four adult coccinellid species in wheat and found no differences. We compared the second most abundant coccinellid species, *Hippodamia convergens* Guérin-Ménéville, to *H. axyridis* and the  $b$  values were not significantly different ( $P = 0.0516$ ). We were unable to compare the other coccinellid species because data sets were not large enough. In addition, adults of coccinellid species are often misidentified (Gardiner et al. 2012). For these reasons, we developed a sampling plan for all aphidophagous coccinellid

species combined. However, there was a significant difference between the b values for larval and adult stages of the combined species of Coccinellidae ( $t = -2.19$ ;  $df = 141$ ;  $P = 0.0300$ ); therefore, further sampling analyses were performed separately for larvae and adults.

The ASN of soybean plants decreased with increasing density of coccinellid larvae per plant and when desired precision was relaxed (Figure 2.3). For coccinellid larvae, a desired precision of 0.10 provided an ASN of 713 (range: 200-869) soybean plants (Table 2.3; Figures 2.3A and 2.3B). The original simulation output for Green's sequential sampling plan resulted in a better average precision level than the desired precision levels of 0.25. A relaxed desired precision level of 0.26 provided an average precision level of 0.25 with an ASN of 108 (range: 59-180) soybean plants (Table 2.3; Figure 2.3C and 2.3D). Targeting a desired ASN of 38 soybean plants resulted in an average precision level of 0.40 (Table 2.3; Figures 2.3E and 2.3F). The ASN of soybean plants decreased with increasing density of coccinellid adults per plant and desired precision (Figure 2.4). For coccinellid adults, a desired precision of 0.10 provided an ASN of 623 (range: 180-815) soybean plants (Table 2.3; Figures 2.4A and 2.4B). The original simulation output for Green's sequential sampling plan resulted in a better average precision level than the desired precision levels of 0.25. A relaxed desired precision level of 0.26 provided an average precision level of 0.25 with an ASN of 102 (range: 47-188) soybean plants (Table 3; Figures 2.4C and 2.4D). Targeting a desired ASN of 38 soybean plants resulted in an average precision level of 0.40 (Table 2.3; Figures 2.4E and 2.4F). Comparing our sampling plan for Coccinellidae larvae and adults in soybean is difficult due to the differences in sampling method from existing

plans. Michels et al. (1997) focused on using sweep-net samples and timed counts to estimate larval and adult coccinellids in wheat. For coccinellid adults, Elliot et al. (1997) used required time to achieve precision levels of 0.10, 0.25 and 0.40 and Knutson et al. (2008) investigated the required beat-bucket samples to achieve a fixed-precision level of 0.35.

### **Conclusion**

Surveys have shown that scouting for aphids is the most common practice soybean growers use to make insecticide application decisions (Olson et al. 2008) and that growers recognize the importance of natural enemies (Rodas and O'Neil 2006). Therefore, creating sampling plans for natural enemies that can be used with *A. glycines* scouting would be useful. Currently, there is a mobile application called Aphid Advisor, which asks users to count 10 soybean plants and enter in the number of observed natural enemies and *A. glycines* (Hallett et al. 2014, [www.aphidapp.com](http://www.aphidapp.com)). Based on data input by the user the application advises users whether a foliar application is necessary. While this application is one of the first to incorporate natural enemies into *A. glycines* management, the level of precision provided by the associated scouting recommendations is unknown. Our findings showed that with a precision of 0.10 and 0.25 an ASN of 398-713 and 64-108 soybean plants, respectively, is required. But for a sample size of 38 plants, the precision levels are between 0.32-0.40.

An objective of IPM is to reduce the amount of insecticide being applied in a field and a sampling plan can provide the information for reducing insecticide applications (Bechinski and Pedigo 1981). Implementing a sampling plan is a fundamental component

to estimating the density of insect populations and can become a tool for making informed management decisions (Castle and Naranjo 2009). The present study provides the first sampling plans for *A. glycines* natural enemies and can provide the fundamental components for further investigation to improve management of the *A. glycines*. Further research should incorporate larger soybean fields and other natural enemy species.

**Table 2.1:** Total number of *A. glycines* predators observed through non-destructive, visual whole-plant counts in Rosemount, Minnesota from 2006-2007 and 2013-2015.

<b>Predators Order/Family</b>	<b>Species</b>	<b>Life Stage</b>	<b>Total</b>
<b>Araneae</b>	Unidentified	Adults	47
<b>Opilinoes</b>	Unidentified	Adults	22
<b>Coleoptera</b>			
Coccinellidae	<i>Coleomegilla maculata</i>	Adults	3
	<i>Cycloneda munda</i>	Adults	8
	<i>Coccinella septumpunctata</i>	Adults	10
	<i>Hippodamia convergens</i>	Adults	33
	<i>Hippodamia parenthesis</i>	Adults	3
	<i>Hippodamia tredecimpunctata</i>	Adults	1
	<i>Hippodamia variegata</i>	Adults	27
	<i>Harmonia axyridis</i>	Larvae	300
		Adults	257
	Unidentified	Larvae	603



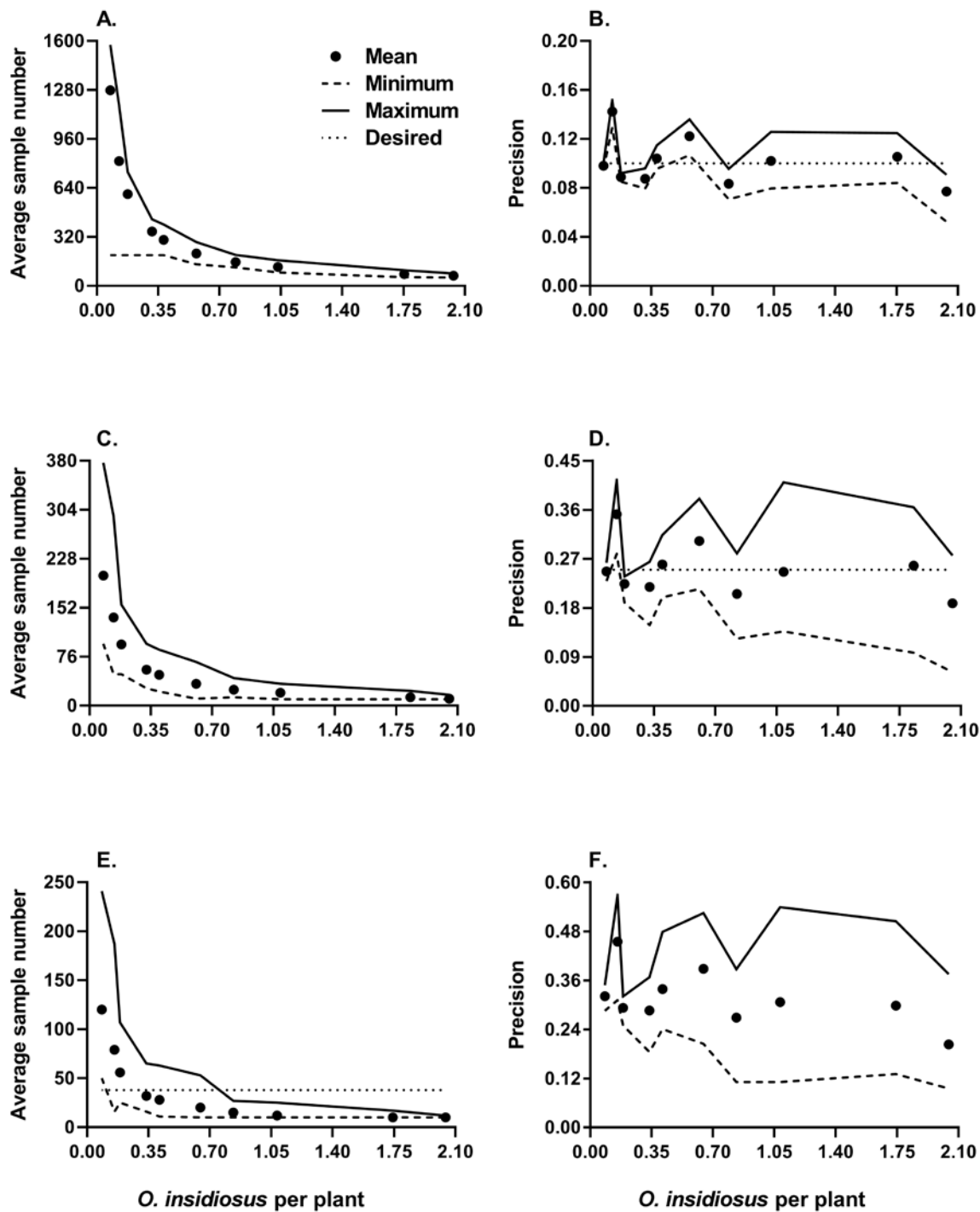
<b>Predators Order/Family</b>	<b>Species</b>	<b>Life Stage</b>	<b>Total</b>
<b>Diptera</b>			
Cecidomyiidae	<i>Aphidoletes aphidimyza</i>	Larvae	347
Syrphidae	Unidentified	Larvae	480
		Adults	9
<b>Hemiptera</b>			
Anthrocoridae	<i>Orius insidiosus</i>	Nymphs	1022
		Adults	437
Nabidae	<i>Nabis</i> spp.	Nymphs	25
		Adults	19
<b>Neuroptera</b>			
Chrysopidae	Unidentified	Larvae	72
		Adults	24
Hemerobiidae	Unidentified	Larvae	51
		Adults	4

**Table 2.2:** Results of Taylor’s power law ( $s^2 = am^b$ ) parameters for the different life stages of *Orius insidiosus*, *Harmonia axyridis* and all Coccinellidae observed in soybean fields from 2006-2007 and 2013-2015 in Rosemount, Minnesota.

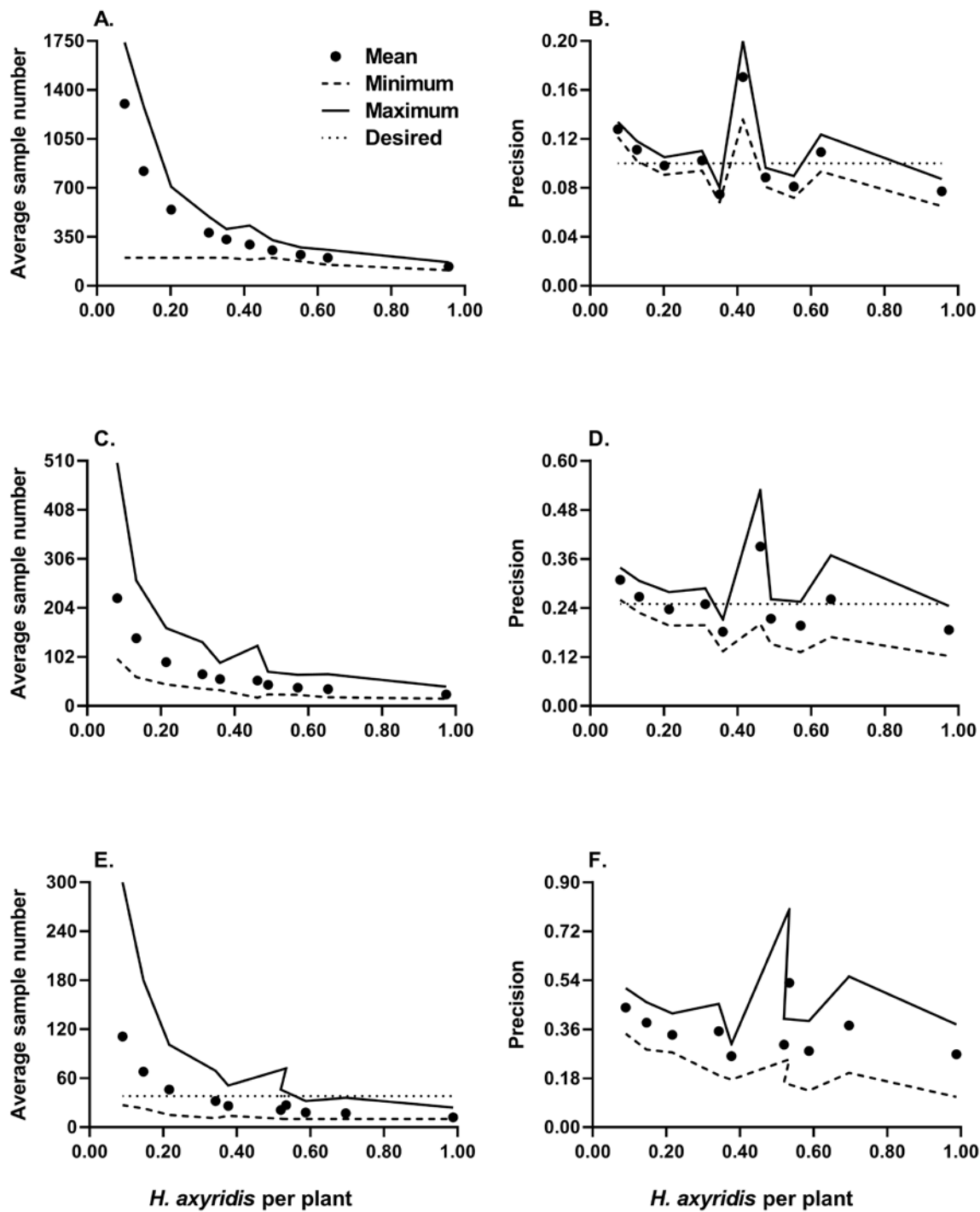
<b>Taxon</b>	<b>Stage</b>	<b><i>a</i> (95% CI)</b>	<b><i>b</i> (95% CI)</b>	<b><i>n</i></b>	<b><i>r</i><sup>2</sup></b>
<i>Orius insidiosus</i>	Nymphs	1.31 (1.11, 1.54)	1.08 (1.00, 1.15)	32	0.97
	Adults	1.31 (1.01, 1.71)	1.05 (0.96, 1.15)	50	0.91
	Combined	1.50 (1.29, 1.74)	1.10 (1.04, 1.16)	61	0.95
<i>Harmonia axyridis</i>	Larvae	1.69 (1.37, 2.09)	1.16 (1.07, 1.25)	43	0.94
	Adults	1.51 (1.19, 1.92)	1.10 (1.02, 1.19)	41	0.94
	Combined	1.59 (1.39, 1.82)	1.12 (1.07, 1.18)	71	0.96
Coccinellidae	Larvae	2.25 (1.78, 2.84)	1.24 (1.12, 1.37)	76	0.85
	Adults	1.46 (1.25, 1.70)	1.10 (1.05, 1.15)	69	0.96

**Table 2.3:** Summary results for validation of Green’s sequential sampling plan using resampling software and desired fixed-precision levels of 0.10 and 0.25, and a desired average sample number of 38 soybean plants for *Orius insidiosus* combined life stages (nymphs and adults), *Harmonia axyridis* combined life stages (larvae and adults) and coccinellid larvae and adults in soybean fields.

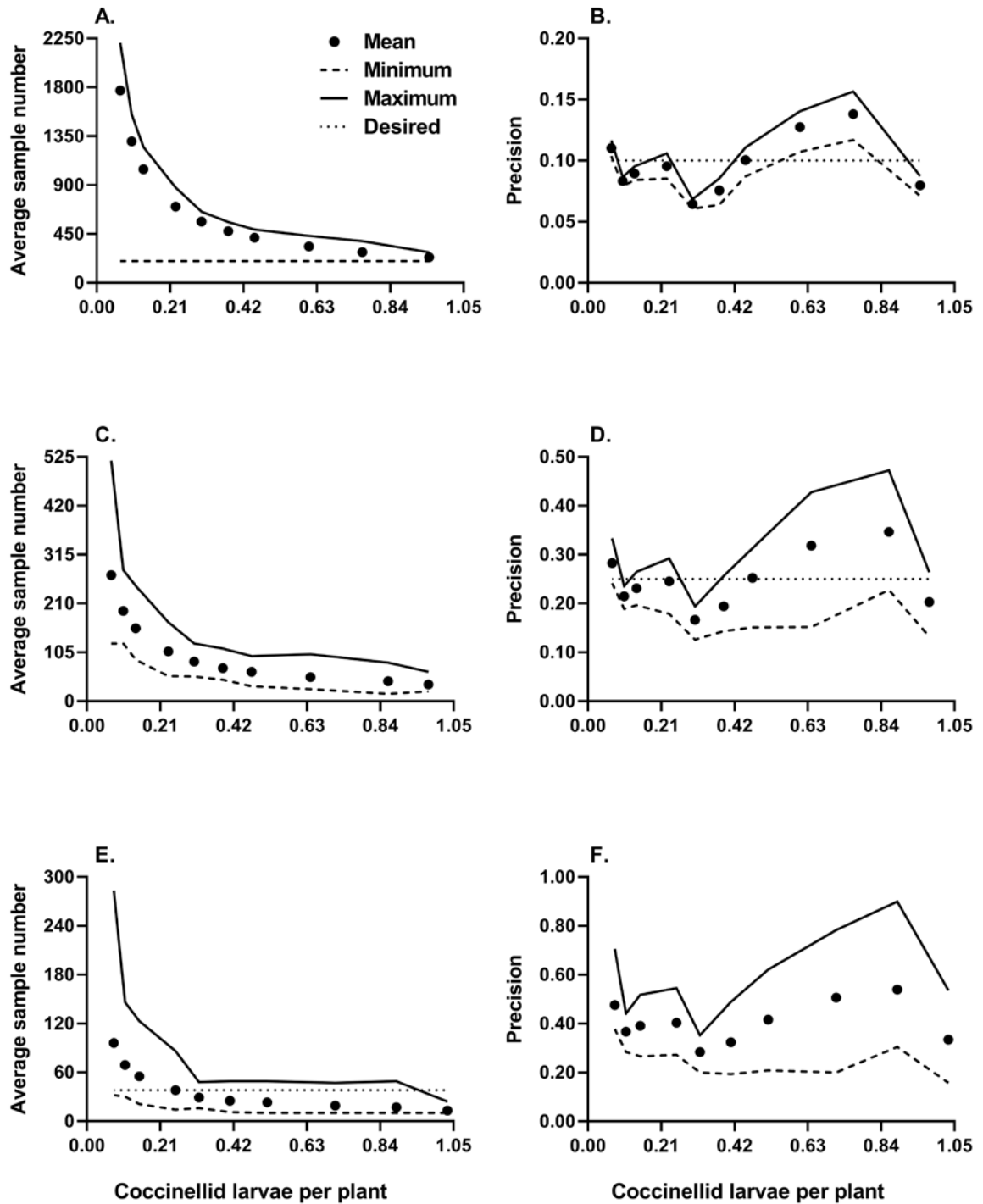
Taxon	Observed mean density	Average statistics for 500 sequential sampling iterations						
		Density	Precision (D)			Average sample number (ASN)		
		Mean	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Desired D=0.10								
<i>Orius insidiosus</i>	0.72	0.73	0.10	0.09	0.11	398	146	517
<i>Harmonia axyridis</i>	0.41	0.41	0.10	0.09	0.11	449	183	609
Coccinellidae larvae	0.39	0.40	0.10	0.09	0.11	713	200	869
Coccinellidae adults	0.41	0.41	0.10	0.09	0.11	623	180	815
Desired D=0.25								
<i>Orius insidiosus</i>	0.72	0.75	0.25	0.17	0.32	64	30	120
<i>Harmonia axyridis</i>	0.41	0.43	0.25	0.18	0.31	77	37	152
Coccinellidae larvae	0.39	0.42	0.25	0.17	0.31	108	59	180
Coccinellidae adults	0.41	0.43	0.25	0.19	0.30	102	47	188
Desired ASN=38								
<i>Orius insidiosus</i>	0.72	0.75	0.32	0.19	0.44	38	17	80
<i>Harmonia axyridis</i>	0.41	0.45	0.35	0.21	0.47	38	14	91
Coccinellidae larvae	0.39	0.45	0.40	0.25	0.59	38	16	90
Coccinellidae adults	0.41	0.45	0.40	0.24	0.53	38	13	98



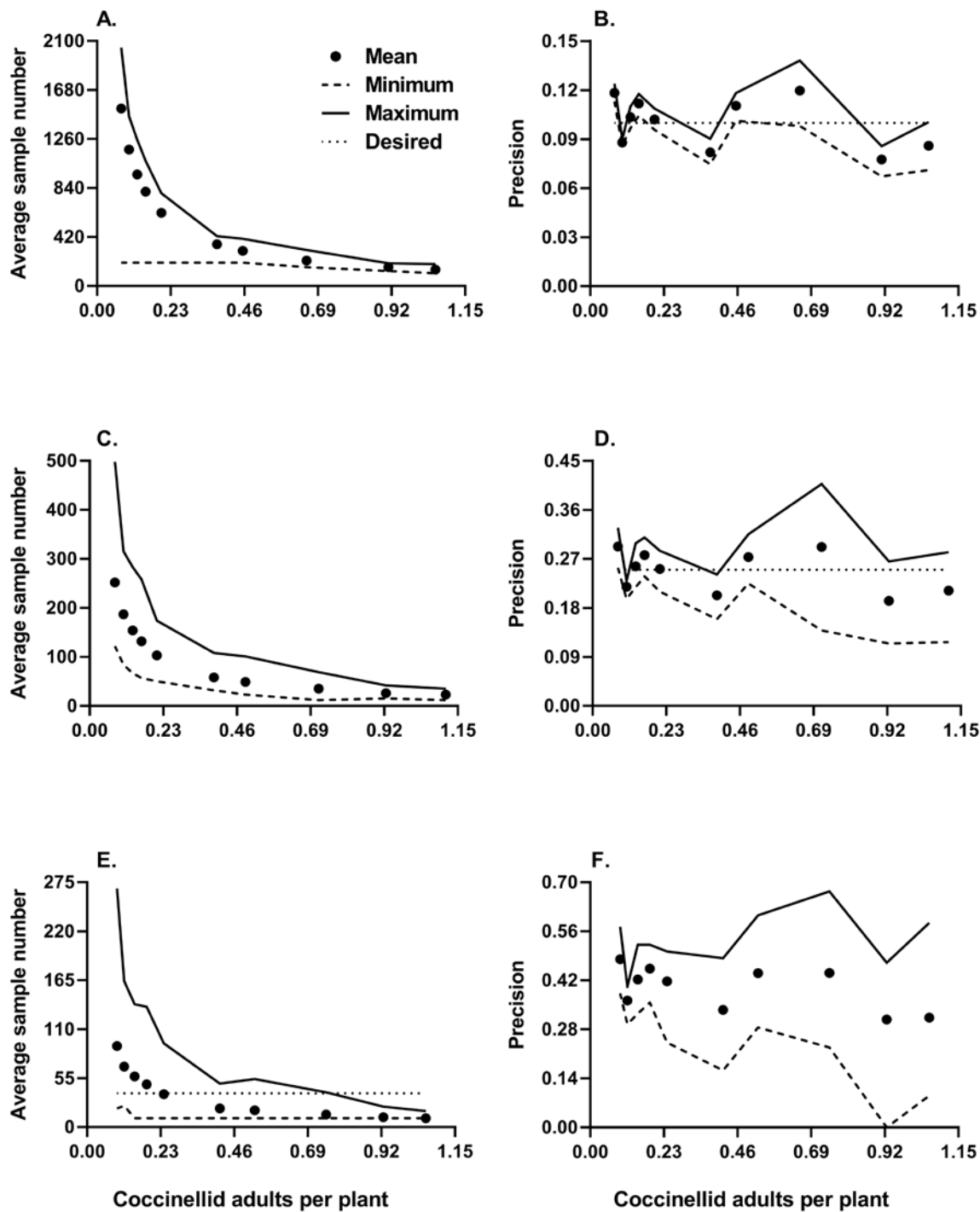
**Figure 2.1:** Summary of the resampling validation analysis under Green’s sequential sampling plan for combined life stages (nymphs and adults) of *Orius insidiosus* on soybean plants with average sample numbers (A, C and E) and average precision levels (B, D and F). Simulation parameters were set to a desired precision of 0.10 (A and B) and 0.25 (C and D), and desired average sample number of 38 (E and F). The dotted horizontal line (B, D, and E) indicates the desired values.



**Figure 2.2:** Summary of the resampling validation analysis under Green’s sequential sampling plan for combined life stages (larvae and adults) of *Harmonia axyridis* on soybean plants with average sample numbers (A, C and E) and average precision levels (B, D and F). Simulation parameters were set to a desired precision of 0.10 (A and B) and 0.25 (C and D), and desired average sample number of 38 (E and F). The dotted horizontal line (B, D, and E) indicates the desired values.



**Figure 2.3:** Summary of the resampling validation analysis under Green’s sequential sampling plan for coccinellid larvae on soybean plants with average sample numbers (A, C and E) and average precision levels (B, D and F). Simulation parameters were set to a desired precision of 0.10 (A and B) and 0.25 (C and D), and desired average sample number of 38 (E and F). The dotted horizontal line (B, D, and E) indicates the desired values.



**Figure 2.4:** Summary of the resampling validation analysis under Green’s sequential sampling plan for coccinellid adult on soybean plants with average sample numbers (A, C and E) and average precision levels (B, D and F). Simulation parameters were set to a desired precision of 0.10 (A and B) and 0.25 (C and D), and desired average sample number of 38 (E and F). The dotted horizontal line (B, D, and E) indicates the desired values.

## Bibliography

- (APHIS) Animal and Plant Health Inspection Service. 2016.** Environmental assessments; availability: Biological control agent for soybean aphid in the continental United States. (<http://www.regulations.gov/document?D=APHIS-2014-0004-0004>).
- (GBIF) Global Biodiversity Information Facility. 2016.** *Aphis glycines* Matsumura, 1917. (<http://www.gbif.org/species/2072221>).
- (NASS) United States Department of Agriculture-National Agriculture Statistic Service. 2016.** <http://www.nass.usda.gov/>. (<https://www.nass.usda.gov/>).
- (US EPA) United State Environmental Protection Agency. 2016.** Data requirements for pesticide registration. (<http://www.epa.gov>).
- (USDA ERS) United States Department of Agriculture-Economic Research Service. 2016.** Soybeans & Oil Crops. (<http://www.ers.usda.gov/topics/crops/soybeans-oil-crops/background.aspx>).
- Alves, T. M., A. K. Tran, W. A. Rich, and R. L. Koch. 2016.** Efficacy of foliar insecticides for management of grape colaspis, 2015. *Arthropod Management Tests*. 40: tsw036.
- Babcock, J. M., C. B. Gerwick, J. X. Huang, M. R. Loso, G. Nakamura, S. P. Nolting, R. B. Rogers, T. C. Sparks, J. Thomas, G. B. Watson, and Y. M. Zhu. 2010.** Biological characterization of sulfoxaflo, a novel insecticide. *Pest Management Science*. 67: 328–334.
- Bannerman, J. A., A. C. Costamagna, B. P. McCornack, and D. W. Ragsdale. 2015.** Comparison of relative bias, precision, and efficiency of sampling methods for natural enemies of soybean aphid (Hemiptera: Aphididae). *Journal of Economic Entomology*. 108: 1381–1397.
- Bartlett, B. R. 1964.** Integration of chemical and biological control, pp. 489–511. *In* DeBach, P. (ed.), *Biological Control of Insect Pests and Weeds*. Reinhold Pub. Corp., New York, NY.
- Bechinski, E. J., and L. P. Pedigo. 1981.** Population dispersion and development of sampling plans for *Orius insidiosus* and *Nabis* spp. in soybeans. *Environmental Entomology*. 10: 956–959.



- Beckendorf, E. A., M. A. Catangui, and W. E. Riedell. 2008.** Soybean aphid feeding injury and soybean yield, yield components, and seed composition. *Agronomy Journal*. 100: 237–246.
- Bennet, J. M., D. R. Hicks, and S. L. Naeve. 1999.** Minnesota soybean field book, 1st ed. University of Minnesota Extension Service, St. Paul, MN.
- Binns, M. R. 1994.** Sequential sampling for classifying pest status, pp. 137–205. *In* Pedigo, L.P., Buntin, G.D. (eds.), *Handbook of Sampling Methods for Arthropods in Agriculture*.
- Binns, M. R., J. P. Nyrop, and W. Van Der Werf. 2000.** Sampling and monitoring in crop protection: the theoretical basis for developing practical decision guides. CAB International, New York, NY.
- Blackman, R. L., and V. F. Eastop. 2000.** Aphids of the world's crops: An identification and information guide, 2nd ed. Wiley, Chichester, New York.
- Burkness, E., and W. Hutchison. 1997.** Development and validation of a binomial sequential sampling plan for striped cucumber beetle (Coleoptera: Chrysomelidae) in cucurbits. *Journal of Economic Entomology*. 90: 1590–1594.
- Castle, S., and S. E. Naranjo. 2009.** Sampling plans, selective insecticides and sustainability: The case for IPM as “informed pest management.” *Pest Management Science*. 65: 1321–1328.
- Clark, A. J., and K. L. Perry. 2002.** Transmissibility of field isolates of soybean viruses by *Aphis glycines*. *Plant Disease*. 86: 1219–1222.
- Costamagna, A. C., and D. A. Landis. 2007.** Quantifying predation on soybean aphid through direct field observations. *Biological Control*. 42: 16–24.
- Costamagna, A. C., D. A. Landis, and M. J. Brewer. 2008.** The role of natural enemy guilds in *Aphis glycines* suppression. *Biological Control*. 45: 368–379.
- Costamagna, A. C., D. A. Landis, and C. D. DiFonzo. 2007.** Suppression of soybean aphid by generalist predators results in a trophic cascade in soybeans. *Ecological Applications*. 17: 441–451.
- Cutler, P., R. Slater, A. J. F. Edmunds, P. Maienfisch, R. G. Hall, F. G. P. Earley, T. Pitterna, S. Pal, V. L. Paul, J. Goodchild, M. Blacker, L. Hagmann, and A. J. Crossthwaite. 2013.** Investigating the mode of action of sulfoxaflor: A fourth-

- generation neonicotinoid. *Pest Management Science*. 69: 607–619.
- Dana, L. A., T. E. Hunt, and D. K. Zahn. 2012.** Control of soybean aphid with foliar insecticides in soybeans, 2011. *Arthropod Management Tests*. 37: F72.
- DeBach, P. 1964.** The scope of biological control, pp. 3–20. *In Biological Control of Insect Pests and Weeds*.
- Desneux, N., A. Decourtye, and J. Delpuech. 2007.** The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology*. 52: 81.
- Desneux, N., R. J. O’Neil, and H. J. S. Yoo. 2006.** Suppression of population growth of the soybean aphid, *Aphis glycines* Matsumura, by predators: The identification of a key predator and the effects of prey dispersion, predator abundance, and temperature. *Environmental Entomology*. 35: 1342–1349.
- Dixon, A. F. G. 1973.** *Biology of aphids*. London.
- Dixon, A. F. G. 2000.** *Insect predator-prey dynamics: ladybird beetles and biological control*. Cambridge University Press, New York, NY.
- Dogramaci, M., J. W. Shrefler, K. Giles, and J. V Edelson. 2006.** Spatial pattern and sequential sampling of squash bug (Heteroptera: Coreidae) adults in watermelon. *Journal of Economic Entomology*. 99: 559–67.
- Dutcher, J. D. 2007.** A review of resurgence and replacement causing pest outbreaks in IPM, pp. 27–43. *In* Ciancio, A., Mukerji, K.G. (eds.), *General Concepts in Integrated Pest and Disease Management, Integrated Management of Plants Pests and Diseases*. Springer Netherlands.
- Elbert, A., M. Haas, B. Springer, W. Thielert, and R. Nauen. 2008.** Applied aspects of neonicotinoid uses in crop protection. *Pest Management Science*. 64: 1099–1105.
- Elliot, N. C., G. J. Michels, R. W. Kieckhefer, and B. W. French. 1997.** Sequential sampling for adult coccinellids in wheat. *Entomologia Experimentalis et Applicata*. 84: 267–273.
- Fehr, W. R., and C. E. Cavines. 1977.** *Stages of soybean development*. Iowa State University of Science and Technology, Ames, Iowa.
- Fox, T. B., D. A. Landis, F. F. Cardoso, and C. D. DiFonzo. 2004.** Predators suppress *Aphis glycines* Matsumura population growth in soybean. *Environmental Entomology*. 33: 608–618.

- Fox, T. B., D. A. Landis, F. F. Cardoso, and C. D. Difonzo. 2005.** Impact of predation on establishment of the soybean aphid, *Aphis glycines* in soybean, *Glycine max*. *Biocontrol*. 50: 545–563.
- Galvan, T. L., E. C. Burkness, and W. D. Hutchison. 2007.** Enumerative and binomial sequential sampling plans for the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in wine grapes. *Journal of Economic Entomology*. 100: 1000–1010.
- Galvan, T. L., R. L. Koch, and W. D. Hutchison. 2005.** Toxicity of commonly used insecticides in sweet corn and soybean to multicolored Asian lady beetle (Coleoptera: Coccinellidae). *Journal of Economic Entomology*. 98: 780–789.
- Gardiner, M. M., L. L. Allee, P. M. J. Brown, J. E. Losey, H. E. Roy, and R. R. Smyth. 2012.** Lessons from lady beetles: Accuracy of monitoring data from US and UK citizen-science programs. *Frontiers in Ecology and the Environment*. 10: 471–476.
- Gardiner, M. M., D. A. Landis, C. Gratton, C. D. DiFonzo, M. E. O’Neal, J. M. Chacon, M. T. Wayo, N. P. Schmidt, E. E. Mueller, and G. E. Heimpel. 2009.** Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. *Ecological Applications*. 19: 143–154.
- Garzón, A., P. Medina, F. Amor, E. Viñuela, and F. Budia. 2015.** Toxicity and sublethal effects of six insecticides to last instar larvae and adults of the biocontrol agents *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Adalia bipunctata* (L.) (Coleoptera: Coccinellidae). *Chemosphere*. 132: 87–93.
- Ghosh, A., A. Das, A. Samanta, M. L. Chatterjee, and A. Roy. 2013.** Sulfoximine: A novel insecticide for management of rice brown planthopper in India. *African Journal of Agricultural Research*. 8: 4798–4803.
- Giles, K., and J. Obrycki. 1997.** Reduced insecticide rates and strip-harvesting: Effect on arthropod predator abundance in first-growth alfalfa. *Journal of the Kansas Entomological Society*. 70: 160–168.
- Gore, J., D. Cook, A. Catchot, B. R. Leonard, S. D. Stewart, G. Lorenz, and D. Kerns. 2013.** Cotton aphid (Heteroptera: Aphididae) susceptibility to commercial and experimental insecticides in the southern United States. *Journal of Economic Entomology*. 106: 1430–9.

- Grafton-Cardwell, E. E., L. D. Godfrey, W. E. Chaney, and W. J. Bentley. 2008.** Various novel insecticides are less toxic to humans, more specific to key pests. *California Agriculture*. 59: 29–34.
- Gray, M. E., S. T. Ratcliffe, and M. E. Rice. 2009.** The IPM paradigm: Concepts, strategies and tactics, pp. 1–13. *In* Radcliffe, E.B., Hutchison, W.D., Cancelado, R.E. (eds.), *Integrated Pest Management: Concepts, Tactics, Strategies, and Case Studies*. Cambridge University Press, Cambridge, MA.
- Grevstad, F. S. 1999.** Factors influencing the chance of population establishment: Implications for release strategies in biocontrol. *Ecological Applications*. 9: 1439–1447.
- Hall, R. W., and L. E. Ehler. 1979.** Rate of establishment of natural enemies in classical biological control. *ESA Bulletin*. 25: 280–283.
- Hallett, R. H., C. A. Bahlai, Y. Xue, and A. W. Schaafsma. 2013.** Incorporating natural enemy units into a dynamic action threshold for the soybean aphid, *Aphis glycines* (Homoptera: Aphididae). *Pest Management Science*. 70: 879–888.
- Hanafi, A., E. B. Radcliffe, and D. W. Ragsdale. 1989.** Spread and control of potato leafroll virus in Minnesota. *Journal of Economic Entomology*. 82: 1201–1206.
- Hanson, A. A., J. H. Orf, and R. L. Koch. 2016.** Sources of soybean aphid resistance in early-maturing soybean germplasm. *Crop Science*. 56: 154–163.
- Hardin, M. R., B. Benrey, M. Coll, W. O. Lamp, G. K. Roderick, and P. Barbosa. 1995.** Arthropod pest resurgence: An overview of potential mechanisms. *Crop Protection*. 14: 3–18.
- Hassan, S. A., F. Bigler, H. Bogenschütz, E. Boller, J. Brun, J. N. M. Calis, J. Coremans-Pelseneer, C. Duso, A. Grove, U. Heimbach, N. Helyer, H. Hokkanen, G. B. Lewis, F. Mansour, L. Moreth, L. Polgar, L. Samsøe-Petersen, B. Sauphanor, A. Stäubli, G. Sterk, A. Vainio, M. van de Veire, G. Viggiani, and H. Vogt. 1994.** Results of the sixth joint pesticide testing programme of the IOBC/WPRS-working group «pesticides and beneficial organisms». *Entomophaga*. 39: 107–119.
- Heimpel, G. E., R., G. E. Heimpel, D. W. Ragsdale, R. Venette, K. R. Hopper, R. J. O’Neil, C. E. Rutledge, and Z. Wu. 2004.** Prospects for importation biological

- control of the soybean aphid: Anticipating potential costs and benefits. *Annals of the Entomological Society of America*. 97: 249–258.
- Heimpel, G. E., Y. Yang, J. D. Hill, and D. W. Ragsdale. 2013.** Environmental consequences of invasive species: Greenhouse gas emissions of insecticide use and the role of biological control in reducing emissions. *PLoS ONE*. 8: 1–7.
- Herron, G. A., B. J. Langfield, D. R. Bogema, and Y. Chen. 2014.** Baseline susceptibility and cross-resistance in *Aphis gossypii* Glover (Aphididae: Hemiptera) to phorate and sulfoxaflor. *Austral Entomology*. 53: 32–35.
- Hesler, L. S., M. V. Chiozza, M. E. O’Neal, G. C. MacIntosh, K. J. Tilmon, D. I. Chandrasena, N. A. Tinsley, S. R. Cianzio, A. C. Costamagna, E. M. Cullen, C. D. DiFonzo, B. D. Potter, D. W. Ragsdale, K. Steffey, and K. J. Koehler. 2013.** Performance and prospects of *Rag* genes for management of soybean aphid. *Entomologia Experimentalis et Applicata*. 147: 201–216.
- Higley, L. G., and L. P. Pedigo. 1996.** The EIL concept, pp. 9–21. *In* Higley, L.G., Pedigo, L.P. (eds.), *Economic Thresholds for Integrated Pest Management*. University of Nebraska Press, Lincoln.
- Hirano, H., K. I. Honda, and S. Miyai. 1996.** Effects of temperature on development, longevity and reproduction of the soybean aphid, *Aphis glycines* (Homoptera: Aphididae). *Applied Entomology and Zoology*. 31: 178–180.
- Hodgson, E. W., E. C. Burkness, W. D. Hutchison, and D. W. Ragsdale. 2004.** Enumerative and binomial sequential sampling plans for soybean aphid (Homoptera: Aphididae) in soybean. *Journal of Economic Entomology*. 97: 2127–2136.
- Hodgson, E. W., B. P. McCornack, K. Tilmon, and J. J. Knodel. 2012.** Management recommendations for soybean aphid (Hemiptera: Aphididae) in the United States. *Journal of Integrated Pest Management*. 3: 1–10.
- Hodgson, E. W., and G. VanNostrand. 2012.** Soybean aphid efficacy screening program, 2011. *Arthropod Management Tests*. 37: F80.
- Hutchison, W. D. 1994.** Sequential sampling to determine population density, pp. 207–244. *In* Pedigo, L.P., Buntin G. David (eds.), *Handbook of Sampling Methods for Arthropods in Agriculture*. Boca, Florida.
- Hutchison, W. D., B. Flood, and J. A. Wyman. 2004.** Advances in United States sweet

- corn and snap bean insect pest management, pp. 247–278. *In* Horowitz, A.R., Ishaaya, I. (eds.), *Insect Pest Management: Field and Protected Crops*. Springer-Verlag Berlin Heidelberg.
- Irwin, M. E., and M. Shepard. 1980.** Sampling predaceous Hemiptera on soybean, pp. 505–531. *In* Kogan, M., Herzog, D.C. (eds.), *Sampling Methods in Soybean Entomology*. Springer-Verlag, New York, NY.
- Ishaayn, I., and J. E. Casida. 1981.** Pyrethroid esterase(s) may contribute to natural pyrethroid tolerance of larvae of the common green lacewing. *Environmental Entomology*. 10: 681–684.
- Jalali, M. A., T. Van Leeuwen, L. Tirry, and P. De Clercq. 2009.** Toxicity of selected insecticides to the two-spot ladybird *Adalia bipunctata*. *Phytoparasitica*. 37: 323–326.
- Johnson, K. D., M. E. O’Neal, D. W. Ragsdale, C. D. Difonzo, S. M. Swinton, P. M. Dixon, B. D. Potter, E. W. Hodgson, and A. C. Costamagna. 2009.** Probability of cost-effective management of soybean aphid (Hemiptera: Aphididae) in North America. *Journal of Economic Entomology*. 102: 2101–2108.
- Johnson, M. W., and B. E. Tabashnik. 1999.** Enhanced biological control through pesticide selectivity, pp. 297–317. *In* Thomas, S.B., Fisher, T.W., Caltagirone, L.E., Dahlsten, D.L., Gordh, G., Huffaker, C.B. (eds.), *Handbook of Biological Control*. Academic Press, San Diego, California.
- Joshi, N. K., and V. K. Sharma. 2009.** Efficacy of imidacloprid (Confidor 200 SI) against aphids infesting wheat crop. *Journal of Central European Agriculture*. 10: 217–222.
- Kaiser, M. E., T. Noma, M. J. Brewer, K. S. Pike, J. R. Vockeroth, and S. D. Gaimari. 2007.** Hymenopteran parasitoids and dipteran predators found using soybean aphid after its midwestern United States invasion. *Annals of the Entomological Society of America*. 100: 196–205.
- Kendall, M. G. 1946.** Sequential analysis of statistical data. *Nature*. 157: 642.
- Knutson, A. E., M. A. Muegge, L. T. Wilson, and S. E. Naranjo. 2008.** Evaluation of sampling methods and development of sample plans for estimating predator densities in cotton. *Journal of Economic Entomology*. 101: 1501–1509.

- Koch, R. L. 2003.** The multicolored Asian lady beetle, *Harmonia axyridis*: A review of its biology, uses in biological control, and non- target impacts. *Journal of Insect Science*. 3: 32.
- Koch, R. L., E. C. Burkness, and W. D. Hutchison. 2006.** Spatial distribution and fixed-precision sampling plans for the ladybird *Harmonia axyridis* in sweet corn. *BioControl*. 51: 741–751.
- Koch, R. L., and A. C. Costamagna. 2016.** Reaping benefits from an invasive species: role of *Harmonia axyridis* in natural biological control of *Aphis glycines* in North America. *BioControl*.
- Koo, H. N., J. J. An, S. E. Park, J. Il Kim, and G. H. Kim. 2014.** Regional susceptibilities to 12 insecticides of melon and cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae) and a point mutation associated with imidacloprid resistance. *Crop Protection*. 55: 91–97.
- Koss, A. M., A. S. Jensen, A. Schreiber, K. S. Pike, and W. E. Snyder. 2005.** Comparison of Predator and Pest Communities in Washington Potato Fields Treated with Broad-Spectrum, Selective, or Organic Insecticides. *Environmental Entomology*. 34: 87–95.
- Lewis, S. 2015.** Sulfoxaflor - Final cancellation order.  
([http://www.epa.gov/sites/production/files/2015-11/documents/final\\_cancellation\\_order-sulfoxaflor.pdf](http://www.epa.gov/sites/production/files/2015-11/documents/final_cancellation_order-sulfoxaflor.pdf)).
- Longhurst, C., J. M. Babcock, I. Denholm, K. Gorman, J. D. Thomas, and T. C. Sparks. 2013.** Cross-resistance relationships of the sulfoximine insecticide sulfoxaflor with neonicotinoids and other insecticides in the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum*. *Pest Management Science*. 69: 809–813.
- Magalhaes, L. C., T. E. Hunt, and B. D. Siegfried. 2008.** Development of methods to evaluate susceptibility of soybean aphid to imidacloprid and thiamethoxam at lethal and sublethal concentrations. *Entomologia Experimentalis et Applicata*. 128: 330–336.
- Marston, N. L., C. E. Morgan, G. D. Thomas, and C. M. Ignoffo. 1976.** Evaluation of four techniques for sampling soybean insects. *Journal of the Kansas Entomological Society*. 49: 389–400.

- McCornack, B. P., D. W. Ragsdale, and R. C. Venette. 2004.** Demography of soybean aphid (Homoptera: Aphididae) at summer temperatures. *Journal of Economic Entomology*. 97: 854–61.
- Medina, P., F. Budia, P. Del Estal, A. Adán, and E. Viñuela. 2004.** Toxicity of fipronil to the predatory lacewing *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Biocontrol Science and Technology*. 14: 261–268.
- Miao, J., K. Wu, K. R. Hopper, and G. Li. 2007.** Population dynamics of *Aphis glycines* (Homoptera: Aphididae) and impact of natural enemies in northern China. *Biological Control*. 36: 840–848.
- Michels, G. J., N. C. Elliott, R. L. Romero, and W. B. French. 1997.** Estimating populations of aphidophagous coccinellidae (Coleoptera) in winter wheat. *Environmental Entomology*. 26: 4–11.
- Moon, R. D., and L. T. Wilson. 2009.** Sampling for detection, estimation and IPM decision making, pp. 75–89. *In* Radcliffe, E.B., Hutchison, W.D., Cancelado, R.E. (eds.), *Integrated Pest Management: Concepts, Tactics, Strategies, and Case Studies*. Cambridge University Press.
- Musser, F. R., J. P. Nyrop, and A. M. Shelton. 2004.** Survey of predators and sampling method comparison in sweet corn. *Journal of Economic Entomology*. 97: 136–44.
- Naranjo, S. E. 2001.** Conservation and evaluation of natural enemies in IPM systems for *Bemisia tabaci*. *Crop Protection*. 20: 835–852.
- Naranjo, S. E., P. C. Ellsworth, C. C. Chu, and T. J. Henneberry. 2002.** Conservation of predatory arthropods in cotton: role of action thresholds for *Bemisia tabaci* (Homoptera: Aleyrodidae). *Journal of Economic Entomology*. 95: 682–691.
- Naranjo, S. E., P. C. Ellsworth, and G. B. Frisvold. 2015.** Economic Value of Biological Control in Integrated Pest Management of Managed Plant Systems. *Annual Review of Entomology*. 60: 1–25.
- Naranjo, S. E., and W. D. Hutchison. 1997.** Validation of arthropod sampling plans using a resampling approach: Software and analysis. *American Entomologist*. 43: 48–57.
- Nauen, R., and A. Elbert. 2003.** European monitoring of resistance to insecticides in *Myzus persicae* and *Aphis gossypii* (Homoptera: Aphididae) with special reference to



- imidacloprid. *Bulletin of Entomological Research*. 93: 47–54.
- Nielsen, C., and A. E. Hajek. 2005.** Control of invasive soybean aphid, *Aphis glycines* (Hemiptera: Aphididae), populations by existing natural enemies in New York State, with emphasis on entomopathogenic fungi. *Environmental Entomology*. 34: 1036–1047.
- Noma, T., C. Gratton, M. Colunga-Garcia, M. J. Brewer, E. E. Mueller, K. A. G. Wyckhuys, G. E. Heimpel, and M. E. O’Neal. 2010.** Relationship of soybean aphid (Hemiptera: Aphididae) to soybean plant nutrients, landscape structure, and natural enemies. *Environmental Entomology*. 39: 31–41.
- Obrycki, J. J., J. D. Harwood, T. J. Kring, and R. J. O’Neil. 2009.** Aphidophagy by coccinellidae: Application of biological control in agroecosystems. *Biological Control*. 51: 244–254.
- Ohnesorg, W. J., K. D. Johnson, and M. E. O’Neal. 2009.** Impact of reduced-risk insecticides on soybean aphid and associated natural enemies. *Journal of Economic Entomology*. 102: 1816–1826.
- Olson, K., T. Badibanga, and C. DiFonzo. 2008.** Farmers’ awareness and use of IPM for soybean aphid control: Reports of survey results for the 2004, 2005, 2006, and 2007 crop years, Staff Paper P08-12. Department of Applied Economics, University of Minnesota, St. Paul, MN.
- Parajulee, M. N., R. B. Shrestha, and J. F. Leser. 2006.** Sampling methods, dispersion patterns, and fixed precision sequential sampling plans for western flower thrips (Thysanoptera: Thripidae) and cotton fleahoppers (Hemiptera: Miridae) in cotton. *Journal of Economic Entomology*. 99: 568–77.
- Park, Y. L., and J. J. Obrycki. 2004.** Spatio-temporal distribution of corn leaf aphids (Homoptera: Aphididae) and lady beetles (Coleoptera: Coccinellidae) in Iowa cornfields. *Biological Control*. 31: 210–217.
- Pedigo, L. P., and M. E. Rice. 2009.** *Entomology and pest management*, 6th ed. Waveland Press, Inc., Long Grove, IL.
- Pérez, M. P., M. G. Isas, A. R. Salvatore, G. Gastaminza, and E. V. Trumper. 2015.** Optimizing a fixed-precision sequential sampling plan for adult *Acrotomopus atropunctellus* (Boheman) (Coleoptera: Curculionidae), new pest on sugarcane.

- Crop Protection. 74: 9–17.
- Pezzini, D. T., and R. L. Koch. 2015.** Compatibility of flonicamid and a formulated mixture of pyrethrins and azadirachtin with predators for soybean aphid (Hemiptera: Aphididae) management. *Biocontrol Science and Technology*. 25: 1024–1035.
- Pree, D. J., D. E. Archibald, and R. K. Morrison. 1989.** Resistance to insecticides in the common green lacewing *Chrysoperla carnea* (Neuropter: Chrysopidae) in Southern Ontario. *Journal of Economic Entomology*. 82: 29–34.
- Qu, Y., D. Xiao, J. Li, Z. Chen, A. Biondi, N. Desneux, X. Gao, and D. Song. 2015.** Sublethal and hormesis effects of imidacloprid on the soybean aphid *Aphis glycines*. *Ecotoxicology (London, England)*. 24: 479–87.
- Qui, L. J., and R. Z. Chang. 2010.** The origin and history of soybean. In Singh, G. (ed.), *The Soybean: Botany, Production and Uses*.
- R Core Team. 2015.** R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.r-project.org/>.
- Ragsdale, D. W., D. A. Landis, J. Brodeur, G. E. Heimpel, and N. Desneux. 2011.** Ecology and management of the soybean aphid in North America. *Annual Review of Entomology*. 56: 375–399.
- Ragsdale, D. W., B. P. McCornack, R. C. Venette, B. D. Potter, I. V MacRae, E. W. Hodgson, M. E. O’Neal, K. D. Johnson, R. J. O’Neil, C. D. DiFonzo, T. E. Hunt, P. A. Glogoza, and E. M. Cullen. 2007.** Economic threshold for soybean aphid (Hemiptera: Aphididae). *Journal of Economic Entomology*. 100: 1258–1267.
- Ragsdale, D. W., D. J. Voegtlin, R. J. O’Neil, D. W. Ragsdale, D. J. Voegtlin, and R. J. O’Neil. 2004.** Soybean aphid biology in North America. *Annals of the Entomological Society of America*. 97: 204–208.
- Ripper, W. E., R. M. Greenslade, and G. S. Hartley. 1951.** Selective insecticides and biological control. *Journal of Economic Entomology*. 44: 448–459.
- Rodas, S., and R. J. O’Neil. 2006.** A Survey of Indiana soybean producers following the introduction of a new invasive pest, the soybean aphid. *American Entomologist*. 52: 146–149.
- Rodríguez-del-Bosque, L. A., and A. P. Terán. 2015.** *Melanaphis sacchari* (Hemiptera : Aphididae): A new sorghum insect pest in Mexico. *Southwestern*

Entomologist. 40: 433–434.

- Roubos, C. R., C. Rodriguez-Saona, R. Holdcraft, K. S. Mason, and R. Isaacs. 2014.** Relative toxicity and residual activity of insecticides used in blueberry pest management: Mortality of natural enemies. *Journal of Economic Entomology*. 107: 277–285.
- Ruesink, W. G. 1980.** Introduction to sampling plan, pp. 61–78. *In* Kogan, M., Herzog, D.C. (eds.), *Sampling Methods in Soybean Entomology*. Springer-Verlag.
- Rutledge, C. E., and R. J. O’Neil. 2005.** *Orius insidiosus* (Say) as a predator of the soybean aphid, *Aphis glycines* Matsumura. *Biological Control*. 33: 56–64.
- Rutledge, C. E., R. J. O’Neil, T. B. Fox, and D. A. Landis. 2004.** Soybean aphid predators and their use in integrated pest management. *Annals of the Entomological Society of America*. 97: 240–248.
- Sazo, L., C. Merino, H. Sepúlveda, and M. Prieto. 2015.** Effect of sulfoxaflor and spirotetramat application timing on the control of San Jose scale (*Diaspidiotus perniciosus* Comstock) in apples. *Chilean Journal of Agriculture and Animal Science*. 31: 149–153.
- Schmidt, N. P., M. E. O’Neal, and P. M. Dixon. 2008.** Aphidophagous predators in Iowa soybean: A community comparison across multiple years and sampling methods. *Annals of the Entomological Society of America*. 101: 341–350.
- Shepard, M. 1980.** Sequential sampling plans for soybean arthropods, pp. 79–93. *In* Kogan, M., Herzog, D.C. (eds.), *Sampling Methods in Soybean Entomology*. Springer-Verlag.
- Shepard, M., G. R. Carner, and S. G. Turnipseed. 1974.** A comparison of three sampling methods for arthropods in soybeans. *Environmental entomology*. 3: 227–232.
- Shi, X., L. Jiang, H. Wang, K. Qiao, D. Wang, and K. Wang. 2011.** Toxicities and sublethal effects of seven neonicotinoid insecticides on survival, growth and reproduction of imidacloprid-resistant cotton aphid, *Aphis gossypii*. *Pest Management Science*. 67: 1528–1533.
- Shipp, J. L., N. Zariffa, and G. Ferguson. 1992.** Spatial patterns of and sampling methods for *Orius* spp. (Hemiptera: Anthocoridae) on greenhouse sweet pepper. *The*

- Canadian Entomologist. 124: 887–894.
- Siebert, M. W., J. D. Thomas, S. P. Nolting, B. R. Leonard, J. Gore, A. Catchot, G. M. Lorenz, S. D. Stewart, D. R. Cook, L. C. Walton, R. B. Lassiter, R. A. Haygood, and J. D. Siebert. 2012.** Field evaluations of sulfoxaflor, a novel insecticide, against tarnished plant bug (Hemiptera: Miridae) in cotton. *Journal of Cotton Science*. 143: 129–143.
- Smith, M. C. 2005.** Plant resistance to arthropods: Molecular and conventional approaches. Springer Netherlands, Dordrecht, The Netherlands.
- Song, F., and S. M. Swinton. 2009.** Returns to integrated pest management research and outreach for soybean aphid. *Journal of Economic Entomology*. 102: 2116–2125.
- Song, F., S. M. Swinton, C. Difonzo, M. O’Neal, D. W. Ragsdale, M. O. Neal, and D. W. Ragsdale. 2006.** Profitability analysis of soybean aphid control treatments in three North-Central states. *Agricultural Economics*. 22.
- Southwood, T. R. E., and P. A. Henderson. 2000.** Ecological methods, Third. ed. Blackwell Science, Inc., Malden, MA.
- Sparks, T. C., G. B. Watson, M. R. Loso, C. Geng, J. M. Babcock, and J. D. Thomas. 2013.** Sulfoxaflor and the sulfoximine insecticides: Chemistry, mode of action and basis for efficacy on resistant insects. *Pesticide Biochemistry and Physiology*. 107: 1–7.
- Studebaker, G. E., and T. J. Kring. 2003.** Effects of insecticides on *Orius insidiosus* (Hemiptera: Anthocoridae), measured by field, greenhouse and petri dish bioassays. *Florida Entomologist*. 86: 178–185.
- Tang, Q., M. Xiang, H. Hu, C. An, and X. Gao. 2015.** Evaluation of sublethal effects of sulfoxaflor on the green peach aphid (Hemiptera: Aphididae) using life table parameters. *Journal of Economic Entomology*.
- Taylor, L. R. 1961.** Aggregation, variance and the mean. *Nature*. 189: 732–735.
- Tilmon, K. J., E. W. Hodgson, M. E. O’Neal, and D. W. Ragsdale. 2011.** Biology of the soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) in the United States. *Journal of Integrated Pest Management*. 2: 1–7.
- Tollerup, K. E., D. Marcum, R. Wilson, and L. Godfrey. 2013.** Binomial and enumerative sampling of *Tetranychus urticae* (Acari: Tetranychidae) on peppermint

- in California. *Journal of Economic Entomology*. 106: 1707–1715.
- Tran, A. K., T. M. Alves, and R. L. Koch. 2016.** Potential for sulfoxaflor to improve conservation biological control of *Aphis glycines* (Hemiptera: Aphididae) in soybean. *Journal of Economic Entomology*. 5: 2105–2114.
- Varenhorst, A. J., and M. E. O’Neal. 2012.** The response of natural enemies to selective insecticides applied to soybean. *Environmental Entomology*. 41: 1565–1574.
- Wanumen, A. C., G. A. Carvalho, P. Medina, E. Viñuela, and Á. Adán. 2016.** Residual acute toxicity of some modern insecticides toward two mirid predators of tomato pests. *Journal of Economic Entomology*. 1–7.
- Watson, G. B., M. R. Loso, J. M. Babcock, J. M. Hasler, T. J. Letherer, C. D. Young, Y. Zhu, J. E. Casida, and T. C. Sparks. 2011.** Novel nicotinic action of the sulfoximine insecticide sulfoxaflor. *Insect Biochemistry and Molecular Biology*. 41: 432–439.
- Wedberg, J. L., C. R. Grau, N. C. Kurtzweil, J. M. Gaska, D. B. Hogg, and T. H. Klubertanz. 2001.** Lessons on soybean aphid in 2000 and 2001. *In Proceedings: Wisconsin Fertilizer, Agrilime & Management Conference*. University of Wisconsin.
- Weinzierl, R. A. 2009.** Integrating pesticides with biotic and biological control for arthropod pest management, pp. 179–191. *In Radcliffe, E.B., Hutchison, W.D., Cancelado, R.E. (eds.), Integrated Pest Management: Concepts, Tactics, Strategies, and Case Studies*. Cambridge University Press, Cambridge, MA.
- Woltz, J. M., R. Isaacs, and D. A. Landis. 2012.** Landscape structure and habitat management differentially influence insect natural enemies in an agricultural landscape. *Agriculture, Ecosystems and Environment*. 152: 40–49.
- Wu, Z., D. Schenk-hamlin, W. Zhan, D. W. Ragsdale, and G. E. Heimpel. 2004.** The soybean aphid in China: A historical review. *Annals of the Entomological Society of America*. 97: 209–218.
- Wyckhuys, K. A. G., R. L. Koch, R. R. Kula, and G. E. . Heimpel. 2009.** Potential exposure of a classical biological control agent of the soybean aphid, *Aphis glycines*, on non-target aphids in North America. *Biological Invasions*. 11: 857–871.
- Xue, Y., C. A. Bahlai, A. Frewin, M. K. Sears, A. W. Schaafsma, and R. H. Hallett.**

**2009.** Predation by *Coccinella septempunctata* and *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Aphis glycines* (Homoptera: Aphididae). *Environmental Entomology*. 38: 708–714.

**Zhu, Y., M. R. Loso, G. B. Watson, T. C. Sparks, R. B. Rogers, J. X. Huang, B. C. Gerwick, J. M. Babcock, D. Kelley, V. B. Hegde, B. M. Nugent, J. M. Renga, I. Denholm, K. Gorman, G. J. DeBoer, J. Hasler, T. Meade, and J. D. Thomas.**  
**2010.** Discovery and characterization of sulfoxaflor, a novel insecticide targeting sap-feeding pests. *Journal of Agricultural and Food Chemistry*. 59: 2950–2957.