

**Community Characterization and Development of a Sampling Plan for Stink Bugs  
(Hemiptera: Pentatomidae) in Soybean in the North Central Region of the U.S.**

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## **Dedication**

To God:

He has been the source of my strength throughout this journey and on His wings only have I soared.

To my dad, Nilton A. Pezzini:

Who taught me the importance of knowledge and always provided support.

To my mom, Domingas S. Pezzini:

Who gave me wings to fly and was my strong pillar and my source of understanding.

## Abstract

Stink bugs are an emerging threat for soybean production in the North Central Region of the U.S., which accounts for 80% of the country's total soybean production. However, there is a lack of literature on stink bug community composition and management recommendations for the region. Therefore, my research aimed to fill the gap of region-specific literature and management recommendations for stink bugs by using data from 9 states of the North Central Region of the U.S.

My first chapter characterized the species composition, abundance and temporal dynamics of stink bugs in the region. We demonstrated that the stink bug community is dominated by *Euschistus* spp. and *Chinavia hilaris*, and the invasive *Halyomorpha halys* was among the most abundant species in the eastern part of the region. Economically damaging infestations of stink bugs occurred in soybean fields in southern areas, and higher densities occurred at later soybean growth stages. Further aspects of stink bug management are addressed in my second chapter, where I assessed spatial patterns and developed fixed-precision sequential sampling plans for stink bugs in the North Central soybean. Results showed that stink bugs were aggregated, but extent of aggregation varied by species, life stage and location. We showed that 40 sample units (i.e., sets of 25 sweeps) would be needed for reliable stink bug estimation under the conditions experienced across the region. However, sample size recommendations could vary significantly by state depending on densities of stink bugs typically encountered. Also, we demonstrated that current sampling recommendations of 6 to 10 sample units may provide unreliable density estimation in some states.

Overall, this is the first study to 1) characterize the stink bug community composition in soybean across the region and 2) develop cost-effective, research-based scouting recommendations for these pests. Results obtained from this thesis will provide important insights for the implementation of IPM for stink bugs in soybean in the North Central Region of the U.S.

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## Introduction

Soybean, *Glycine max* (L.) Merrill, is the most valuable bean in the world, due to its high nutritional value (NASS, 2018). Soybean seed composition is approximately 18% oil and 38% protein, making it the largest source of protein for animal feed and the second largest source of vegetable oil in the world (NASS, 2018). The U.S. is the largest global soybean producer, and within the U.S., the North Central Region is responsible for over 80% of the country's soybean production (NASS, 2018). Stink bugs (Hemiptera: Pentatomidae) are among the most important insect pests damaging soybean (McPherson and McPherson, 2000). Herbivorous stink bugs with economic importance belong to the subfamily Pentatominae, where approximately 40 genera and 180 species can be encountered worldwide (McPherson and McPherson, 2000; Panizzi et al., 2000). In soybean in the North Central Region, approximately 23 stink bug species can be found (McPherson and McPherson, 2000; Panizzi et al., 2000; Koch et al., 2017). Stink bugs are generally polyphagous insects which can be recognized by their shield-shaped body, 5-segmented antennae and a well-developed scutellum (McPherson and McPherson, 2000; Panizzi et al., 2000; Koch et al., 2017).

Stink bugs cause injury to soybean by using piercing-sucking mouthparts to inject powerful digestive enzymes into the plant and remove plant fluids. Stink bugs prefer feeding on reproductive parts (i.e., developing pods and developing seeds) of soybean. However, the timing of their feeding can result in differential levels of damage (McPherson and McPherson, 2000). Beginning pod set to beginning seed set (R3 – R5) are the most sensitive soybean growth stages, when feeding of stink bugs can result in more severe damage (McPherson and McPherson, 2000; Koch et al., 2017). Growth stages from full seed set (R6) onward are generally less affected by stink bug feeding (McPherson and McPherson, 2000; Musser et al., 2011). Injury leads to reduction in seed quality and yield (McPherson and McPherson, 2000). Feeding of stink bugs can also result in deformation and discoloration of soybean seeds, delayed plant maturity (e.g., stay-green syndrome) and transmission of pathogens that cause disease to soybean (Boethel et al.,

2000; Vyavhare et al., 2015). Moreover, stink bug feeding can result in decreased carbohydrate and lipid content of soybean seeds, and punctures from feeding may affect the potential germination of the seeds (Jensen and Newsom, 1972; Todd and Turnipseed, 1974; McPherson and McPherson, 2000).

Historically, stink bugs have presented an economic problem for soybean and other major crops in southern states of the U.S. (McPherson and McPherson, 2000; Panizzi et al., 2000), but not in much of the North Central Region. However, increased attention has recently been given to stink bugs in northern states. First, an invasive species, the brown marmorated stink bug, *Halyomorpha halys* (Stål), is invading the region. This polyphagous pest, which originated from Asia, was first detected in North America in Pennsylvania in 1996 (Hoebeke and Carter, 2003; Leskey et al., 2012). Since its detection, this pest and its impacts are spreading westward in the U.S. (Northeastern IPM Center, 2018). *Halyomorpha halys* has been detected in 44 states in the U.S. and has caused severe agriculture and nuisance problems in 9 states, primarily in the mid-Atlantic region (Northeastern IPM Center, 2018). The second reason of increasing concern about stink bugs is due to the reported increasing abundance of native species such as the green, *Chinavia hilaris* (Say), brown, *Euschistus servus* (Say), onespotted, *E. variolarius* (Palisot de Beauvois), and redshouldered, *Thyanta custator acerra* McAtee stink bugs in crops (Hunt et al., 2011, 2014; Michel et al., 2015; Koch and Pahs, 2014). Because there is not a history of economic infestations of stink bugs in crops in much of the North Central Region, there is a lack of region-specific literature about the stink bug community in soybean. Stink bug biology and behavior has been well reviewed by McPherson and McPherson (2000), Panizzi et al. (2000), Koch et al. (2017), and McPherson (2017). Stink bug species host range (McPherson and McPherson, 2000; Panizzi et al., 2000; Koch et al., 2017), potential damage to soybean (Sosa-Gómez and Moscardi, 1995; Depieri and Panizzi, 2011; Corrêa-Ferreira and De Azevedo, 2002) and susceptibility to pesticides (Willrich et al., 2003; Snodgrass et al., 2005) are reported to vary across species, which may result in different management recommendations for stink bug species.

Therefore, research is needed to quantify stink bug abundance, diversity and phenology, and determine the pest status of *H. halys* in soybean in the North Central Region.

Stink bug dispersal and movement between agricultural and non-agricultural habitats is associated with the availability of host sources and overwintering habitats in the agricultural landscape (Reay-Jones, 2010; Reeves et al., 2010; Venugopal et al., 2014). Agricultural and non-agricultural habitats surrounding soybean fields can influence the stink bug community and consequently the resulting stink bug damage in these fields (Gurr et al., 2012; Venugopal et al., 2014; Rice et al., 2017). Stink bugs overwinter as adults and have a preference for overwintering in protected areas such as woodlands (i.e., under tree bark and leaf litter), under crop residues in the field and in man-made structures (Jones and Sullivan, 1981; McPherson and McPherson, 2000; Rice et al., 2014). In general, stink bug species are reported as univoltine (i.e., one generation per year) in northern states and as bivoltine (i.e., two generations per year) in more southern states (McPherson and McPherson, 2000; Koch et al., 2017). Early in the season, overwintering adults emerge and start to colonize early-maturing hosts such as wheat and some plants, where they build up their population size (McPherson and McPherson, 2000; Olson et al., 2012; Venugopal et al., 2014). Later in the season, with decreasing suitability of early-maturity hosts, stink bugs migrate to soybean fields where host quality is increasing for stink bugs (McPherson and McPherson, 2000; Olson et al., 2012; Venugopal et al., 2014). In addition, stink bug populations are often encountered in greater density in the field edges compared to the field interior (Reay-Jones, 2010; Tillman et al., 2014; Reeves et al., 2010; Leskey et al., 2012).

Scouting for pests in fields is an underlying concept of integrated pest management (IPM) (Moon and Wilson, 2008; Pedigo and Rice, 2009). Current management recommendations for stink bugs in soybean are based on combined counts of nymphs and adults of all herbivorous stink bug species (Boyd and Bailey, 2000; McPherson and McPherson, 2000; Stewart et al., 2010; Cullen, 2012; Greene, 2017; Koch et al., 2017; Raudenbush et al., 2017; Reisig, 2017; Tilmon, 2017). Application of foliar insecticides for management of stink bugs in soybean the North

Central Region is justified when pest density reaches 5 stink bugs / 25 sweeps for soybean grown for grain and 10 stink bugs / 25 sweeps for soybean grown for seed production (Kogan, 1976; Koch et al., 2017). If stink bug densities reach these economic thresholds in fields, broad-spectrum insecticides (i.e., pyrethroids, organophosphates, carbamates and neonicotinoid/pyrethoid mixtures) are commonly used as a therapeutic measure (Willrich et al., 2003; Bergmann and Raupp, 2014; Cira et al., 2017). Reliable estimates of pest density are necessary to avoid incorrect decision-making for pest management (Naranjo and Hutchison, 1997; Pedigo and Rice, 2009). Incorrect estimates can result in extra costs with unnecessary control measures or yield losses due to pest damage (Naranjo and Hutchinson, 1997; Moon and Wilson, 2008). However, sampling recommendations to estimate stink bug density in soybean fields vary greatly among states and lack quantification of associated precision levels (Boyd and Bailey, 2000; Stewart et al., 2010; Cullen, 2012; Biles, 2017; Krupke and Obermeyer, 2017; Greene, 2017; Reisig, 2017; Tilmon, 2017; Mississippi State University, 2018). Therefore, development of research-based scouting recommendations (i.e., sampling plans) for stink bugs in soybean in the North Central Region are needed.

This thesis aims to address the general lack of knowledge and region-specific management recommendations for stink bugs in soybean in the North Central Region, as concerns about stink bugs increase. Chapter 1 examines stink bug species composition, abundance and temporal dynamics in soybean in the North Central Region. This information will provide an important baseline about the stink bug community in soybean in the North Central Region and will document the pest status of *H. halys* in the region. Also, because management of stink bugs can vary by species, it is important to identify species of stink bugs that are present in soybean in the North Central Region for development of management recommendations. Chapter 2 develops a fixed-precision sequential sampling plan for stink bugs in soybean in the North Central Region. This sampling plan will provide cost-effective, research-based scouting recommendations for stink bugs in the region. In general, results obtained from this thesis will

provide important insights for the implementation of IPM for stink bugs in soybean in the North Central Region of the U.S.

## **Chapter 1**

### **Community Composition, Abundance and Temporal Dynamics of Stink Bugs (Hemiptera: Pentatomidae) in Soybean in the North Central Region of the U.S.**

## Summary

Stink bugs are an emerging threat for soybean production in the North Central Region of the U.S., which accounts for 80% of the country's total soybean production. However, there is a lack of knowledge about the community of stink bugs in soybean across this region. Characterization of the stink bug community is essential for development of management programs for these pests. This study aimed to fill this gap of knowledge by using data from 9 states over two years. Specifically, we characterized the relative abundance, richness and diversity of this community, and assessed seasonal differences in abundance of herbivorous and predatory stink bugs. Overall, the stink bug community was dominated by *Euschistus spp.* and *Chinavia hilaris*. *Euschistus variolarius*, *C. hilaris* and *Halyomorpha halys* were more abundant in the northwest, southeast and east, respectively. Species richness differed across states, while diversity was the same across the region. Herbivorous and predatory species were more abundant at later soybean growth stages. Economically significant infestations occurred in fields in southern states. Our results represent the first regional characterization of the stink bug community in soybean fields and will be fundamental for development of state- and region-specific management programs for these emerging pests in the North Central Region of the U.S.

## Introduction

Soybean, *Glycine max* (L.) Merrill (Fabales: Fabaceae), is the most valuable bean in the world due to its high nutritional value (Wilcox, 2004). The U.S. is the largest global soybean producer, and the North Central Region of the U.S. is responsible for 80% of the country's total soybean production (NASS, 2018). Stink bugs (Hemiptera: Pentatomidae) are among the most important insect pests of this crop, because they feed primarily on pods, causing irreversible direct damage to developing seeds (McPherson and McPherson, 2000). Stink bugs, though historically insignificant in the North Central Region of the U.S., pose a new threat in soybean production in this region, because of recent changes to the community of these insects.

Invasive stink bugs are particularly important new threats to soybean because of their polyphagy and ability to adapt to new environments (Panizzi, 2015; McPherson, 2018). The brown marmorated stink bug, *Halyomorpha halys* (Stål), was first detected in Pennsylvania around 1996 (Hoebeke and Carter, 2003). Since its introduction, *H. halys* has spread to 44 U.S. states, including detections in 11 of the 12 states of the North Central Region (Northeastern IPM Center, 2018). In the Mid-Atlantic Region of the U.S. where *H. halys* is well established, soybean growers have reported up to 50% yield reductions in soybean due to this pest (Leskey et al., 2012). In addition, the redbanded stink bug, *Piezodorus guildinii* (Westwood), an important pest of soybean in the South America, has been detected in soybean fields in Missouri (Tindall et al., 2011). In the Southeastern Region of the U.S., *P. guildinii* has been detected in many states (Panizzi and Slansky, 1985; McPherson et al., 1993; Smith et al., 2009) and, in Louisiana, it represents the most abundant stink bug species in soybean (Temple et al., 2013). Furthermore, the painted bug, *Bagrada hilaris* (Burmeister), was recently detected in Minnesota (Koch et al., 2018). Although this species has preference for feeding on plants of the family *Brassicaceae*, it may also feed on soybean (Reed et al., 2013).

In addition to the new threats posed by invasive species to soybean, increasing abundance of native species such as the green stink bug, *Chinavia hilaris* (Say), brown stink bug, *Euschistus*



servus (Say), onespotted stink bug, *E. variolarius* (Palisot de Beauvois), and redshouldered stink bug, *Thyanta custator acerra* McAtee has been reported in soybean fields in the North Central Region of the U.S. (Hunt et al., 2011, 2014; Michel et al., 2015; Koch et al., 2017). The factors contributing to the increase in abundance of native species is unknown, but it may be due to increasing temperatures and transition from conventional to reduced-tillage cultivation systems (Panizzi, 2015). Because of their historical insignificance as pests of soybean in much of the region, little is known about the stink bug community in this key soybean producing region. Therefore, assessment of community composition of stink bugs in the North Central Region is needed.

In the North Central Region, approximately 67 stink bug species are encountered, and 23 species are potentially found in soybean (Packauskas, 2012; Sites et al., 2012; Swanson, 2012; Koch et al., 2014, 2017). Herbivorous stink bugs with economic importance belong to the subfamily Pentatominae and are well recognized as pests of many crops (McPherson and McPherson, 2000). In addition to pests, the family Pentatomidae also includes beneficial (i.e., predatory) species, which belong to the subfamily Asopinae. This subfamily comprises some well-known predators of insect pests of economic importance (De Clercq, 2000; McPherson and McPherson, 2000) and within the U.S., the spined soldier bug, *Podisus maculiventris* (Say), is reported as the most common predatory species (De Clercq, 2000; McPherson, 1982).

The objective of this study was to characterize the community composition and temporal dynamics of stink bugs in soybean in the North Central Region of the U.S. Because host range (McPherson and McPherson, 2000; Panizzi et al., 2000; Koch et al., 2017), potential damage to soybean (Sosa-Gómez and Moscardi, 1995; Depieri and Panizzi, 2011; Corrêa-Ferreira and Azevedo, 2002) and susceptibility to pesticides (Kamminga et al., 2009; Willrich et al., 2003; Snodgrass et al., 2005) are reported to vary across species of stink bugs, it is important to identify species of stink bugs that are present in soybean in the North Central Region for development of management recommendations for this pest complex. Furthermore, data on stink bug community

composition in soybean will provide baseline information for documentation of potential species shifts resulting from invasive species, climate change and changes in production practices.

## **Materials and Methods**

### ***Study sites and data collection***

Stink bugs were sampled following a standardized protocol in soybean fields distributed across nine states in the North Central Region of the U.S. from 2016 to 2017 (Fig. 1.1). From the first year, data were used from study sites located in Indiana, Kansas, Minnesota, Missouri, Nebraska, North Dakota and South Dakota. From the second year, data were used from study sites located in Indiana, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio and South Dakota (Fig. 1.1). A total of 50 and 51 soybean fields ranging from 0.5 to 120 ha (mean $\pm$ SEM: 17.9 $\pm$ 2.1 ha) in size were sampled in 2016 and 2017, respectively. In each state fields were located at one to four sites, with sites separated by 13 to 368 km. Fields were located at university research stations or cooperating farms, with soybean row spacing of 76.2 cm. Soybean varieties, planting dates and management practices were representative of the respective states.

Stink bugs were sampled with sweep nets (net diameter = 39 cm) from R1 (i.e., “beginning bloom”) to R8 (i.e., “full maturity”) soybean growth stages (Fehr and Caviness, 1977). Fields included in this study were sampled over at least four sample dates over the growing season to capture seasonal dynamics of the fauna. Generally, 12 sample units were collected from fields on each sample date; however, larger sample sizes were occasionally collected. A sample unit consisted of a set of 25 sweeps. Once a sample unit was collected, contents of the net were transferred to a labeled 20.3  $\times$  25.4-cm zippered plastic bag, which was placed in a cooler and transported to the laboratory. The season-long number of sample units (i.e., sets of 25 sweeps) collected per field ranged from 60 – 96 in South Dakota, 48 – 84 in Michigan, 60 – 72 in Kansas, 48 – 60 in Ohio, 60 – 72 in North Dakota, 48 – 96 in Nebraska, 72 – 236 in

Minnesota, 60 – 72 in Missouri, and 96 – 120 in Indiana. Rough sorting of the samples to remove plant material and non-target insects was performed in laboratories in each state. Samples containing stink bugs were sent to the University of Minnesota, where, for each specimen, life stage was determined, and nymphs were identified to genus or species and adults were identified to species or subspecies. Identifications were based on Decoursey and Esselbaugh (1962) and Evans (1985) for nymphs and McPherson and McPherson (2000), Rider (2012), and Paiero et al. (2013) for adults. Nymphs of *Euschistus* were identified to genus, because the species of this genus are difficult to distinguish in this life stage. Voucher specimens of each species were placed in the University of Minnesota Insect Collection.

### ***Data analyses***

For all analyses, species or subspecies of adults and genera of nymphs that accounted for less than 0.5% of the total number of individuals for the respective life stage were removed from analyses (Table 1.1). This approach was taken because occurrence of these rare species may be incidental, and they may be of less relevance as pests or natural enemies (e.g., Winfree et al., 2015; Kleijn et al., 2015).

### ***Relative abundance***

We examined differences in relative abundances of species to compare the stink bug community composition and determined the most important species in soybean fields within states of the North Central Region. Analyses of relative abundance were performed separately for nymphs and adults by state with R version 3.4.4 (R Core Team, 2018). Friedman's nonparametric test [package, *code*: *rcompanion*, *friedman.test* (Mangiafico, 2018)] was used to compare the relative abundance of the predominant taxa of stink bug adults and nymphs for each state and *post hoc* multiple comparison was performed [*rcompanion*, *pairwiseSignTest* (Mangiafico, 2018)]. Friedman's test was used to take account for the lack of independence among taxa in a relative abundance data.

### ***Species richness and diversity***

Species richness (i.e., number of species) and diversity of the stink bug community was estimated for each field over the season. For these analyses, stink bug subspecies were combined within species because of the similar biology of subspecies within a given species (McPherson, 1982) and to avoid overestimation of species richness and diversity when subspecies of the same species were detected in the same field. For assessment of richness, the presence or absence of adults of each species (i.e., 9 total) was determined for each sample unit (i.e., sets of 25 sweeps). A nonparametric method, Chao2, was used for estimation of species richness for each field (Chao, 1987; Colwell and Coddington, 1994). Chao2 estimates species richness for sample-based incidence (presence/absence) data based on occurrence of “singletons” (i.e., species that occur in only one sample unit across the season) and “doubletons” (i.e., species that occur in two sample units across the season) (Colwell and Coddington, 1994). Chao2 is a robust species richness estimator that has been used in other systems (Colwell and Coddington, 1994; Barcellos et al., 2008; Baz et al., 2014; Mokam et al., 2014; Acosta et al., 2017), and it was chosen as the richness estimator in this study to account for the uneven number of sample units taken over the season for different fields.

For assessment of diversity, the number of adults of each species was summed within sample units. Species diversity was estimated by the Simpson inverse diversity index using Estimate S<sup>TM</sup> (version 9) (Colwell et al., 2012). This is a robust index that accounts for both number of species and abundance of each species, applying more weight to the most abundant species (Simpson, 1949). Because the total sampling effort was not the same for each field, rarefaction curves, resampled 100 times, were created using EstimateS<sup>TM</sup> (Colwell et al., 2012). Then, calculation of Simpson’s index at 48 sample units (i.e., the lowest total number of sample units collected from a field) was used to standardize sampling effort for different fields. The software could not compute richness and diversity estimates for fields where the most abundant species had fewer than 2 individuals; therefore, 1 such field was removed from analysis.

Generalized linear mixed models (GLMM) were used to test the effect of years (i.e., 2 years) and state (i.e., 9 states) on stink bug species richness (Chao2) or diversity (Simpson's index), with site as a random effect [*lme4*, *lmer* (Bates et al., 2015)]. Analyses were performed with R version 3.4.4 (R Core Team, 2018) and assumptions were checked by inspection of the residual plots. Tukey-Kramer-adjusted pairwise comparisons of least square means were used to determine significant differences ( $\alpha = 0.05$ ) among treatments for analyses of stink bug species richness (Chao2) and diversity (Simpson's index).

### ***Temporal dynamics and maximum abundance***

Temporal dynamics of stink bug abundance was assessed to determine which soybean growth stages are more likely to have damaging economic infestations by herbivorous species and higher potential biological control by predatory species. Stink bug temporal abundance was summarized by averaging the mean densities of the nymph and adult life stages of herbivorous or predatory stink bugs by soybean growth stage across fields and years.

Maximum mean abundance of herbivorous and predatory stink bugs was calculated as the peak mean density (i.e., stink bugs / 25 sweeps) for each field across the season. GLMMs were used to test the effect of year (i.e., 2 years) and state (i.e., 9 states) on maximum mean abundance ( $\log x+1$ ) of herbivorous or predatory stink bugs, with site as a random effect. Analyses were performed with R version 3.4.4 (R Core Team 2018) and assumptions were checked by inspection of the residual plots. Tukey-Kramer-adjusted pairwise comparisons of least square means were used to determine significant differences ( $\alpha = 0.05$ ) among treatments for herbivorous and predatory stink bug mean maximum abundance [*multcomp*, *glht* (Hothorn et al., 2008)].

## **Results**

Analyses were performed on a total of 704 samples (i.e., 9,457 sample units of 25 sweeps) collected from 101 soybean fields across 9 states and 2 years. From these samples, a total of 5,794 stink bug specimens were collected, of which 1,968 were adults and 3,826 were nymphs.

### ***Relative abundance***

Across years and states, a total 19 species and subspecies of adults (i.e., 17 herbivores and 2 predators) (Table 1.1) and 7 genera of nymphs (i.e., 6 herbivores and 1 predator) were collected (Table 1.2). For adults, *E. variolarius* was the most abundant species followed by *C. hilaris*, *Euschistus servus* (i.e., *E. s. servus*, *E. s. euschistoides* and *E. s. hybrid*), *P. maculiventris*, *E. tristigma* (i.e., *E. t. tristigma* and *E. t. luridus*), *T. c. acerra* and *H. halys* (Table 1.1). A similar pattern was observed for nymphs, where *Euschistus* spp. were the most abundant, followed by *C. hilaris*, *H. halys*, *Podisus* spp. and *Thyanta* spp. (Table 1.2). Overall, combining nymphs and adults, 10 stink bug taxa accounted for 99.2% of individuals in soybean fields (Tables 1.1–1.2). Ten additional taxa accounted for less than 0.8% of the total number of stink bugs collected in this study (Tables 1.1–1.2).

Relative abundance of species and subspecies of stink bug adults (i.e., after exclusion of rare species) differed significantly within each state [Friedman test results: Indiana ( $\chi^2 = 83.2$ ,  $df = 9$ ,  $P = <0.001$ ), Ohio ( $\chi^2 = 41.5$ ,  $df = 9$ ,  $P = <0.001$ ), Minnesota ( $\chi^2 = 98.2$ ,  $df = 9$ ,  $P = <0.001$ ), North Dakota ( $\chi^2 = 70.9$ ,  $df = 9$ ,  $P = <0.001$ ), South Dakota ( $\chi^2 = 42.6$ ,  $df = 9$ ,  $P = <0.001$ ), Nebraska ( $\chi^2 = 96.1$ ,  $df = 9$ ,  $P = <0.001$ ), Kansas ( $\chi^2 = 35.3$ ,  $df = 9$ ,  $P = <0.001$ ), Missouri ( $\chi^2 = 65.0$ ,  $df = 9$ ,  $P = <0.001$ ), Michigan ( $\chi^2 = 51.7$ ,  $df = 9$ ,  $P = <0.001$ )] (Fig. 1.2). For adults, *E. variolarius* was generally more abundant in states in the northwestern part of the region and *C. hilaris* was generally more abundant in states in the southeastern part of the region (Fig. 1.2). Similarly, the relative abundance of genera of nymphs differed significantly within each state [Friedman test results: Indiana ( $\chi^2 = 50.4$ ,  $df = 4$ ,  $P = <0.001$ ), Ohio ( $\chi^2 = 22.6$ ,  $df = 4$ ,  $P = <0.001$ ), Minnesota ( $\chi^2 = 49.6$ ,  $df = 4$ ,  $P = <0.001$ ), North Dakota ( $\chi^2 = 40.9$ ,  $df = 4$ ,  $P = <0.001$ ), South Dakota ( $\chi^2 = 16$ ,  $df = 4$ ,  $P = <0.01$ ), Nebraska ( $\chi^2 = 43.6$ ,  $df = 4$ ,  $P = <0.001$ ), Kansas ( $\chi^2 =$

16.1, df = 4, P = <0.01), Missouri ( $\chi^2 = 45.4$ , df = 4, P = <0.001), Michigan ( $\chi^2 = 25.1$ , df = 4, P = <0.001)] (Fig. 1.3). For nymphs, *Euschistus* spp. were generally more abundant in states in the northwestern part of the region and *C. hilaris* was generally more abundant in states in the southeastern part of the region (Fig. 1.3). *Halymorpha halys* adults and nymphs were more abundant in states in the eastern part of the region (e.g., Indiana and Michigan), and were rare or not detected in states in the western part of the region (Fig. 1.2–1.3). The exotic stink bugs *P. guildinii* and *B. hilaris* were not detected in this study.

### ***Species richness and diversity***

Chao2 richness indices differed significantly across states ( $\chi^2 = 24.327$ , df = 8, P < 0.01), but not between years ( $\chi^2 = 0.040$ , df = 1, P = 0.841) (Fig. 1.4A). Across years, Missouri had the highest index for species richness ( $4.5 \pm 0.4$ ) (Fig. 1.4A). Indiana ( $4.0 \pm 0.3$ ) had the second highest index for species richness, but it did not differ from those of Minnesota ( $3.2 \pm 0.3$ ) or Missouri (Fig. 1.4A). To compare species diversity across states, sample-based rarefaction curves were created for each field and data were standardized by interpolating to a sample effort of 48 sample units. Simpson's diversity indices did not differ significantly across states ( $\chi^2 = 8.783$ , df = 8, P = 0.360) or years ( $\chi^2 = 0.054$ , df = 1, P = 0.814).

### ***Temporal dynamics and maximum abundance***

Across years and locations, mean density (i.e., stink bugs / 25 sweeps) of herbivorous adult stink bugs peaked in the “full bloom” (R2) soybean growth stage (Fig. 1.5A). Mean density of herbivorous nymphs and total herbivorous stink bugs (i.e., nymphs and adults) peaked in the “beginning maturity” (R7) soybean growth stage (Fig. 1.5A). For predatory stink bugs, mean density of adults peaked in the “full pod set” (R4) soybean growth stage (Fig. 1.5B). Mean density of predatory nymphs and total predatory stink bugs (i.e., nymphs and adults) peaked in the “beginning maturity” (R7) soybean growth stage (Fig. 1.5B).

Across states, herbivorous stink bug maximum mean density per field ranged from 0.0 – 15.25 stink bugs / 25 sweeps in 2016, and from 0.0 – 4.10 stink bugs / 25 sweeps in 2017. For

herbivores, comparison of stink bug maximum mean density across states and years showed that there was a significant difference of stink bug maximum mean density across states and it was higher in 2016 than in 2017 (i.e., significant interaction) ( $\chi^2 = 29.974$ ,  $df = 5$ ,  $P < 0.001$ ) (Fig. 1.6A–B). Except for Ohio and Kansas, higher herbivorous maximum mean density occurred in southern states. In both years, the highest herbivorous maximum mean abundance was observed in Missouri (2016:  $7.0 \pm 1.4$  bugs / 25 sweeps; 2017:  $2.8 \pm 0.6$ ), and it was not different from Nebraska (2016:  $4.7 \pm 0.6$ ; 2017:  $1.6 \pm 0.4$ ) and, in 2017, it was not different from Indiana ( $2.0 \pm 0.3$ ) and Michigan ( $1.8 \pm 0.4$ ).

For predatory stink bugs, maximum mean density ranged from 0.0 – 0.83 stink bugs / 25 sweeps in 2016, and from 0.0 – 0.2 stink bugs / 25 sweeps in 2017. Maximum mean abundance of predatory stink bugs was significantly different across states ( $\chi^2 = 18.484$ ,  $df = 8$ ,  $P < 0.05$ ) and it was significantly higher in 2016 than in 2017 ( $\chi^2 = 5.677$ ,  $df = 1$ ,  $P < 0.05$ ) (Fig. 1.6C–D). In contrast to herbivores, predatory stink bug maximum mean abundance did not show a pattern of geographical distribution across states. For predators in 2016, Nebraska ( $0.21 \pm 0.07$ ) had the highest maximum mean abundance of stink bugs and it did not differ from South Dakota ( $0.12 \pm 0.07$ ), Minnesota ( $0.07 \pm 0.01$ ) and Missouri ( $0.09 \pm 0.12$ ). In 2017, Missouri ( $1.8 \pm 0.4$ ) had the highest maximum mean abundance of predatory stink bugs, but it was not different from all other states.

## **Discussion**

Stink bugs are an emerging threat to soybean in the North Central Region of the U.S. (Hunt et al., 2011, 2014; Michel et al., 2015; Koch et al., 2017). However, because of their historically minor significance as pests in soybean in much of the region, there is a lack of region-specific knowledge about this pest complex. Here, we present the first regional characterization of the stink bug community in soybean fields across the North Central Region of the U.S.



A total of 20 stink bug species and subspecies (nymphs and adults combined) were collected across states and years sampled (Table 1.1), and all these taxa have been previously reported to occur in the North Central Region of the U.S. (McPherson, 1982; Packauskas, 2012; Rider, 2012; Sites et al., 2012; Swanson, 2012; Koch et al., 2014; Koch et al., 2017). Among these species, the herbivores *E. variolarius*, *E. tristigmus*, *E. servus*, *C. hilaris*, *H. halys*, *T. c. acerra* and the predator *P. maculiventris* accounted for over 98% of the stink bug community across the region. Similar results have been observed in more focused studies in Minnesota soybean (Koch and Pahs, 2014; Koch and Rich, 2015). Management recommendations for stink bugs in soybean in the North Central Region should consider differences in biology and pest potential among these species (McPherson and McPherson, 2000; Koch et al., 2017). The biology, identification and management of these species has been detailed in the literature (McPherson and McPherson, 2000; Panizzi et al., 2000; Koch et al., 2017). Susceptibility to pesticides is reported to vary between the native species *E. servus* and *C. hilaris* (Kamminga et al., 2009; Willrich et al., 2003; Snodgrass et al., 2005), while damage caused to soybean is similar among *C. hilaris*, *E. servus* and *E. tristigmus* (McPherson et al., 1979; Jones and Sullivan, 1982; McPherson and McPherson, 2000). However, these studies do not include *E. variolarius*, which is the most abundant species in the North Central Region. Future studies should compare the susceptibility of *E. variolarius* to pesticides and its damage to soybean compared to that of other species.

In addition, several species (i.e., *A. cynicus*, *B. dimidiata*, *Chlorochroa* spp., *C. delius*, *C. lintneriana*, *H. limbolarius*, *M. lugens*, *O. pugnax*, *T. calceata* and *T. semivittata*) were detected infrequently in soybean across the region. The generally low abundance of these species in soybean fields may be due to their preferences for other hosts (i.e., grasses, small grain, wild hosts, ornamental plants and small fruits) over soybean. It is likely that their presence in soybean was incidental, as it was reported by others in the region (McPherson and McPherson, 2000; Koch and Pahs, 2014; Koch et al., 2017; Pezzini et al., 2018).

The composition of the stink bug community in soybean in the North Central Region of the U.S. differed from that reported in soybean in other regions of the U.S. For example, in the Mid-Atlantic Region, *H. halys* is documented as the most abundant stink bug species in soybean (Nielsen and Hamilton, 2009). In southern U.S., the stink bug community is comprised mostly of *Nezara viridula* (Linnaeus), *C. hilaris* and *E. servus* (McPherson et al., 1993; Smith et al., 2009) and, more recently, the exotic *P. guildinii* became the most abundant species in Louisiana soybean (Temple et al., 2013).

Across years, our results demonstrated that stink bug species richness (i.e., Chao2) differed across states, while diversity (i.e., Simpson's index) was the same across the region. The lack of differences in diversity across states may be attributed to the observed dominance of few species in our study (e.g., 2 adult species accounted for over 75% of the total) (Table 1.1). Simpson's index is heavily weighted toward the most abundant species (Magurran, 2004). Similarly, other studies have observed a lack of difference in Simpson's index for communities with a few dominant species (Mokam et al., 2014; Gogoi et al., 2017).

Trends observed for relative abundance of adult stink bug species and subspecies across states of the North Central Region of the U.S. (Fig. 1.2–1.3) were similar to those reported in the literature. For example, *E. variolarius* is reported as the most common species in northern states (Koch and Pahs, 2014; Koch et al., 2017) and it is relatively uncommon in southern states of the U.S. (i.e., below 37° latitude) (McPherson et al., 1993; Smith et al., 2009; Temple et al., 2013). In contrast, *C. hilaris* is generally more abundant in southern states (Smith et al., 2009; Tindall and Fothergill, 2011; Temple et al., 2013), and northern populations of this species appear to result from semi-migratory populations from southern states (Panizzi et al., 2000). *Chinavia hilaris* has a preference for feeding on soybean and green beans on its southern distribution; however, it feeds on a wider range of hosts in northern areas (Panizzi et al., 2000), which may result in the populations being more dispersed over the landscape and consequently less abundant in any given habitat. Furthermore, *H. halys*, the new invasive species, was more abundant in eastern states

such as Michigan, Indiana and Ohio (2016 data not shown), than western states in the region. In fact, although it has been reported through much of the region, abundant, agriculturally damaging populations of *H. halys* are still restricted to the more eastern states (Northeastern IPM Center, 2018). The higher abundance of *H. halys* in eastern areas can be attributed to its spread westward from the mid-Atlantic region, where it was first detected (Hoebeke and Carter, 2003). Results presented here indicate that *H. halys* has surpassed the relative abundance of several native species in soybean Michigan and Indiana. As much of the North Central Region of the U.S. is suitable for establishment of *H. halys* (Zhu et al., 2012; Wallner et al., 2014), continued survey efforts are required to document resulting species shifts and to inform growers of the presence and potential impacts of this new pest.

In general, differences in community of stink bugs across states within the North Central Region of the U.S. and in comparison to other regions may be driven by differences in geographic distributions and overwintering abilities of the species (McPherson and McPherson, 2000), and compositions of agricultural landscapes (Tillman et al., 2009; Reay-Jones, 2010; Reeves et al., 2010; Herbert and Toews, 2011; Venugopal et al. 2014). Although most stink bugs are reported to feed on a wide variety of plants, details such as host preferences, host range and overwintering sites differ somewhat among species (McPherson and McPherson, 2000; Koch et al., 2017). Because stink bugs colonize other hosts prior to migrating to soybean, the composition of surrounding habitats may influence the stink bug species that will occupy soybean fields later in the season (Tillman et al., 2009; Reay-Jones, 2010; Reeves et al., 2010; Herbert and Toews, 2011; Venugopal et al., 2014). Moreover, studies have shown that fields adjacent to wooded areas and to preferred host plants for stink bugs may experience higher stink bug densities because these may provide overwintering sites and extended food sources (Reay-Jones, 2010; Tillman et al., 2009; Reeves et al., 2010; Leskey et al., 2012; Rice et al., 2014; Venugopal et al., 2014). Differences of landscape composition may contribute to differences observed across states and at regional levels, where the landscape of the North Central Region of the U.S. is predominantly

composed of soybean and corn while the mid-Atlantic and southern regions have more diverse agricultural landscapes (NASS, 2017). Therefore, future work should evaluate the effects of surrounding habitats on stink bug species composition and abundance in soybean fields in the North Central Region of the U.S.

Results of herbivorous stink bug maximum mean density in our study showed that economic infestations occurred on southern states of the region. In 2016, 2 fields (i.e., located in Missouri) reached the economic threshold for stink bugs for soybean grown for grain (i.e., 10 stink bugs / 25 sweeps), and 9 fields (i.e., 5 in Missouri, 3 in Nebraska and 1 in Indiana) reached the economic threshold for stink bugs for soybean grown for seed (i.e., 5 stink bugs / 25 sweeps). In 2017, no field reached the economic threshold for stink bugs. Differences of maximum mean abundance of stink bugs observed across states may be attributed to warmer temperatures in southern states. Year to year variability in abundance may be attributed to abiotic factors such as precipitation, winter temperatures and accumulation of degree-days across the season.

Patterns of seasonal abundance of herbivorous stink bugs observed in soybean fields in the present study (Fig. 1.5A) may be attributed to landscape-level movement of stink bugs in search of food resources (McPherson and McPherson, 2000; Venugopal et al., 2014). Similar seasonal patterns have been observed in other surveys of soybean (Smith et al., 2009; Tillman, 2011; Tillman et al., 2009; Koch and Pahs, 2014; Koch and Rich, 2015). Overwintering adults of herbivorous stink bugs emerge in the spring and feed on early-maturing wild and cultivated hosts. As the season progresses, the suitability of early-maturing hosts decreases, and the suitability of soybean increases with development of pods and seeds (McPherson and McPherson, 2000; Panizzi et al., 2000).

Populations of predatory stink bugs increased at later soybean growth stages (Fig. 1.5B). To our knowledge, no studies exist on the seasonal abundance of predatory stink bugs in soybean fields. *Podisus maculiventris*, the most abundant predatory species in our study, is a generalist predator feeding on more than 70 insect species and it can be found in a wide range of habitats

including woods, shrubs, orchards and crops (De Clercq, 2000). Specifically, *P. maculiventris* is an important predator of field crop pests, including larvae of several noctuids (Lepidoptera: Noctuidae) and the Colorado potato beetle (Coleoptera: Chrysomelidae) (Marston et al., 1978; De Clercq, 2000). The use of this generalist predator as a biological control of crop pests as well as its ability to find and consume prey has been well reviewed (Waddill and Shephard, 1975; Marston et al., 1978; De Clercq, 2000; Gyawaly and Park, 2013). Information presented here on seasonal dynamics and maximum mean abundance of *P. maculiventris* across states may improve integration of this predator as a biological control agent in soybean pest management programs in the North Central Region of the U.S. In addition, management recommendations should take in consideration that the susceptibility of the predatory *P. maculiventris* to insecticides may be similar or greater than that of herbivorous stink bug species (Tillman and Mullinix, 2004).

### **Conclusion**

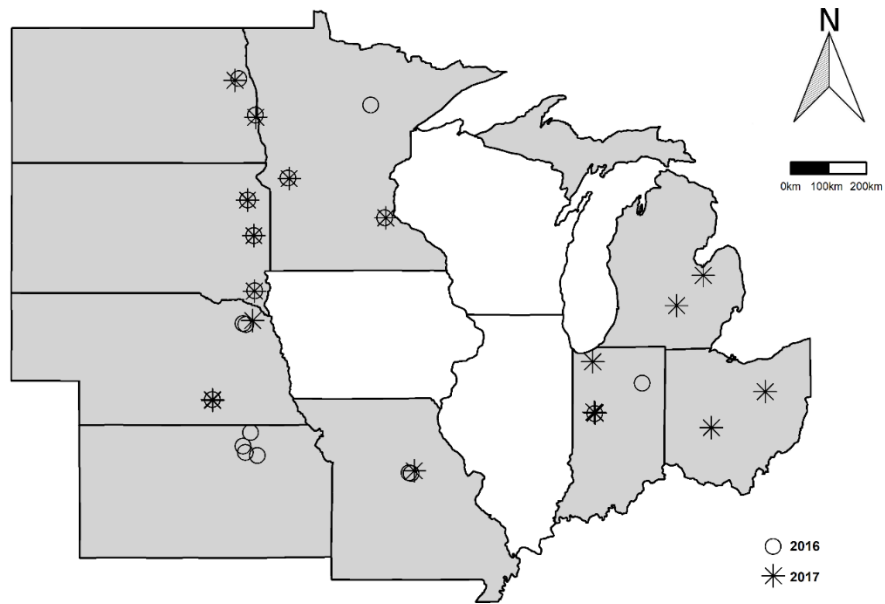
Results presented here provide a foundation for development of integrated pest management programs for stink bugs in soybean in the North Central Region of the U.S. Specifically, we provided important basic information on the community composition and seasonal abundance of herbivorous and predatory stink bugs that can be used by growers and researchers. More attention should be given to stink bugs in soybean fields in southern areas of the region, where stink bugs more often reached economically damaging infestations. In contrast, areas located on northern areas of the region experienced relatively low levels of risk from stink bugs to soybean production. In general, we demonstrated that the stink bug community of the region is dominated by *Euschistus* spp. in the northwestern and *C. hilaris* in the southeastern parts of the region. Furthermore, our study documents that the invasive *H. halys* was among the most abundant species in the eastern part of the region. Therefore, future studies should evaluate the potential impact of and management tactics for *Euschistus* spp., *C. hilaris* and *H. halys* since these are likely the most problematic species in the region.

**Table 1.1:** Relative abundance of taxa of stink bug adults collected across states and years in the North Central Region of the U.S.

Species	Relative Abundance (%)
Subfamily: Pentatominae	
<i>Banasa dimidiata</i>	0.05
<i>Chinavia hilaris</i>	25.81
<i>Coenus delius</i>	0.35
<i>Cosmopepla lintneriana</i>	0.25
<i>Euschistus servus servus</i>	2.35
<i>Euschistus servus euschistoides</i>	6.93
<i>Euschistus servus</i> hybrid	0.77
<i>Euschistus tristigmus luridus</i>	1.96
<i>Euschistus tristigmus tristigmus</i>	0.77
<i>Euschistus variolarius</i>	50.79
<i>Halyomorpha halys</i>	1.80
<i>Holcostethus limbolarius</i>	0.18
<i>Mormidea lugens</i>	0.03
<i>Oebalus pugnax</i>	0.19
<i>Thyanta calceata</i>	0.18
<i>Thyanta custator accerra</i>	2.75
<i>Trichopepla semivittata</i>	0.02
Subfamily: Asopinae	
<i>Apoecilus cynicus</i>	0.09
<i>Podisus maculiventris</i>	4.75

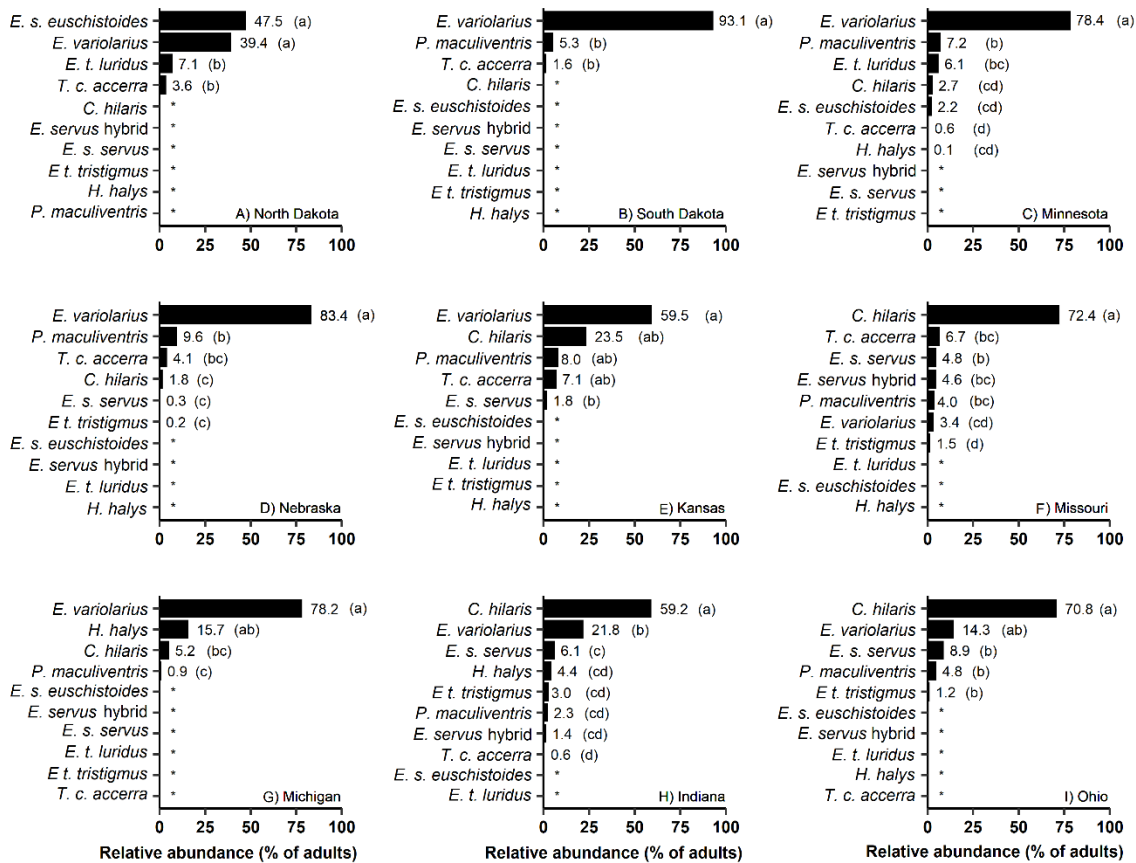
**Table 1.2:** Relative abundance of taxa of stink bug nymphs collected across states and years in the North Central Region of the U.S.

Species	Relative Abundance (%)
Subfamily: Pentatominae	
<i>Chinavia hilaris</i>	21.93
<i>Chlorochroa</i> spp.	0.33
<i>Euschistus</i> spp.	73.75
<i>Halyomorpha halys</i>	2.18
<i>Oebalus pugnax</i>	0.02
<i>Thyanta</i> spp.	0.64
Subfamily: Asopinae	
<i>Podisus</i> spp.	1.15

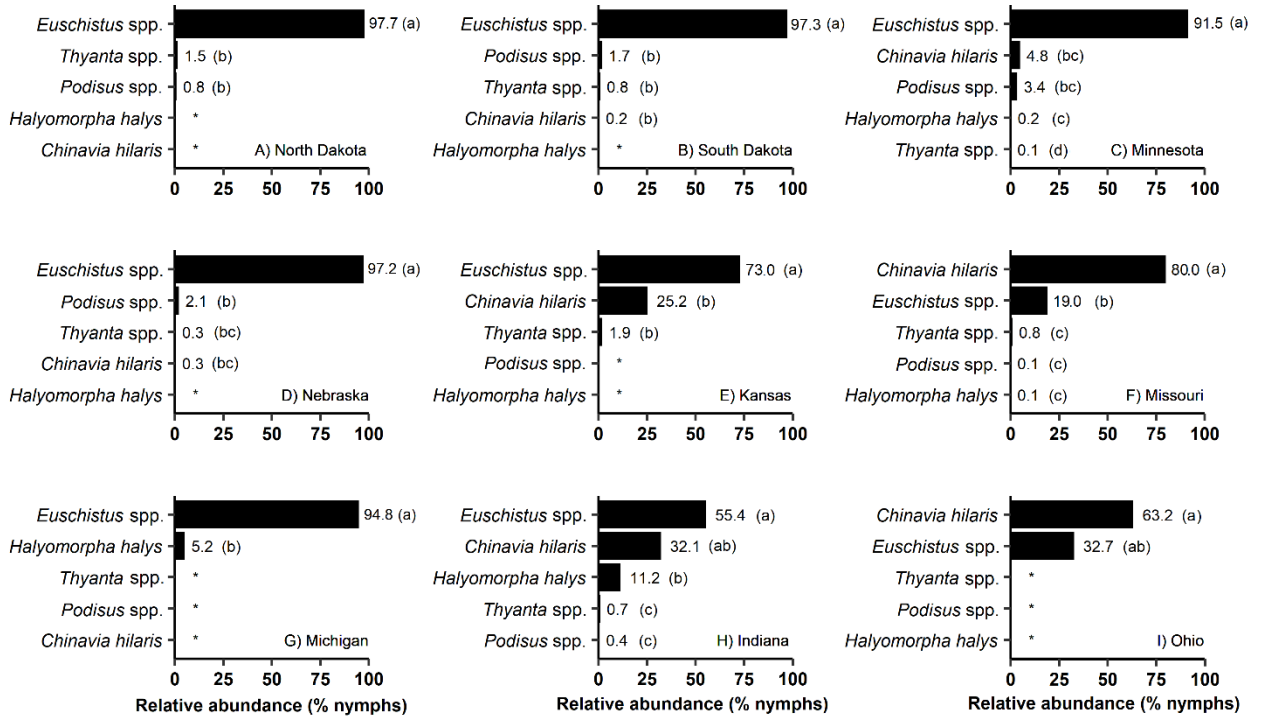


**Figure 1.1:** Location of sites where soybean fields were sampled for stink bugs in the North Central Region of the U.S. in 2016 and 2017. Shading indicates states included in this research. In each year, one to four soybean fields were sampled for stink bugs at each site.

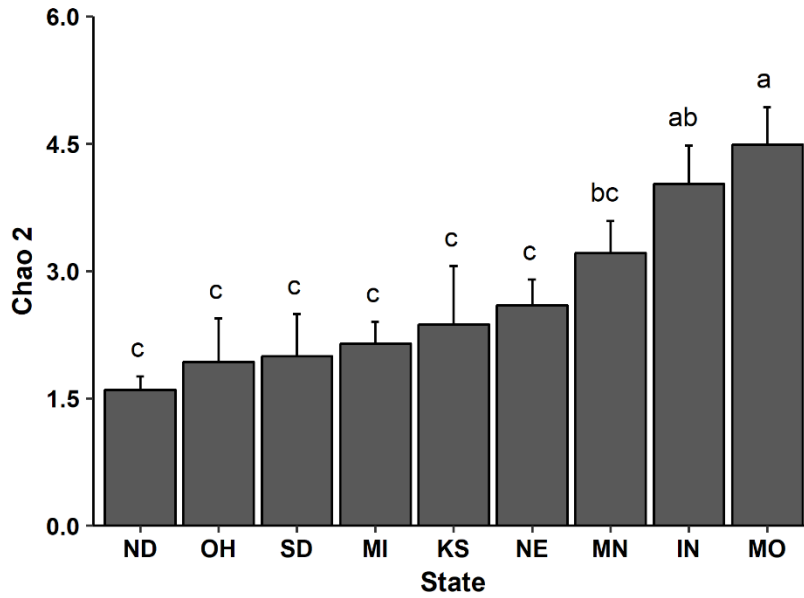




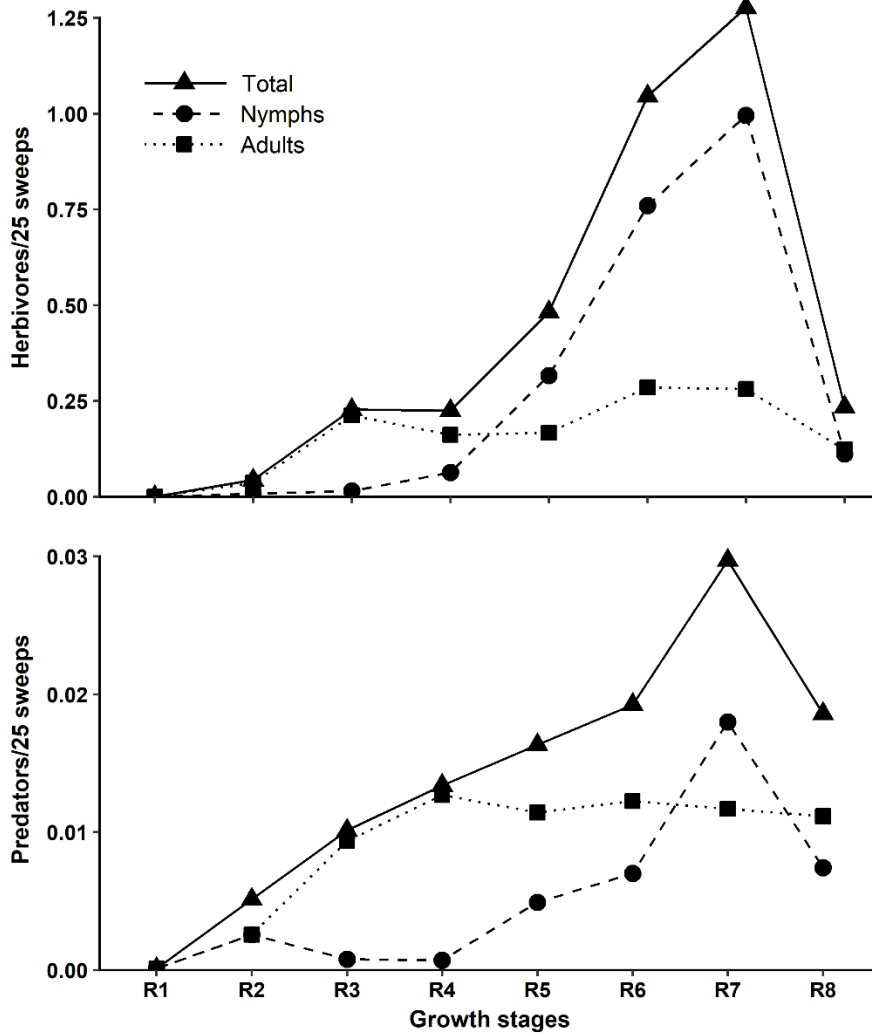
**Figure 1.2:** Mean relative abundance (%) of species of stink bug adults in soybean across years and fields for states in the North Central Region of the U.S. Numbers after bars indicate mean values. Means followed by the same letter were not significantly different at  $\alpha = 0.05$  using Friedman test and *post-hoc* multiple comparison. Asterisks (\*) indicate that the species or subspecies was not detected in the state.



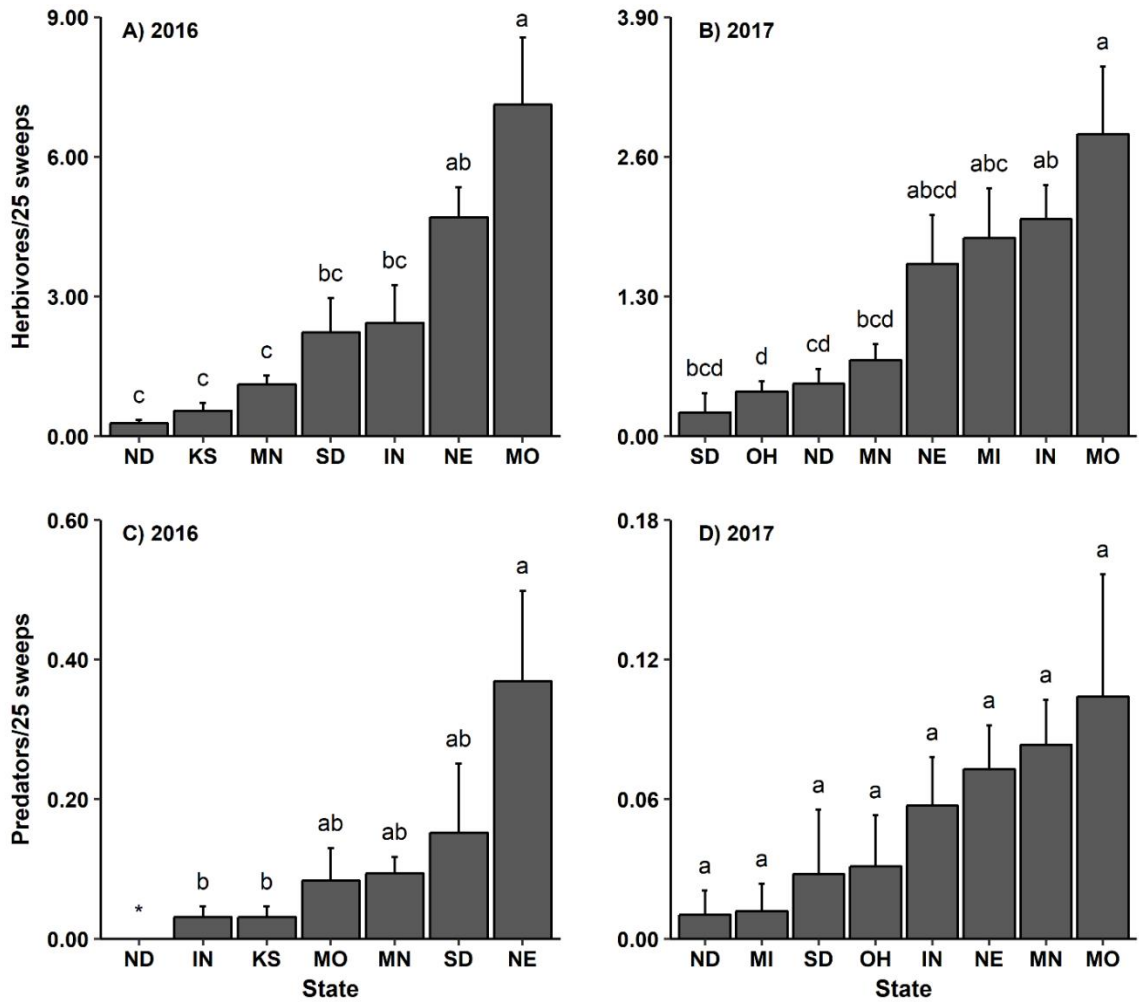
**Figure 1.3:** Mean relative abundance (%) of genera of stink bug nymphs in soybean across years and fields for states in the North Central Region of the U.S. Numbers after bars indicate mean values. Means followed by the same letter were not significantly different at  $\alpha = 0.05$  using Friedman test and *post-hoc* multiple comparison. Asterisks (\*) indicate that the stink bug genus was not detected in the state.



**Figure 1.4:** Mean ( $\pm$ SEM) Chao2 species richness for stink bug communities sampled from soybean across years and fields for states in the North Central Region of the U.S. (IN=Indiana, KS=Kansas, MI=Michigan, MN=Minnesota, MO=Missouri, NE=Nebraska, ND=North Dakota, OH=Ohio, SD=South Dakota). Means with same letters were not significantly different at  $\alpha = 0.05$  using Tukey-Kramer-adjusted pairwise comparisons of least square means.



**Figure 1.5:** Mean abundance of herbivorous (A) and predatory (B) stink bugs from beginning-bloom (R1) through full maturity (R8) soybean growth stages across years and fields in the North Central Region of the U.S.



**Figure 1.6:** Mean ( $\pm$ SEM) maximum abundance of herbivorous (A, B) and predatory (C, D) stink bugs in soybean fields for states in the North Central Region of the U.S. (IN=Indiana, KS=Kansas, MI=Michigan, MN=Minnesota, MO=Missouri, NE=Nebraska, ND=North Dakota, OH=Ohio, SD=South Dakota) in 2016 (A, C) and 2017 (B, D). Means with same letters were not significantly different at  $\alpha = 0.05$  using Tukey-Kramer-adjusted pairwise comparisons of least square means. Asterisks (\*) indicate zero.

## **Chapter 2**

### **Spatial Patterns and Sequential Sampling Plans for Stink Bugs (Hemiptera: Pentatomidae) in Soybean in the North Central Region of the U.S.**

## Summary

Stink bugs (Heteroptera: Pentatomidae) are an emerging threat to soybean in the North Central Region of the U.S. Consequently, region-specific scouting recommendations for stink bugs are needed. The aim of this study was to characterize the spatial pattern of stink bugs in soybean field and develop sampling plans. In 2016 and 2017, a total of 125 fields distributed across 9 states were sampled using sweep nets. The spatial patterns of stink bug species (i.e., *Chinavia hilaris* and *Euschistus* spp.), life stages (i.e., nymphs and adults) and locations in the field (i.e., edge and interior) were assessed using Taylor's power law. Results showed that stink bugs were aggregated, but extent of aggregation varied by species, life stage and location. Sequential sampling plans were developed for each combination of species, life stage and location. Furthermore, a more practical combined sequential sampling plan was developed to be used by growers for management purposes. Validation of the combined sampling plan showed that an average of 40 sample units would be necessary to achieve precision of 0.25 for stink bug densities commonly encountered across the region. However, based on the gradient of stink bug densities found across the region, more practical sample sizes (5–10 sample units) would be sufficient in states in the southeastern part of the region where stink bug densities were higher, whereas impractical sample sizes (>100 sample units) may be required in the northwestern part of the region where densities were lower. Our findings provide research-based sampling recommendations for research and management of these emerging pests of soybean.

## Introduction

The North Central Region of the U.S. ranks first for soybean, *Glycine max* (L.) Merr., production, accounting for 82% of the national soybean acreage (NASS, 2018). The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is currently the most important insect pest of soybean in the region (Ragsdale et al., 2007; Hurley and Mitchell, 2014). However, stink bugs (Hemiptera: Pentatomidae) are an increasing concern for soybean in this region for several reasons (Koch et al., 2017). The brown marmorated stink bug, *Halyomorpha halys* (Stål), a significant pest of fruit, vegetable and field crops on the East Coast, is currently invading the North Central Region (Leskey et al., 2012; Rice et al., 2014). In addition, there has been an increase in native stink bug species, including green (*Chinavia hilaris* (Say)), brown (*Euschistus servus* (Say)), onespotted (*E. variolarius* (Palisot de Beauvois)), and redshouldered (*Thyanta custator acerra* McAtee) stink bugs in field crops (Hunt et al., 2011, 2014; Michel et al., 2015). Stink bugs feed on soybean seeds and pods using piercing-sucking mouthparts, which results in lower seed quality and yield (McPherson and McPherson, 2000). Due to the increasing concern with stink bugs in the North Central Region of the U.S., region-specific management recommendations are needed (Koch et al., 2017).

Integrated pest management (IPM) programs rely upon accurate sampling to estimate pest densities in the field (Naranjo and Hutchison, 1997; Moon and Wilson, 2008; Pedigo and Rice, 2009). Indeed, approximately 88% of the soybean acreage in the North Central Region is currently scouted for insects (NASS, 2018). An underlying concept of IPM is that no action is taken against a pest, unless that pest is present at a density that is likely to result in economic crop loss (Pedigo and Rice, 2009). Sampling methods for estimating stink bug density in soybean include the use of sweep nets, which is reported to be more cost-effective than other traditional sampling methods (Todd and Herzog, 1980; Sane et al., 1999) and more efficient at lower insect



densities (Reay-Jones et al., 2009). In the North Central Region, 23 species of stink bugs are likely to occur in soybean fields (Koch et al., 2017), with *C. hilaris* and species of *Euschistus* being the most abundant (Koch and Pahs, 2014; Koch and Rich, 2015; Pezzini, Chapter 1). Because of general similarity of feeding habits in soybean, counts of species of the subfamily Pentatominae (i.e., herbivorous species) are combined for pest management purposes in soybean (McPherson et al., 1979; Jones and Sullivan, 1982; McPherson and McPherson, 2000). The economic threshold for stink bugs (i.e., nymphs and adults combined) in the North Central Region is 5 stink bugs per 25 sweeps for soybean grown for seed and 10 stink bugs per 25 sweeps for soybean grown for grain (Kogan, 1976; Koch et al., 2017). When stink bug density reaches the economic threshold, foliar insecticides are applied to protect soybean yields (Leskey et al., 2012; Bergmann and Raupp, 2014; Cira et al., 2017).

Reliability of sampling plans is determined by the precision level, measured by the variability of sample estimates (i.e., sample standard error) relative to their associated means (Todd and Herzog, 1980; Moon and Wilson, 2008). Sampling plans with poor levels of precision may misrepresent actual insect density, which may result in extra costs and environmental contamination from unnecessary insecticide sprays, yield loss due to unmitigated pest damage, or unreliable estimates for research purposes (Naranjo and Hutchison, 1997; Moon and Wilson, 2008). Currently, sampling recommendations for estimation of stink bug abundance in soybean fields vary across the U.S. (Table 1) and, to our knowledge, associated precision levels have not been characterized for these recommendations.

Sequential sampling for estimation is a sampling method that uses a pre-established precision level to estimate pest density in the field (Naranjo and Hutchinson, 1997; Todd and Herzog, 1980). Because of their flexibility in adjusting sample size relative to the number of insects collected, sequential sampling plans ensure that no more samples are collected than

necessary, by considering the desired precision level aimed to be achieved with sampling (Todd and Herzog, 1980). Sequential sampling plans are widely used because they save time and may achieve a higher precision level than conventional methods (i.e., fixed sample size sampling plans) (Todd and Herzog, 1980; Hodgson et al., 2004; Galvan et al., 2007; Reay-Jones et al., 2009).

The development of appropriate sampling plans for insects requires an understanding of the spatial pattern of the target pest, which may vary by species, abundance, life stage and landscape composition (Taylor, 1961; Vinatier et al., 2011). Green's method, a type of fixed-precision sequential sampling for estimation, uses parameters from Taylor's power law regressions to characterize the spatial pattern of the pest for calculation of sampling stop lines (i.e., when sampling achieves the pre-determined precision level) (Green, 1970). Taylor's power law is a robust method that describes the variance-mean relationship of the densities of a subject organism and thereby provides an expression of the spatial pattern of that organism (Taylor, 1961).

Stink bugs are polyphagous pests, and their spatial and temporal dynamics depend on availability of food resources and patterns of field colonization (McPherson and McPherson, 2000; Panizzi et al., 2000). Adults move to soybean fields in mid-summer, when soybeans reach reproductive growth stages (i.e., developing pods and seeds) (McPherson and McPherson, 2000; Panizzi et al., 2000; Koch and Rich, 2015). Within fields, stink bug densities tend to be greater at field edges (Tillman et al., 2009; Reeves et al., 2010; Leskey et al., 2012). During the colonization process, stink bug females lay clusters of eggs on leaves (Panizzi et al., 2000). First instars emerging from these eggs generally do not feed on the plants and remain aggregated near the egg mass, likely for symbiont acquisition (McPherson and McPherson, 2000). The subsequent four instars disperse to feed on plant material, but remain relatively close to the oviposition site

until they reach the winged adult stage that readily colonizes other parts of the field or other habitats (McPherson and McPherson, 2000).

Therefore, to improve pest management programs for stink bugs in soybean in the North Central Region of the U.S., the goals of this study were to characterize the spatial pattern of stink bugs in soybean fields, and to develop and validate a sequential sampling plan for stink bugs in the region. Results of this study are essential to improve sampling and management recommendations as stink bugs increase in number and importance in this key soybean production area of the U.S.

## **Materials and Methods**

### ***Field sites***

A standardized protocol was used to sample stink bugs in soybean fields distributed across the North Central Region of the U.S. In 2016, 63 soybean fields were sampled in 8 states: North Dakota, South Dakota, Nebraska, Kansas, Minnesota, Missouri, Indiana and Ohio (Table 2). In 2017, 62 fields were sampled from the same 8 states, plus Michigan. In general, fields in each state were at one to four separate sites, with one to four fields per site. Sites were 13 to 368 km apart within states and were located at university research stations or cooperating farms. Field sizes ranged from 0.3 to 120 ha (mean  $\pm$  SEM:  $17.6 \pm 1.8$  ha), row spacing was 76.2 cm, and soybean varieties, planting dates and management practices were representative of the respective states. Fields were generally sampled on a weekly basis from beginning-bloom (R1) through full maturity (R8) soybean growth stages (Fehr and Caviness, 1977), which spanned approximately mid-July to late September. A grand total of 125 fields were sampled during individual visits in the two years combined.

### ***Data collection***

In each field, sampling was stratified with sample units collected from a field edge (i.e., less than 10 m into the field) and from the field interior (i.e., more than 10 m into the field). A sample unit consisted of a set of 25 pendulum-style sweeps through the upper canopy of two adjacent soybean rows using a 39-cm diameter sweep net. Each pass of the net counted as one sweep, and consecutive sweeps were arranged in a “Lazy-8” pattern (Kogan and Pitre, 1980). Sample units were spaced at least 10 m apart. In most locations, 4 sample units were collected from the field edge and 8 sample units were collected from field interior on each sample date. However, in two locations in Minnesota, additional sample units (up to 50 sample units per field per sample date) were occasionally collected. After each set of 25 sweeps was collected, the net was emptied into a 20.3 × 25.4-cm plastic zipper-locking bag (Reloc Zippit™, Lima, Ohio) and labeled by date and location.

Bags were frozen soon after collection, rough sorted to extract stink bugs, and then specimens were shipped to the University of Minnesota for identification and quantification. Nymphs were identified to genus using DeCoursey and Esselbaugh (1962) and Evans (1985), and adults were identified to species and subspecies using keys in McPherson and McPherson (2000), Rider (2012), and Paiero et al. (2013). Voucher specimens of each species were placed in the University of Minnesota Insect Collection.

### *Assessment of spatial patterns*

Based on Kogan and Herzog (1980), we use the term “spatial pattern” as opposed to other commonly used terminology, such as “spatial distribution” or “spatial dispersion”. Comparisons of the spatial pattern of stink bugs by species, life stage and location were performed by calculating arithmetic means and variances of the number of stink bugs for each set of samples (i.e., collection of sample units from a given field on a given sample date). The relationships between matching sample variances and means were analyzed using Taylor’s power law.

Taylor's power law is  $s^2 = am^b$  from a linear regression of  $\log_{10}$ -transformed variances and  $\log_{10}$ -transformed means, where  $s^2$  is sample variance,  $m$  is sample mean,  $a$  is the antilog of the intercept and  $b$  is the slope of the regression. The term  $b$  is the relationship between sample variance on y-axis and sample mean on x-axis, which can range from  $b < 1$  to  $b = 1$  to  $b > 1$ , and indicates uniform, random or aggregated spatial patterns (Taylor, 1961). Excluding samples from fields where stink bugs were absent, there was a total of 1,416 data sets involving different field locations, stink bug species, and life stages.

Regression analyses were accomplished by fitting a generalized linear mixed model (GLMM) using the *lme4* package (Bates et al., 2015) in R version 3.4.3 (R Core Team, 2018), with log-variance as the response variable and fixed effects for log-mean, stink bug species, location in field (i.e., interior and edge), life stage (i.e., adult and nymph), and two- and three-way interactions. Random effects included year and state. A significant interaction between a regression's slope (i.e., log-mean) and any of the other fixed effects indicated slopes varied among corresponding species, life stages, or locations. Backward elimination was used to omit non-significant ( $P > 0.05$ ) fixed effects or interactions. Marginal R-squared ( $r^2$ ) for each regression was calculated using the package "MuMIn" (Barton, 2018).

### ***Sampling plan development***

Stop lines for sequential sampling plans were developed from 1,416 data sets (i.e., samples split by species, life stage and location) from individual field visits. Green's method defines stop lines as  $T_n \geq (an^{1-b}/D^2)^{1/(2-b)}$ , where  $T_n$  is cumulative number of stink bugs,  $n$  is growing number of sample units,  $D$  is desired precision (SEM/mean), and  $a$  and  $b$  are parameters of Taylor's power law (Green, 1970). Desired precision was set at 0.25, a recommended level for pest management purposes (Southwood, 1978), which has been used to develop sampling plans by others (Naranjo and Hutchison, 1997; Hodgson et al., 2004). Stop lines were calculated for a

minimum of  $n = 4$  sample units, which is the smallest sample size recommended for sampling stink bugs in rice, *Oryza sativa* L. (Poales: Poaceae) (Espino et al., 2008), in cotton, *Gossypium arboreum* L. (Malvales: Malvaceae) (Reay-Jones et al., 2009), and more generally for arthropods in soybean (Kogan and Pitre, 1980).

We compared stop lines for different stink bug species, life stages and field location by examining their respective 83% confidence intervals for overlap (Payton et al., 2003). This approach was used to account for the inflation of errors when multiple treatments with similar standard errors are compared (Payton et al. 2003). Confidence intervals were calculated with the *boot* procedure (Canty and Ripley, 2012)] in R, with sample sizes of 1,000 estimates of Taylor's power law parameters from relevant subsets of the original sample data, resampled with replacement. In turn, we calculated 8.5th and 91.5th percentiles for  $T_n$ s over a realistic range of chosen sample sizes. Because few of the stop lines among species, life stages and field strata were judged to be different based on overlap of confidence intervals, the data sets were combined, and a single sequential sampling plan was developed for more practical use by growers and researchers. Stop lines for the combined sequential sampling plan were developed from 526 of the 549 data sets from individual field visits (i.e., samples) and followed the same steps described above. The remaining 23 data sets were used for subsequent validation of the sampling plan.

### ***Sampling plan validation***

The combined sampling plan was validated using Resampling for Validation of Sampling Plans (RVSP) v. 2.0, following methods of Naranjo and Hutchison (1997). Simulations were performed by randomly selecting samples with replacement from the validation data sets until the corresponding stop line was exceeded. If resulting average precision values were better than the desired precision values, desired precision was relaxed to obtain the average desired precision (Burkness and Hutchison, 1997; Galvan et al., 2007; Tran and Koch, 2017). Resampling analysis

to determine the sample sizes required to attain the desired precision was based on 500 resampling iterations of each data set. The resulting sample sizes were averaged across validation data sets to obtain the average sample number (ASN) for estimating stink bugs in soybean fields under the ranges of densities of stink bugs experienced in the 23 validation data sets (i.e., samples). In addition, based on the above sampling plan, the currently recommended sample sizes for stink bugs (Table 1) were assessed for resulting levels of precision. In particular, simulations were performed by adjusting desired precision levels to achieve a targeted ASN of 6 and 10 sample units.

## **Results and Discussion**

A total of 373 and 381 samples were collected in 2016 and 2017, respectively. Across years, a total of 1,973 adults and 3,857 nymphs of *Euschistus* spp. and *C. hilaris* were collected from samples. Analyses and sample plan development focused on *C. hilaris* and *Euschistus* spp. because these species have potential damage to soybean in the North Central Region of the U.S. (Koch et al., 2017) and they represent over 95% of all individual stink bugs collected from soybean in the region (Pezzini, Chapter 1). *Euschistus* spp. are more prevalent in northwestern parts of the region and *C. hilaris* is more prevalent in southeastern parts of the region (Pezzini, Chapter 1). *Euschistus* spp. comprised 6 species and sub-species (*E. variolarius*, *E. servus servus* (Say), *E. s. euschistoides* (Vollenhoven), *E. servus* hybrid, *E. tristigmus tristigmus* (Say) and *E. t. luridus* Dallas). Additional stink bug species observed on our study were low in abundance and therefore were not included in our analyses. Results of species abundance observed here were consistent with earlier reports (McPherson and McPherson, 2000; Koch et al., 2017; Pezzini, Chapter 1).

### ***Assessment of spatial patterns***

Slopes (i.e., indication of spatial pattern) and intercepts (i.e., a sampling parameter) of Taylor's power law regressions from the 1,416 data sets (i.e., samples split by species, life stage and location) differed significantly among the two principal stink bug species (*Euschistus* spp. versus *C. hilaris*), their life stages (nymphs versus adults), and locations within fields (edge versus interior) (Table 3). These differences justified separation of the data for species-, stage- and location-specific assessments of spatial patterns (Table 4). Regression models for each species-, life stage- and location-specific analysis had slopes ( $b$ ) that were statistically greater than one ( $P < 0.05$ ), which indicated aggregated spatial patterns (Table 4).

Aggregated spatial patterns were previously described for stink bugs in soybean (Todd and Herzog, 1980), rice (Espino et al., 2008) cotton (Tillman et al., 2009; Reay-Jones et al., 2009) and corn, *Zea mays* L. (Babu and Reisig, 2018). In our study, both *C. hilaris* and *Euschistus* spp. nymphs and adults were aggregated (i.e., slopes greater than one) in field edges and interiors, but they exhibited different levels of aggregation (Table 4). Between species, *C. hilaris* were more aggregated than *Euschistus* spp. ( $P < 0.001$ ), and comparing life stages across species, nymphs were more aggregated than adults ( $P < 0.001$ ) (Table 4). Except for *Euschistus* spp. adults, stink bugs were more aggregated in field interiors than in field edges (Table 4).

Spatial pattern of an insect species is primarily determined by behavior (Davis, 1994), and the level of aggregation can differ among insect life stages (Taylor et al., 1961). For example, aggregation of *Nezara viridula* (L.) adults in rice fields was related to the sexual attraction of males to females (Nakasuji et al., 1965). In addition, aggregation is also mediated by species-specific aggregation pheromones produced by males that attracts males, females and nymphs (Aldrich et al., 1991; Tillman et al., 2010), and substrate-borne vibrational signaling (Harris and Todd, 1980; Lampson et al., 2013). The observed differences in spatial patterns between stink bug adults and nymphs is likely mediated by the ovipositional behavior of stink bug females and



differences in dispersal capacity of the life stages. Specifically, females lay eggs in clumps and resulting nymphs have limited mobility until later instars compared to mobile winged adults (Kiritani et al., 1965; Reay-Jones et al., 2009). Different levels of aggregation among stink bug species has also been observed in various crops such as wheat (*Triticum* L.) (Poales: Poaceae), cotton, peanut (*Arachis hypogaea* L.) (Fabales: Fabaceae), soybean and corn (Reay-Jones, 2014; Pilkay et al., 2015). Differences in the level of aggregation across locations in the field are likely driven by differences in the spatial and temporal colonization pattern and flight capacity of stink bug species in search of high quality of food sources (Tillman et al., 2009; Reay-Jones, 2014; Pilkay et al., 2015; Babu and Reisig, 2018).

### ***Sampling plan development***

To use sequential sampling plans for estimation, a crop scout tracks the cumulative number of stink bugs ( $T_n$ ) and the cumulative number of sample units ( $n$ ) in the Figures 2.1 and 2.2, and sampling ceases when the critical stop-line is reached. In our study, Taylor's power law results supported the separation of data by species, life stage and location in the field. Therefore, sampling plans for each combination of these covariates were developed for a fixed precision of 0.25 (Fig. 2.1). Estimating the density of stink bugs by species, life stage and/or location in the field may be of use for researchers with specific interests. Also, the availability of species-specific sampling plans is important because of the relative differences in geographical distribution and abundance of each species across the North Central Region (Pezzini, Chapter 1).

Species-, life stage-, and location-specific sampling plans may have value for detailed research purposes, but more generalized sampling recommendations are desirable for pest management purposes. Though statistical differences in spatial patterns existed among species, life stages and locations, 83% confidence intervals developed for sequential sampling plan stop lines for each combination generally overlapped (Fig. 2.1), suggesting these sampling plans may

be combined. Furthermore, thresholds for therapeutic treatment of stink bug infestations in soybean are based on counts of nymphs and adults of all herbivorous species combined (Boyd and Bailey, 2000; McPherson and McPherson, 2000; Stewart et al., 2010; Cullen, 2012; Greene, 2017; Koch et al., 2017; Raudenbush et al., 2017; Reisig, 2017; Tilmon, 2017). Such recommendations are supported by studies that show different stink bug species (i.e., *E. servus*, *N. viridula*, *C. hilaris*) cause similar damage to soybean (Blickenstaff and Huggins, 1962; McPherson et al., 1979; Jones and Sullivan, 1982; McPherson and McPherson, 2000). Therefore, a single sampling plan for a fixed-precision level of 0.25 was developed to estimate stink bug density across species (i.e., *C. hilaris* and *Euschistus* spp.), life stages (i.e., nymph and adult) and locations in fields (i.e., edge and interior) (Fig. 2.2).

### ***Sampling plan validation***

The combined sampling plan was validated through resampling of 23 field-collected data sets (i.e., samples) (Fig. 2.3, Table 5). Overall, required sample sizes decreased with increasing stink bug density (Fig. 2.3). During resampling simulations, average resulting precision exceeded the desired precision level of 0.25. Therefore, desired precision level was relaxed to 0.28 to achieve a resulting precision level of 0.25, as was done by Burkness and Hutchison (1997), Galvan et al. (2007) and Tran and Koch (2017). Results showed that an ASN of 40 sample units (i.e., sets of 25 sweeps) would be required to achieve a desired precision of 0.25 for stink bug densities ranging from 0.05 – 10.4 stink bugs / 25 sweeps (Fig. 2.3A, Table 4). Because collection of 40 sample units may be impractical for most applications, we calculated precision levels expected from commonly recommended sample sizes of 6 and 10 sample units (Table 1), which resulted in average precision levels of 0.54 and 0.43, respectively, across the range of stink bug densities observed in this 9-state study (Fig. 2.3B–D, Table 2.4). Because the commonly recommended sample sizes resulted in precision levels greater than 0.25, the use of those

sampling recommendations may provide unreliable estimates for the average range of stink bug densities commonly observed across the region.

Although the objective of our study was to create a common sampling plan for stink bugs in soybean fields across the North Central Region of the U.S., our results show that required sample sizes changed abruptly with stink bug mean densities, which varies considerably among different states and soybean growth stages (Pezzini, Chapter 1). For relatively low stink bug densities (i.e., < 0.5 stink bugs / 25 sweeps) often present in early soybean growth stages (i.e., vegetative through early reproductive stages) and in northern states such as North Dakota, South Dakota and Minnesota, as many as 109 sample units may be required to obtain an average precision level of 0.25. For intermediate stink bug densities (i.e., 1 to 3 stink bugs / 25 sweeps), sometimes observed in later soybean reproductive growth stages (i.e., beginning seed-set through beginning maturity) in states like Ohio and Michigan, fewer than 20 sample units would be required to achieve an average precision level of 0.25. Under relatively high stink bug densities (i.e., > 3 stink bugs / 25 sweeps), which were sometimes observed in Nebraska, Missouri and Indiana during later soybean reproductive stages (i.e., full seed set), as few as 5 sample units were needed achieve an average precision level of 0.25.

Comparison of the present sample size requirements with previously published sampling plans is difficult because of the lack of studies using the same sampling methods (Souza et al., 2014; Babu and Reisig, 2018). However, based on our results, currently recommended sample sizes (Table 1) will likely result in inadequate precision to estimate the density of herbivorous stink bugs in some soybean fields. The contrast between statistically-based sampling plans versus practical scouting recommendations is not unusual. A fixed-precision sampling plan for aster leafhopper, *Macrostelus quadrilineatus* Forbes (Hemiptera: Cicadellidae), in carrot, *Daucus carota* (L.) (Apiales: Apiaceae), estimated that 86 10-sweep sample units and 5 10-sweep sample

units were required to estimate low and high leafhopper densities at a precision level of 0.25, compared to an extension recommendation of 100 total-sweeps (O'Rourke et al., 1998). In cotton, 88 samples of 25 sweeps were estimated as necessary to reach a precision level of 0.30 for a density of 1 stink bug / 25 sweeps, but no sample size recommendation was available previously (Reay-Jones et al., 2009).

### **Conclusion**

This is the first study to examine spatial patterns of stink bugs in soybean in the North Central Region of the U.S., and to develop and validate sampling plans for these pests in that crop. Results showed that stink bug spatial patterns were aggregated, but varied by species, life stage and location in the field. Such knowledge is necessary for understanding the basic biology of these pests and for development of sequential sampling plans for research (Fig. 2.1) and more practically for pest management purposes (Fig. 2.2). Here, we provided a sequential sampling plan that can be used by growers to make reliable estimates of stink bug density in soybean fields in the North Central Region of the U.S. (Fig. 2.2). Also, we used field data to validate sampling plans and found that for stink bug densities commonly encountered across the region, an average sample size of 40 units (i.e., 40 x 25 sweeps) was necessary to achieve a precision of 0.25 (Fig. 2.3A).

Based on the gradient of stink bug densities found across the region (Pezzini, Chapter 1), sampling in soybean in southeastern states, where stink bug densities are higher, may attain acceptable precision levels with relatively small sample sizes (6 – 20 sample units), which matches some of the current recommendations for stink bug scouting (Table 1). In contrast, in the northwestern part of the region where lower stink bug densities are generally encountered, current sampling recommendations for stink bugs (Table 1) may provide an inadequate level of precision

and prohibitively large sample sizes (~100 sample units) would be required to achieve acceptable levels of precision (Fig. 2.3B-D). To estimate stink bug densities for making management decisions (i.e., economic thresholds of 5 to 10 stink bugs per 25 sweeps), as few as 6 sample units may be sufficient when densities are close to damaging levels ( $> 3$  stink bugs / 25 sweeps). However, researchers aiming to make reliable estimates of stink bug densities in some parts of the region will need to invest considerable sampling effort (i.e., large sample sizes). Also, practitioners should consider an arbitrary maximum number of sample units to balance cost (i.e., time) and reliability of estimates (i.e., precision), and to prevent never-ending sampling bouts when stink bug densities are very low. Further efforts should focus on evaluating different sample unit sizes (i.e., number of sweeps per sample unit) and cost-effectiveness (i.e., time spent sampling) of sampling plans for stink bugs.

**Table 2.1:** Scouting recommendations for stink bug sampling in soybean in the U.S.

Entity	Sample size <sup>a</sup>	Sample unit size <sup>b</sup>
Clemson University <sup>1</sup>	2	10
Mississippi State University <sup>2</sup>	-	100
NCSRP <sup>3</sup>	5	10
North Carolina State University <sup>4</sup>	12	15
Ohio State University <sup>5</sup>	“Several”	10
Purdue University <sup>6</sup>	-	20
Texas A&M University <sup>7</sup>	-	100
University of Kentucky <sup>8</sup>	Increases with field size	50
University of Missouri <sup>9</sup>	“Several”	-
University of Tennessee <sup>10</sup>	-	100
University of Wisconsin <sup>11</sup>	5	20

<sup>a</sup>Number of sample units.

<sup>b</sup>Number of sweeps / sample unit.

Hyphen (-) means information not indicated.

Sources: <sup>1</sup>Greene 2017, <sup>2</sup>Mississippi State University 2018, <sup>3</sup>Raudenbush et al. 2017, <sup>4</sup>Reisig 2017, <sup>5</sup>Tilmon 2017, <sup>6</sup>Krupke and Obermeyer 2017, <sup>7</sup>Texas A&M University

(<https://agrilife.org/extensionto/resources/management-guides/managing-soybean-insects-in-texas/soybean-insects-late-season-pests-r1-to-r7/>), <sup>8</sup>University of Kentucky

(<https://ipm.ca.uky.edu/content/green-stinkbug-soybeans>), <sup>9</sup>Boyd and Bailey 2000, <sup>10</sup>Stewart et al. 2010, and <sup>11</sup>Cullen 2012.

**Table 2.2:** Number of soybean fields sampled for stink bugs with sweep nets in cooperating states in the North Central Region of the U.S., by study year.

<b>State</b>	<b>2016</b>	<b>2017</b>
Indiana	8	8
Kansas	8	7
Michigan	0	8
Minnesota	12	8
Missouri	8	4
Nebraska	7	8
North Dakota	8	8
Ohio	8	8
South Dakota	4	3
Total	63	62

**Table 2.3:** Taylor’s power law regressions performed as analysis of covariance (ANCOVA) of sample variances (log-transformed) matched with sample means (log-transformed) of numbers of stink bugs per 25 sweeps from soybean fields in the North Central Region of the U.S., grouped by stink bug species (*Chinavia hilaris* vs. *Euschistus* spp.), life stage (nymph vs. adult) and location in field (edge vs. interior).

<b>Variables*</b>	$\chi^2$	<b>Df</b>	<b>P</b>
Log mean	6554.4	1	<0.001
Species	9.6	1	<0.005
Life stage	1.1	1	0.303
Location	10.7	1	<0.005
Log mean $\times$ Species	17.7	1	<0.001
Species $\times$ Location	6.3	1	<0.050
Log mean $\times$ Life stage $\times$ Species	10.2	1	<0.005

\*Non-significant interactions of fixed effects were removed from table.



**Table 2.4:** Summaries of Taylor’s power law parameters (*a*: intercept and *b*: slope) for counts of *Chinavia hilaris* and *Euschistus* spp. nymphs and adults in edges and interiors of soybean fields in the North Central Region of the U.S.

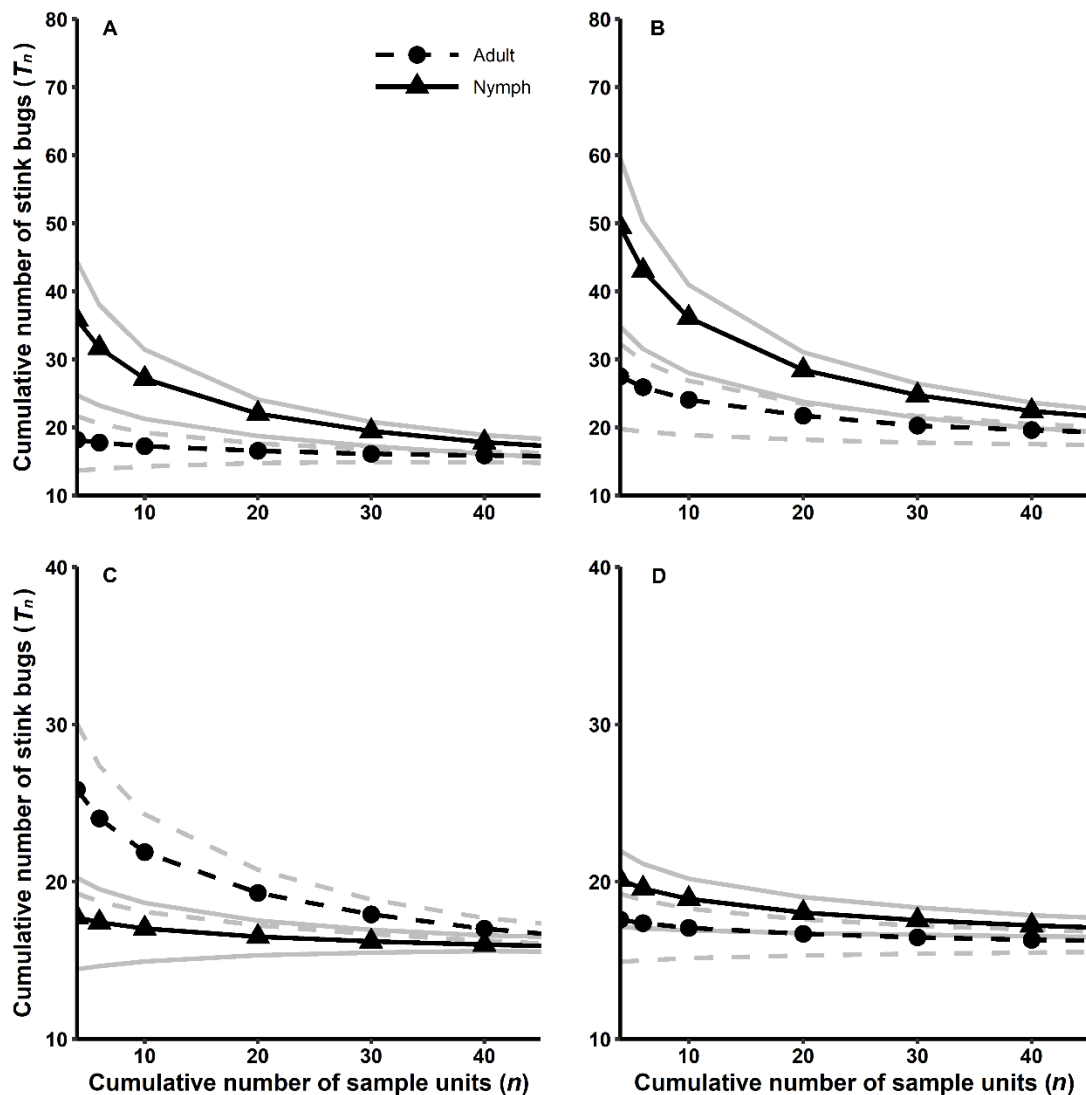
Species	Life stage	Location	<i>a</i> ± SE	<i>b</i> ± SE	N <sup>a</sup>	<i>r</i> <sup>2</sup>
<i>Chinavia hilaris</i>	Adult	Edge	0.019 ± 0.024	1.056 ± 0.051	110	0.71
		Interior	0.128 ± 0.036	1.128 ± 0.053	97	0.82
	Nymph	Edge	0.129 ± 0.022	1.233 ± 0.040	90	0.84
		Interior	0.211 ± 0.027	1.256 ± 0.042	83	0.92
<i>Euschistus</i> spp.	Adult	Edge	0.084 ± 0.023	1.154 ± 0.043	223	0.81
		Interior	0.021 ± 0.030	1.032 ± 0.038	262	0.88
	Nymph	Edge	0.017 ± 0.017	1.043 ± 0.033	271	0.72
		Interior	0.055 ± 0.016	1.063 ± 0.024	280	0.87
Combined <sup>b</sup>			0.146 ± 0.013	1.155 ± 0.014	549	0.93

<sup>a</sup>Number of data sets.

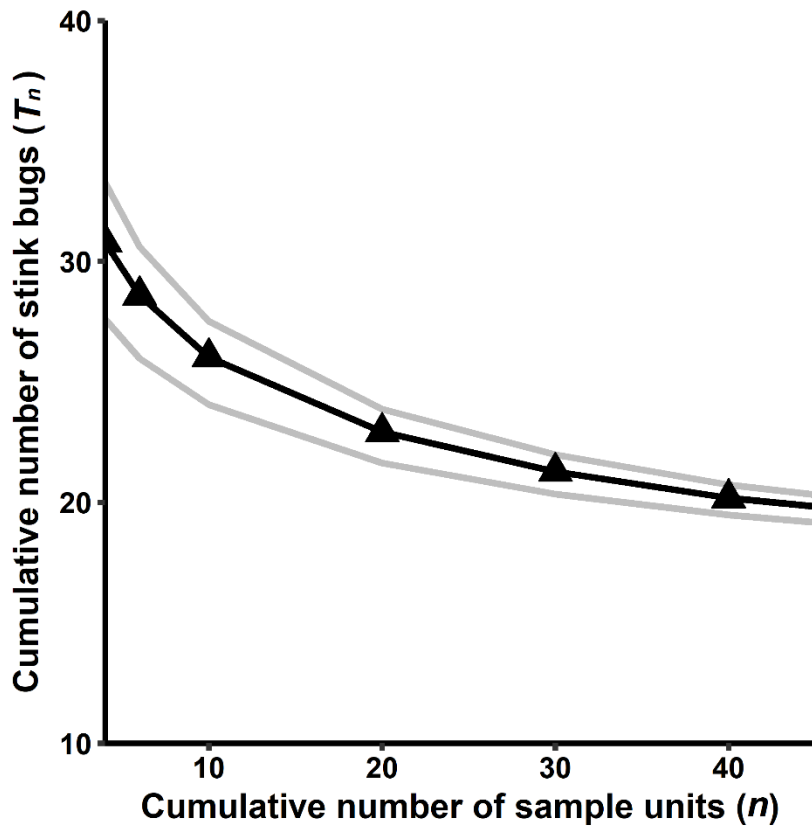
<sup>b</sup>Data combined across species, life stages and locations.

**Table 2.5:** Summary results for resampling validation of Green’s sequential sampling plan for estimating mean density of stink bugs in soybean in the North Central Region of the U.S., with desired fixed precision level = 0.25 for species, life stages and field locations combined, and a desired average sample number (ASN) of 6 and 10 sample units at an observed mean density of 1.8 stink bugs / 25 sweeps.

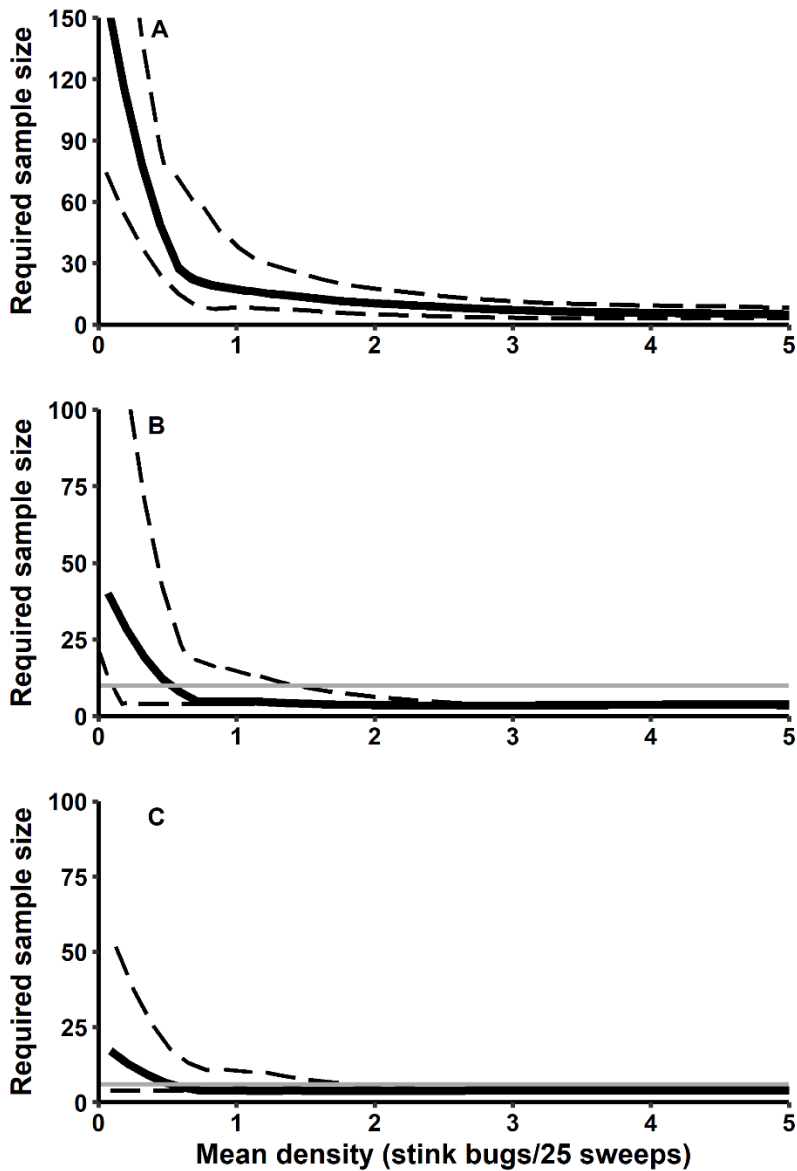
<b>Precision</b>			<b>ASN</b>		
D	Dmin	Dmax	N	Nmin	Nmax
<b>0.25</b>	0.12	0.35	40	19	72
0.43	0.04	0.77	<b>10</b>	4	34
0.53	0.06	0.90	<b>6</b>	4	21



**Figure 2.1:** Green's sequential sampling plans for estimating mean density of herbivorous stink bugs in soybean in the North Central Region of the U.S., by species, life stage and location in field, with 25-sweep sample units and precision level = 0.25. Sampling plans for (A) *Chinavia hilaris* in field edges and (B) interiors, and for (C) *Euschistus* spp. in field edges and (D) interiors. Black lines are stop lines for cumulative number of stink bugs ( $T_n$ ) and gray lines indicate bootstrap estimates of upper and lower 83% confidence limits for the stop lines.



**Figure 2.2:** Green's sequential sampling plan for estimating mean density of herbivorous stink bugs in soybean in the North Central Region of the U.S., using 25-sweep sample units, combined across species, life stages and field locations, and with precision level = 0.25. Black line indicates stop line for cumulative number of stink bugs ( $T_n$ ) and gray lines indicate upper and lower 83% confidence limits for the stop line.



**Figure 2.3:** Summary of validation of Green’s sequential sampling plan for all stink bug species, life stages and field locations in soybean in the North Central Region of the U.S. (A) Sampling plan for a desired precision level of 0.25. (B–C) Sampling plans for an average sample number (ASN) of 10 and 6 sample units, respectively. Black solid lines indicate mean required sample size and dashed lines indicate maximum and minimum required sample sizes. Solid gray horizontal lines indicate desired ASN.

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