ABSTRACT

BABU, ARUN. Farmscape Ecology of *Euschistus servus* (Hemiptera: Pentatomidae) in a Corn, Wheat, Soybean Ecosystem and Development of a Sampling Plan in Corn. (Under the direction of Dr. Dominic Reisig).

*Weed Manipulation and E. servus Densities in Corn.* Brown stink bug, *Euschistus servus* (Say), is a damaging pest of corn, *Zea mays* L., in the southeastern United States. In the agroecosystem, weeds serve as a bridge host for overwintered *E. servus* populations until they move to corn. Our objective was to reduce *E. servus* densities in corn by manipulating the weedy field borders with mowing and dicamba herbicide applications. In this farmscape, *E. servus* was the predominant stink bug species in the corn and *E. servus* adult density in the unmanaged weed plots started declining around the second week of May, followed by an increase in density in adjacent corn plots. This movement coincided with the seedling growth of corn. In 2016, dicamba application in the weedy field border resulted in a lower *E. servus* density in herbicide-treated weed plots compared to untreated plots. Despite this difference, weed manipulations did not lead to any significant changes in their density in corn. Further evidence suggests that a prominent external source of *E. servus*, other than field border weeds, in this farmscape is likely driving densities in corn.

*Flight Capacity of Adult E. servus.* In addition to crops, both weedy field borders and the wooded areas of a typical farmscape in the southeastern United States harbor *E. servus* host plants, many of which are temporally and spatially limiting in availability or nutritional suitability. Therefore, local dispersion of *E. servus* is required so that individuals efficiently track and utilize host resources. This research sought to establish the baseline flight capacity of adult *E. servus* across the season in relation to body weight, sex, overwintering status, nutritional status, and plant host using a computer-monitored flight mill system. Across all the flight
sessions, 90.7% of individuals tested flew in a range of 0-1 km, with an individual maximum flight distance of 6.4 km in 22-h. The mean total distance flown, mean flight speed and mean total time spent on actual flight varied across the season. The highest mean flight potential was observed soon after overwintering emergence and a relatively low flight potential was observed during the cropping season. The baseline dispersal potential information generated from this study will help to develop, plan and implement *E. servus* management programs.

*Within-Plant* *E. servus* *Distribution in Corn.* A 2-year study was conducted to quantify the within-plant vertical distribution of adult *E. servus* in field corn, to examine potential plant phenological characteristics associated with their observed distribution, and to select an efficient partial plant sampling method for adult *E. servus* population estimation. Within-plant distribution of adult *E. servus* was influenced by corn phenology. Based on the multiple selection criteria, during V4-V6 corn growth stages, either the corn stalk below the lowest green leaf or basal stratum method could be employed for efficient *E. servus* sampling. Similarly, on reproductive corn growth stages (R1-R4), the plant parts between two leaves above and three leaves below the primary ear leaf were found to be areas to provide the most precise and cost-efficient sampling sites.

*Sampling E. servus in Corn.* Developing a reliable and practical sampling plan for population monitoring of *E. servus* in corn is essential for implementing integrated pest management measures. *E. servus* was sampled from commercial corn fields (*n*=14) in North Carolina in 2016 and 2017. Both the adults and nymphs had a predominantly aggregated spatial distribution. For early vegetative stage corn (V4-V6), using whole plant visual sampling and an economic threshold density of 2 adult stink bugs per 20 plants, 27 sample units were required to estimate population density within 30% of the mean. At the same growth stage, using partial
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Farmscape Ecology of *Euschistus servus* (Hemiptera: Pentatomidae) in a Corn, Wheat, Soybean Ecosystem and Development of a Sampling Plan in Corn

by

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A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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APPROVED BY:

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Dr. James Walgenbach             Dr. Ronnie Heiniger
DEDICATION

To my parents, Babu Abraham and Mary Babu Azhakathu, to my wife Neethu Devachan 
and daughter Catherine Mariam Arun.
BIOGRAPHY

Arun Babu was born to Babu and Mary Babu Azhakathu in 1985 at Calicut, Kerala, India. He received a bachelor’s degree in Agricultural Science from College of Horticulture, Kerala Agricultural University, Thrissur, KL. After the graduation, he worked as a level 1 expert at Kissan Call Center, an agro-advisory service for the growers of Lakshadweep and Kerala State. While working in this capacity, he was fortunate to get accepted to Mississippi State University, MS, USA, to pursue a master’s degree in Entomology under the guidance of Dr. Fred R. Musser. After graduation, he moved to North Carolina to pursue a Ph.D. degree in Entomology from North Carolina State University under the guideship of Dr. Dominic D. Reisig.
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CHAPTER I

INFLUENCE OF WEED MANIPULATION IN FIELD BORDERS ON BROWN STINK BUG (HEMIPTERA: PENTATOMIDAE) DENSITIES AND DAMAGE IN FIELD CORN
1.1 Abstract

Brown stink bug, *Euschistus servus* (Say), is a damaging pest of corn, *Zea mays* L., in the southeastern United States. In North Carolina during the spring, winter planted wheat, *Triticum aestivum* L., serves as the earliest available crop host and *E. servus* seems to prefer this crop over seedling corn. In the absence of wheat in the agroecosystem, weeds serve as a bridge host for overwintered *E. servus* populations until they move to corn. Our objective was to reduce *E. servus* densities in corn by manipulating the weedy field borders with mowing and dicamba herbicide applications. During the study, multiple stink bug species (*n* =16) were found associated with the weed plots. However, *E. servus* was the predominant (>94 %) stink bug species in the corn. In this farmscape, *E. servus* adult density in the unmanaged weed plots started declining around the second week of May, followed by an increase in density in adjacent corn plots. This movement coincided with the seedling growth of corn. In 2016, dicamba application in the weedy field border resulted in a lower *E. servus* density in herbicide-treated weed plots compared to untreated plots. Despite this difference, weed manipulations did not lead to any significant changes in *E. servus* adult density in corn. Further evidence suggests that a prominent external source of *E. servus*, other than field border weeds, in this farmscape is likely driving densities in corn.
1.2 Introduction

In the southeastern United States, the agricultural landscape is comprised of a mosaic of crop fields surrounded by non-crop habitats. In these farmscapes, stink bugs are damaging pests that attack a wide variety of crops, including wheat, *Triticum aestivum* L., corn, *Zea mays* L., cotton, *Gossypium hirsutum* L., and soybean, *Glycine max* L. As polyphagous pests, they depend on multiple hosts that are temporally limiting in nutritional suitability; hence, host switching is common in stink bugs (Panizzi 1997). Accordingly, stink bug population dynamics and movement across the landscape is closely linked to the spatiotemporal distribution, availability, and the suitability of crop and non-crop habitats. Identification of the temporal sequence of hosts, and host switching in response to host quality, can be exploited to manipulate the host or host sequence to avoid or reduce stink bug damage in crops. Stink bug management can be achieved by proactively suppressing the population in attractive early-season crops, or wild hosts, when stink bugs are less numerous or when their distribution is spatially confined in the landscape.

For North Carolina field corn, *Euschistus servus* (Say) is the major damaging stink bug species. *Euschistus servus* overwinters as an adult with a preference for open sites (Jones and Sullivan 1981) and a higher survivorship under weeds along field borders (Rolston and Kendrick 1961). Adults emerging after overwintering in late March through early April (Jones and Sullivan 1981), sustain themselves on wild hosts until nearby agricultural crops become attractive. Winter wheat, when present, can serve as the first feeding and reproductive crop host for post-overwintered *E. servus* adults, and based on movement data from wheat to corn, *E. servus* seems to prefer reproductive stage wheat over seedling corn (Reisig et al. 2013). In the absence of wheat nearby, weeds serve as a bridge host for post-overwintered *E. servus* populations until seedling corn is available as an acceptable host. Consequently, *E. servus* individuals that are present in the early vegetative stages of timely planted corn (typically April
and May in North Carolina) are most likely comprised of overwintered adults that moved directly from overwintering sites (weedy field boundaries) or spring season weed hosts. Targeting *E. servus* management measures in weed hosts during early spring, where *E. servus* populations are spatially concentrated and temporally confined, might reduce the damage by *E. servus* in subsequent crops, including corn (Blinka 2008). However, identifying the major weed hosts that support *E. servus* during this period, as well as understanding the population dynamics of both the pest and associated weed hosts during early spring, is critical for developing pest management strategies.

Timely weed manipulation can result in stink bug control on various crops. For example, Woodside (1947) recommended mowing the understory weed hosts of stink bugs in Virginia peach orchards as a management measure to reduce the cat-facing of fruit. Similarly for North Carolina peach orchards, Killian and Meyer (1984) recommended herbicide application to keep orchards weed-free to reduce the stink bug damage to the fruit, but warned that mowing understory weeds in the orchard after the stink bugs colonized the weed hosts could lead to increased damage to the crop. The potential for *E. servus* management in field corn through the manipulation of early season weedy field borders, which serves as an overwintering site and a source of *E. servus* to seedling corn, remains unexplored.

In the southeastern United States, *E. servus* is bivoltine (Rolston and Kendrick 1961, Herbert and Toews 2011), and individuals from the post-diapause population produce the first generation (*F*₁) from May to July. In North Carolina, winter-planted wheat contributes most of the *F*₁ population (Blinka 2008) when this crop is present in the system. *F*₁ populations that develop in wheat subsequently infest adjacent corn crops, mostly after wheat harvest (Reisig et al. 2013); these individuals may then move to other crops, including cotton or soybean as corn
senesces (Tillman 2011). The F₂ generation is mostly produced in soybean around late September and October (Blinka 2008, Herbert and Toews 2011). Along with non-agronomic hosts (weeds), soybean is suspected as the major contributor of overwintering generation of *E. servus* in this landscape. It has been suggested that the F₁ population produced from wheat, and from weeds, has an impact on the population dynamics of *E. servus* in the in-season crops (Blinka 2008), while the F₂ generation produced from soybean largely influences *E. servus* population dynamics in the subsequent season. Multiple studies have observed a population peak of *E. servus* in soybeans (Gore et al. 2006, Blinka 2008, Pilkay et al. 2015). However, to our knowledge, none of the studies explicitly linked the previous season’s soybean to *E. servus* abundance in weedy borders during the spring.

In our research, we evaluated the influence of two crops, field corn and soybean, on the occurrence and abundance of overwintered *E. servus* on spring season weed hosts. To identify an alternative management method of *E. servus* in corn, we manipulated weedy field borders using a combination of mowing and dicamba herbicide treatments. Major weed hosts of *E. servus* in the weedy field border were also identified.

### 1.3 Materials and Methods

#### 1.3.1 Study Site

The study was conducted in fields of a large (∼18200 ha) contiguous commercial farm located at Carteret Co., NC (34.8941, -76.5668). The farmscape was predominated by large corn and soybean blocks, each consisting of multiple rectangular fields bordered by drainage ditches spaced ≈ 100 m apart. The entire farmscape was surrounded by a pine plantation (*Pinus taeda* L.), and individual fields were surrounded by drainage canals. Drainage canal banks in the field borders supported a wide variety of mostly herbaceous non-agronomic weeds species and were
regarded as suitable overwintering sites for *E. servus* (Jones and Sullivan 1981). In the southeastern United States, harvesting wheat in the late spring can result in large scale movement of *E. servus* to neighboring corn fields (Blinka 2008, Reisig et al. 2013). To avoid the interference of stink bug populations from wheat fields, our study location was chosen such that the nearest wheat field was located at least 5 km away from the experimental plots during the study years (CropScape USDA-NASS 2017).

### 1.3.2 Influence of The Previous Season’s Crop on *E. servus* Number

We wanted to identify the influence of the previous season’s crop on the population density of *E. servus* adults that had emerged from overwintering and were present on spring season weeds hosts. *Euschistus servus* individuals were sampled from the weedy field borders (henceforth referred to as the weed plots) of fallow fields that were planted with either corn or soybean in the previous season. All the fields selected were bordered by a dirt road, followed by drainage canal and pine plantation on one side; *E. servus* observations were taken from weed plots on the canal bank bordering the field. Individual weed plots were approximately 2 to 3 m wide and at least 1000 meters long. Within a weed plot, observations were taken from five sampling points parallel to the crop field, with the first sampling point located at 200 m from the edge and successive sampling points that were separated 200 m apart. At each sampling point, five randomly selected one square meter areas (subsamples) were searched for adult *E. servus*. On 5 April 2016, adult *E. servus* counts were taken from eight weed plots, corresponding to four randomly selected fields each of corn and soybean. The same weed plots were sampled again on 6 April 2017, with four additional randomly selected weed plots, corresponding to two additional fields each of corn and soybean. Before analysis, *E. servus* counts from all the subsamples (n =
25) within a weed plot were pooled together and expressed as mean adult *E. servus* counts per square meter area.

### 1.3.3 Weed Manipulation

To identify if weed habitat manipulation was a viable management method for *E. servus* in field corn in this farmscape, weedy field borders adjacent to corn fields were manipulated using a combination of broadleaf herbicide and mowing treatments. In both 2015 and 2016, several single large adjacent corn fields, where stink bugs were known to be a problem in previous years, were selected for this experiment. In 2015, the fields (≈ 115 ha total) were bordered by corn on two sides and soybean on one side; in 2016, the fields (≈ 170 ha total) were bordered by corn on 3 sides. In both years, one edge was bordered by a dirt road, followed by 4 m wide canal, followed by a pine plantation. The canal had a 2 m wide weedy ditch bank adjacent to the dirt road and 6 to 8 meter wide weedy ditch bank adjacent to the pine plantation. The corn fields (each field was an individual plot) were demarcated by shallow drainage ditches spaced 100 m apart. Sixteen individual adjacent corn fields were selected for the experiment in each year, representing 16 individual plots; these plots were 100 m wide and at least 100 m long. Parallel to each corn plot, weed plots of 100 m in length were marked with flags on both sides of the canal bank.

For weed manipulation, a 2 X 2 factorial experiment was created with four treatment combinations and four replicated blocks, running along the length of canal bank. The treatment factors were mowing and herbicide application, and each factor with two levels, mowing or no mowing, and dicamba herbicide application (0.44 kg ae / ha, Clarity®, BASF corporation, Research Triangle Park, NC) or no herbicide application. The intent of using dicamba was to manage broadleaf weeds from the canal ditch banks while leaving uncultivated grasses intact.
The intent of mowing was to reduce weed height, delay the flowering of weeds, and to maintain vegetative cover for erosion control on the canal bank. In 2015, mowing and herbicide treatments were initiated on 16 March and 2 April, respectively. In 2016, mowing and herbicide treatments were initiated on 25 March and 29 March, respectively. In both years, mowing and/or herbicide applications were carried out at every 30-40 days, depending on the weed growth. The first set of treatments in the weed plots were initiated at least 15 days prior to corn planting date. In 2015, corn plots were planted with DeKalb DKC 66-97, DKC 67-58 (Dekalb Seeds, Monsanto, St Louis, MO) or Pioneer Brand P2089 YHR (Pioneer Hi-bred International, Des Moines, IA). In 2016 all the fields were planted with Pioneer Brand corn hybrid P1197 YHR. Seeding rate in 2015 and 2016 were 74,000 and 75,000 seeds per hectare, respectively. Planting date ranges from 24 to 27 April in 2015 and 14 to 15 April in 2016. In both years, seeds were treated with clothianidin seed treatment at a rate of 1.25 mg a.i. per kernel. No foliar insecticides were applied to either the weeds or the corn plots during this study.

The stink bug population densities in the weed plots were monitored twice a month by randomly searching ten one square meter quadrats in each plot. The observations were taken from late April until the adjacent corn plots reached the R3-R4 growth stages (Ritchie et al. 1989). From each weed plot, adult counts of each stink bug species were recorded. Additionally, when *E. servus* was encountered, the weed host associated with each sighting of adults and nymphs were recorded. To quantify weed coverage and composition, the major species of broadleaf weeds and their percentage coverage, as well as the percentage coverage of Bermudagrass, *Cynodon dactylon* (L.) Pers., and the percentage of grass cover excluding Bermudagrass, were noted from three randomly selected subsamples each of one square meter.
area. The rationale for this type of evaluation was that Bermudagrass was the dominant weed species present (Table 1).

Stink bug counts were recorded from corn plots corresponding to the weed plots using sampling points located on a single transect established at middle of individual corn plots, perpendicular to the corresponding weed plots. On each transect four sampling points were marked at distances of 0, 5, 10, and 20 meters into the corn field, with the first sampling point starting from the field edge (“0 meter”), nearest the weed plot. The above-ground vegetation of thirty corn plants per sampling point was visually inspected for stink bug presence and adult stink bug counts were recorded for each species. Both adult and nymph counts were recorded for *E. servus*. Weekly observations were initiated at two weeks after corn planting until the plants reached the R5 stage (mid-April to mid to late July). Voucher stink bug specimens from both corn and weed plots were deposited in the North Carolina State University Insect Museum in Raleigh, NC.

Stink bug damage to the corn was assessed at the R5 growth stage. For an ear damage assessment, primary ears from 10 random plants per sampling location were hand harvested and the number of kernels with stink bug damage as well as the area of aborted kernel from stink bug damage were recorded. Aborted kernels from the tip of the ear were excluded from the damage rating since unfertilized kernels in that region can be caused by factors other than stink bug damage (Ni et al. 2010). Additionally, the “banana ear” incidence in corn plots was assessed by examining the primary ears of 30 plants from each sampling point. Banana ear is the term used by many growers to describe malformed ears that are missing kernels and crook over, resembling the shape of a banana. In 2016, corn plants in the edge row were highly non-uniform due to
environmental effects unrelated to stink bug incidence; therefore, both the \textit{E. servus} density and ear damage data from the ‘zero’ distance were excluded from all the 2016 analysis.

1.3.4 Data Analysis

The influence of the previous season’s crops on the adult \textit{E. servus} counts in the spring season weed plots were analyzed using a general linear mixed model ANOVA (PROC MIXED, SAS Institute 2011). Analyses were conducted with \textit{E. servus} counts per square meter area of weeds as response variable. The fixed effects in the full models were year (n = 2), previous season crop (n = 2), and their interaction. The random effect was field nested in year. Means were separated using Tukey’s HSD ($\alpha = 0.05$).

From the weed plots, the broadleaf weed hosts of \textit{E. servus} adults and nymphs were identified to at least to the genus level, whereas for the grass species, only Bermudagrass was identified to species level; all other grass species were grouped together into a single category. Across the treatments, the seasonal sum and percentage of total of \textit{E. servus} adults and nymphs captured from each weed host, and the seasonal mean percentage coverage of various weed hosts in the field borders were calculated. Only \textit{E. servus} adults and nymphs recovered from live plants were included in this calculation. The influence of mowing and herbicide treatment on the coverage area of broadleaves, grasses and total weeds in the plots was analyzed as a repeated measures 2 X 2 factorial experiment using a generalized linear mixed model with a Gaussian distribution (PROC GLIMMIX, SAS Institute 2011). The fixed effects in the full model were year (n = 2), mowing (n = 2), herbicide (n = 2), and their interactions. Replication nested in year was a random factor. Sampling date was fitted with an appropriate covariance structure based on model selection using the lowest $\chi^2$ value criterion (Littell et al. 2006). Means were separated using Tukey’s HSD ($\alpha = 0.05$).
To test the effects of weed manipulation on *E. servus*, count data for *E. servus* adults from the weed plots and corn plots were analyzed separately as repeated measures 2 x 2 factorial experiment using a generalized linear mixed model with a Gaussian distribution (PROC GLIMMIX, SAS Institute 2011). The full model analysis indicated that year was significant for *E. servus* adults counts from both the corn plots as well as the weed plots, so subsequent analyses were carried out separately for each year. Because adult *E. servus* count in the 2015 weed plots were so low, parametric data analysis was not possible; therefore, means (± SEM) are reported without statistical analysis. For data from the 2016 weed plots, mowing (n = 2), herbicide (n = 2), and their interaction were modeled as fixed effects. For *E. servus* adult counts from both the 2015 and 2016 corn plots, the fixed effects were mowing (n = 2), herbicide (n = 2), sampling distance (2015; n = 4, 2016; n = 3), and their interactions. Replication was a random factor. Sampling date was fitted with an appropriate covariance structure. Means were separated using Tukey’s HSD (α = 0.05).

Stink bug damage data (stink bug damaged kernels, aborted kernels, and banana ear incidence), from the corn plots were analyzed separately for each year using generalized linear mixed model with a Gaussian distribution (PROC GLIMMIX, SAS Institute 2011), with the same fixed and random effects as those in the *E. servus* count corn plot analyses. The mean area of aborted kernels was natural log transformed before analysis. Means were separated using Tukey’s HSD (α = 0.05). Untransformed means are presented.

Weed plot capacity to act as a source of adult *E. servus* to seedling corn was also assessed. For this purpose, the total *E. servus* adult population density found in the unmanaged weed plots before the population moved to the corn in the spring (27 April 2015 and 19 May 2016; Fig. 1) was compared to the total *E. servus* adult population density found in the
corresponding corn plots immediately after the population moved from weed hosts (25 May 2015 and 23 May 2016; Fig. 1). The total *E. servus* adult population density in unmanaged weeds was calculated by extrapolating counts from the square meter subsamples. However, for the corn plots, our calculation of total *E. servus* adult population was limited to an area located at the first 20 meters into the corn plots, since we only sampled up to this distance.

### 1.4 Results

#### 1.4.1 Influence of the Previous Season’s Crop on Adult *E. servus* Number

During early April, a significantly higher number of *E. servus* adults were counted in weed plots associated with fields that were soybean during the previous year, compared to counts from weed plots associated with fields that were corn during the previous year (*F* = 9.45; df = 1, 8; *P* = 0.0152) (Fig. 2). *Euschistus servus* nymphs were not observed in the weed plots during this time.

#### 1.4.2 Stink Bug Species Complex from Weeds and Corn

Across the study period, a total of 2,951 adult stink bugs (2015; *n* = 1008, and 2016; *n* = 1943), and seven different species were counted from 33,120 corn plants using a whole plant inspection technique. Stink bug species identified in corn included *E. servus*, *Euschistus ictericus* (L.), *Euschistus tristigmus tristigmus* (Say), *Hymenarcys nervosa* (Say), *Oebalus pugnax pugnax* (Fab.), *Podisus maculiventris* (Say), and *Proxys punctulatus* (Palisot de Beauvois). *Euschistus servus* was the most abundant species in corn and accounted for 94.0 and 97.7 % of the total adult stink bugs present in corn in 2015 and 2016, respectively. A total of 140 *E. servus* nymphs (2015; *n* = 37, and 2016; *n* = 103), were also captured from corn. From the weed plots, a total of 16 different stink bug species were captured across the study period. All the stink bug species captured from corn except *E. ictericus* (L.) were also present in weed plots. Additional species
that were recovered exclusively from weed plots included *Chinavia hilaris* (Say), *Chlorochroa persimilis* (Horváth), *Coenus delius* (Say), *Edessa florida* Barber, *Euschistus obscurus* (Palisot de Beauvois), *Mormidea lugens* (Fab), *Neottiglossa cavifrons* Stål, *Stiretrus anchorago* (Fabricius), *Thyanta calceata* (Say), and *Thyanta custator acerra* McAtee.

### 1.4.3 Non-agronomic Host Range of *E. servus*

The season total of *E. servus* adults and nymphs on non-agronomic hosts (weeds), and the seasonal mean percentage land coverage under various *E. servus* host plants in weedy field border are listed in Tables 1 and 2. Across both sampling years and treatments, from 672 m$^2$ of the total area searched, a total of 262 adult *E. servus* were recovered from non-agronomic hosts, which belonged to 10 different plant families. *Euschistus servus* nymph counts were generally low in weed plots (Table 2 and Fig. 1). Across years and treatments, a total of 27 *E. servus* nymphs were found associated with non-agronomic hosts in the weedy field border, which belonged to five different plant families; the majority (85.2%) of nymphs were found in 2016. Among the non-agronomic host plants, a relatively high proportion of *E. servus* nymphs were found on grasses (Table 2). Additionally, during 2016, a high proportion of *E. servus* nymphs were found on common evening primrose, *Oenothera biennis* L.

### 1.4.4 Influence of Treatments on Weed Composition

Overall, dicamba herbicide application altered the weed composition in herbicide-treated plots by reducing broadleaf weeds as well as increasing grasses (Table 3). Furthermore, in comparison to non-herbicide treated plots, dicamba-treated weed plots had less area with weeds (broadleaf + grasses). Overall, mowing had no significant influence on either the grass or total weed area between the mowed and unmowed weed plots. However, in comparison to the unmowed plots, the mowed plots had more broadleaves (Table 3).
1.4.5 Weed Management on *E. servus* Abundance

The overall mean *E. servus* adult population densities in weed plots were significantly higher in 2016 (0.018 ± 0.006 *E. servus* adults/m², mean ± SEM), than in 2015 (0.004 ± 0.003) (\(F = 14.32; \text{df} = 1, 6.39; P = 0.0081\)). During 2016, the overall season long *E. servus* adult counts were not significantly different in the mowed plots or the unmowed plots (\(F = 0.62; \text{df} = 1, 10; P = 0.4478\); Fig. 3). However, dicamba applications significantly reduced the season long mean *E. servus* adult counts compared to non-herbicide treated plots (\(F = 7.74; \text{df} = 1, 10; P = 0.0194\)).

Between the sampling years of 2015 and 2016, *E. servus* adult population dynamics in the corn were different between the sampled fields (Fig. 1), with significantly higher overall mean *E. servus* adults in 2016 (\(F = 37.76; \text{df} = 1, 6.6; P = 0.0006\)). During both 2015 and 2016, neither mowing (2015; \(F = 0.57; \text{df} = 1, 10; P = 0.4695\), 2016; \(F = 0.09; \text{df} = 1, 10; P = 0.7727\)) nor herbicide treatments (2015; \(F = 1.15; \text{df} = 1, 10; P = 0.3094\), 2016; \(F = 0.26; \text{df} = 1, 10; P = 0.6221\)) in the weedy field border significantly influenced overall mean adult *E. servus* counts in the corresponding corn plots (Fig. 3A). However, in both years, overall mean stink bug counts were significantly influenced by sampling distance within corn plots (2015; \(F = 37.85; \text{df} = 3, 557; P = <0.0001\), 2016; \(F = 6.12; \text{df} = 2, 510; P = 0.0024\)). In 2015, significantly fewer adult *E. servus* were observed in the edge row (0 meter) compared to other sampling distances and no significant differences in adult *E. servus* densities were observed among the 5, 10 and 20 meter distances into the corn field. In contrast, in 2016, more adult *E. servus* were observed 5 meters into the corn field compared to 20 meters; *E. servus* counts at 10 meters were not significantly different from those at 5 or 20 meters.
1.4.6 Stink Bug Damage in Corn

The mean number of stink bug damaged corn kernels was different between years, with more kernels damage observed in 2016 ($F = 75.85; \text{df} = 1, 95; P < 0.0001$). In 2015, neither mowing nor herbicide applications in weed plots significantly influenced the mean number of stink bug damaged kernels in the corn plots (mowing; $F = 0.39; \text{df} = 1, 54; P = 0.5364$, herbicide; $F = 0.55; \text{df} = 1, 54; P = 0.4621$; Fig. 4). However, in 2016, corn plots near the herbicide applied weed plots had a significantly higher mean number of stink bug damaged kernels ($F = 4.52; \text{df} = 1, 41; P = 0.0395$); in contrast, the effect of mowing the weed plot was not significant ($F = 1.59; \text{df} = 1, 41; P = 0.2144$). Sampling distance significantly influenced the mean number of stink bug damaged kernels, with more damage observed towards the edge row ($F = 7.22; \text{df} = 2, 41; P = 0.0021$).

The mean area of aborted kernels was different between years ($F = 138.94; \text{df} = 1, 90; P < 0.0001$) and sampling distance ($F = 11.64; \text{df} = 3, 90; P < 0.0001$). During 2016, no differences in mean area of aborted kernels were detected among the corn plots across herbicide and mowing treatments (herbicide; $F = 0.01; \text{df} = 1, 40; P = 0.9285$, mowing; $F = 0.26; \text{df} = 1, 40; P = 0.6120$). However, in 2015, more mean area of aborted kernels per ears was observed in corn plots located near unmowed weed plots compared to mowed plots ($F = 10.74; \text{df} = 1, 56; P = 0.0018$); in contrast, no difference in damage was observed among plots near dicamba-treated and untreated plots ($F = 1.33; \text{df} = 1, 56; P = 0.2541$).

The number of banana ears was not influenced by year, sampling distance, mowing or herbicide (year; $F = 3.89; \text{df} = 1, 5.73; P = 0.0985$, distance; $F = 2.80; \text{df} = 2, 73.7; P = 0.0675$, mowing; $F = 0.05; \text{df} = 1, 75.8; P = 0.8245$, herbicide; $F = 0.01; \text{df} = 1, 74.2; P = 0.9246$), or their interactions (values not presented).
1.4.7 Population Dynamics of *E. servus* in Weeds and Corn

Population dynamics of *E. servus* adults and nymphs in unmanaged weed plots were compared with their corresponding corn plots (Fig. 1). During 2015, the peak *E. servus* adult populations in unmanaged weed plots were observed on 27 April, during the first sampling date, in the weed plots. Adult *E. servus* densities in the weed plots started declining around the first week of May, which coincided with the seedling growth of corn (Fig. 1). We did not detect any *E. servus* nymphs in unmanaged weed hosts during 2015, although four nymphs were observed on 22 June 2015 in the weed hosts of treated plots (Table 2). The appearance of nymphs in weed hosts of treated plots in 2015 also coincided with the summer solstice (21 June 2105). In 2015, adult *E. servus* densities in corn plots varied across corn growth stages with a prominent peak around the V5 growth stage. Nymphs first appeared in corn around 16 June, at the V12 growth stage, and low densities of nymphs were observed in corn across many of the successive sampling dates until the R3 growth stage.

In 2016, adult *E. servus* densities in unmanaged weed plots followed similar population dynamics as those of 2015 (Fig. 1). During 2016, the peak of *E. servus* adult populations in unmanaged weed plots was observed on 9 May, followed by a rapid decline that coincided with the presence of seedling corn. Another small peak in *E. servus* adult population density was observed in unmanaged weed plots on 21 June of this year, which nearly coincided with a peak of the *E. servus* adult population densities in corn plots (28 June) and the 2016 summer solstice (20 June). *Euschistus servus* nymphs in unmanaged weed plots were first observed on 9 May followed by a small population peak of nymphs on 23 May; the nymphs were recovered from multiple weed hosts (Table 2). From the 2016 corn, peak *E. servus* population densities coincided with the reproductive stages of corn (R1-R2). Nymphs were first detected on corn on
31 May and low densities of nymphs were observed in the corn during all the later observations dates.

The capacity of unmanaged weed plots to serve as a source of *E. servus* adults in seedling corn was assessed by comparing the total *E. servus* adult population in unmanaged weed plots to the population in the first 20 meters of their corresponding corn plots (Table 4). Assuming that all the *E. servus* adults present in the first 20 meters of corn were those that were displaced from the adjacent weed plots, during 2015 and 2016, only 11.4 and 69.7% of the total *E. servus* adult population present in the first 20 meter of corn fields could have been explained by the movement of *E. servus* adults from the unmanaged weed plots.

1.5 Discussion

Non-agronomic plants in the weedy strip near corn in this farmscape supported multiple species of stink bugs (*n* = 16), of which, only a limited number of species are known to reach economic pest status in corn, and other crops. Corn plots located near these weedy strips also supported multiple stink bug species (*n* = 7), including the beneficial predatory stink bug, *P. maculiventris*. However, *E. servus* was the most abundant species in corn, comprising 94.0 and 97.7% of the total stink bug populations in 2015 and 2016, respectively. Although our weed treatments of mowing and dicamba applications altered plant species composition in the weedy strips bordering corn, they did not influence *E. servus* densities in adjacent corn and resulted in minimal impacts on stink bug damage in adjacent corn.

During early April of both years, a relatively high abundance of *E. servus* adults was observed in weedy field borders adjacent to fields that were soybean during the previous season, compared to those that were near corn during the previous season. The sampling date of this experiment coincided with the usual spring emergence time (late March to April) of
overwintering *E. servus* populations (Jones and Sullivan 1981). Adults that were observed on the weed plots at this sampling time likely comprised of a mixture of overwintering or freshly overwintered adults, as indicated by their reddish-brown abdominal sternal coloration (Borges et al. 2001). Additional support for this supposition is based on studies that indicate that it is improbable that F₁ adults would be present at this time (Rolston and Kendrick 1961, Jones and Sullivan 1982). Furthermore, in both 2016 and 2017, these observations were taken before the planting of any crops (corn or soybean), in the farmscape, eliminating the possibility of interference from crops during the current season. Our results are also in agreement with the previous findings indicating that *E. servus* overwinters under the weed litter along field borders (Rolston and Kendrick 1961, Jones and Sullivan 1981). Altogether, this suggests that soybean from the previous season is the major contributor of most of the overwintering *E. servus* population in this farmscape. This is not surprising as soybean is often suggested as the crop component that influences *E. servus* population dynamics in crops in the following cropping season (Townsend and Sedlacek 1986, Olson et al. 2018), though, to our knowledge this is the first study that shows a direct link of soybean from the previous season to *E. servus* abundance in weed borders during the spring.

In the southeastern United States, *E. servus* utilizes multiple non-agronomic plants (weeds), as feeding or breeding hosts (Woodside 1947, Tugwell et al. 1973, Jones and Sullivan 1982). In this study, *E. servus* adults were commonly observed on non-agronomic host plants including: Bermudagrass, prickly lettuce, *Lactuca serriola* L., and unidentified grass species during 2015. In contrast, in 2016, *E. servus* adults were commonly observed on common evening primrose, Canadian horseweed, *Conyza canadensis* (L.) Cronquist, and unidentified grass species. Comparing the seasonal percentage capture of *E. servus* counts from a non-agronomic
host to the mean seasonal coverage of that host suggests that *E. servus* prefers certain plant hosts over others (Table 1). For example, 22.2% of *E. servus* adults in 2015 were captured from prickly lettuce despite the fact that coverage by this weed species represented only 0.58% of total weed area; this indicates a high preference of *E. servus* adults for this host. Similarly, 35% of *E. servus* adults in 2016 were recovered from common evening primrose when the average cover from this species was mere 4.22%. Only a limited number of *E. servus* nymphs were observed on non-agronomic hosts in weed plots and the majority of them were from grasses, including Bermudagrass and other unidentified grass species. Finally, in 2016, common evening primrose likely served as preferred breeding host in the last week of May, since nymphs were observed on this plant; hence, this plant could have served as a major broadleaf feeding and breeding host in the weedy field border.

As expected, dicamba, a selective broadleaf herbicide, altered the weed composition in treated plots by reducing the area with broadleaf species and increasing the area with grass species. The observed increase in the mean area under grass species in the dicamba-treated plots was likely due to the near elimination of competition from the broadleaf weed species in the treated plots. In contrast, mowing at repeated intervals resulted in an overall increase in the area with broadleaf weeds. Despite the effectiveness of weed manipulations in the treated plots, overall *E. servus* adult counts in weed plots were influenced only by the 2016 herbicide applications. The reduction of *E. servus* in dicamba-treated weed plots compared to untreated weed plots in 2016 could partially be attributed to the destruction of broadleaf weeds, many of which served as a preferred feeding and breeding hosts for *E. servus* in this farmscape. Moreover, it has been suggested that a mechanical operation, including a pesticide application, can also force the stink bugs to disperse out of a crop field (Tillman et al. 2009). We visually
observed stink bugs dispersing soon after dicamba application, although this is not supported by data in our study. Therefore, we cannot rule out this as a factor resulting in a lower *E. servus* density in the herbicide treated weed plots compared to non-herbicide treated plots during 2016. The effect in weeds seen in 2016 translated to the corn; the movement of *E. servus* from herbicide-treated weed plots to the nearby corn plots resulted in higher kernel damage per ear compared to corn near non-herbicide treated plots.

The sequence and timing of host utilization by *E. servus* depends on the sequence and availability of their preferred crop and non-crop hosts that are in a suitable phenological stage in a farmscape. In this study, a steep decline of *E. servus* adult densities from unmanaged weed plots, followed by a rapid increase in densities in nearby corn plots during mid to late May (Fig. 1), suggests a mass movement of *E. servus* from spring season weed hosts to seedling corn. This movement appears to coincide with the availability of seedling corn as a direct response of adult *E. servus* to the newly available food source. This apparent preference of *E. servus* adults to field corn over weed hosts was further evidenced from the relatively low *E. servus* densities observed in the weed plots throughout most of the corn growing season. Overall this suggests that during the spring, post-overwintered *E. servus* adults use weeds as a bridge host to sustain themselves until nearby corn becomes attractive. Moreover, since there is limited reproduction during this time in the weed hosts, the population of adults that moved to seedling corn in our study probably consisted entirely of individuals from the overwintered generation. In contrast, if there is winter planted wheat in the farmscape during the spring, *E. servus* will prefer reproductive stage wheat over seedling corn in this region (Reisig et al. 2013). Unlike the weed-corn farmscape, where adult *E. servus* moves to a new host (corn) as a response to the newly available food source, in a wheat-corn farmscape *E. servus* will develop the F₁ generation in wheat and
continue to feed until wheat senescence; moreover, most of the emigration from wheat to the nearby corn occurs only after the wheat harvest (Blinka 2008, Reisig et al. 2013). Therefore, the timing and generation (overwintered or $F_1$) of *E. servus* that move to corn in this type of farmscape largely depends on the presence of winter-wheat in the system.

Our further evaluation of unmanaged weeds to serve as a source of *E. servus* adults in the corn suggests that only 11.4 and 69.7% of total *E. servus* adult population present in 2015 and 2016, respectively, were accounted for by *E. servus* adults from weeds, even in the first 20 meters of corn where stink bug density is typically the highest. The first 20 meters into the corn plots represented only a small percentage of total corn area available in the focal fields (2.8 and 1.9% in 2015 and 2016, respectively). Furthermore, *E. servus* reproduction in corn was minimal during the early vegetative stages, and the populations we observed in the corn were composed entirely of individuals from external sources. Therefore, the occurrence of relatively high *E. servus* densities in the corn plots during the *E. servus* mass movement suggests that the prominent source of adult *E. servus* is not the weed plots in this farmscape. Previous research has reported that certain trees, shrubs and vines in woodland habitats can serve as a feeding and breeding host of the *E. servus* in a farmscape (Jones and Sullivan 1982, Tillman et al. 2014, Bakken et al. 2015). Although the pine plantation bordering the weed plots was not surveyed for *E. servus* plant hosts or overwintering *E. servus* individuals, it could be a possible source of overwintering sites or *E. servus* host plants in this farmscape.

Previous research has successfully demonstrated the effectiveness of weed host manipulation for stink bug management in crops (Woodside 1947, Killian and Meyer 1984, Coombs 2000). However, weed host manipulation generally did not influence *E. servus* densities or reduce injury in corn in our study. A closer examination of successful studies (Woodside
revealed that the situations where weed manipulation was effective in managing stink bugs in crops had several commonalities: 1) non-agronomic weed hosts played a major role in the population growth of the stink bugs before they moved into the crops; 2) the crop contributed little, or no population increase of the targeted pest; and 3) movement of stink bugs to the crop from external sources other than the weed hosts being manipulated was minimal. Based on the results of our study, the first condition is probably not true, while the second condition is true for the local farmscape of our study. It is unclear whether the third condition is true, however. Our study conditions, especially those related to first and third conditions, were greatly different from other successful studies, which could partially explain the absence of a positive influence of weed manipulation in our study to reduce *E. servus* density or damage in corn.

Despite the importance of spring weed hosts in *E. servus* crop colonization and its population dynamics in subsequent crops, only a few studies (Woodside 1947, Killian and Meyer 1984) have tried to manage the weed hosts of *E. servus* as a pest control measure. Our results demonstrate that herbicide application in weedy field border may flush *E. servus* from weedy hosts and could result in increased kernel damage by stink bugs in the corn ear. Moreover, the influence of alternative *E. servus* sources other than weedy host borders in this farmscape needs further investigation. The results from this study augment our knowledge on population dynamics of *E. servus* in spring weed hosts and, hopefully, will stimulate future studies for development of ecological based stink bug management tactics.
1.6 Acknowledgments

Allen Scott, Axel David González, Christopher McBennett, Clifton Moore, Dan Mott, Dannyel Akira Nelson, Ian McAreavy and Steven Roberson provided the technical assistance. We thank Antonio Cinti Luciani and Jonathan Peppers of Open Ground Farms, Beaufort, NC, for their collaboration and support in establishing and maintaining the experimental plots. We thank Robert L. Blinn (North Carolina State Insect Museum, Raleigh, NC.) for assisting the stink bug species identification. Partial funding of this project was provided by NC Agricultural and Life Sciences Research Foundation and the Corn Growers Association of North Carolina.
1.7 References Cited


### Tables and Figures

#### Table 1.1
Seasonal sum (and percentage of total) of *E. servus* adults on non-agronomic hosts and the mean seasonal percentage coverage of various non-agronomic host plant species in the weedy field border, North Carolina 2015-2016

<table>
<thead>
<tr>
<th>Plant Family, genus species (common)</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sum (and percentage) of <em>E. servus</em> adults*</td>
<td>Mean percentage weed cover</td>
</tr>
<tr>
<td>Asteraceae, <em>Ambrosia artemisiifolia</em> L. (annual ragweed)</td>
<td>-</td>
<td>0.19</td>
</tr>
<tr>
<td>Asteraceae, <em>Conyza canadensis</em> (L.) Cronquist (Canadian horseweed)</td>
<td>3 (8.3)</td>
<td>3.66</td>
</tr>
<tr>
<td>Asteraceae, <em>Eupatorium capillifolium</em> (Lam.) Small (dogfennel)</td>
<td>1 (2.8)</td>
<td>0.17</td>
</tr>
<tr>
<td>Asteraceae, <em>Lactuca serriola</em> L. (prickly lettuce)</td>
<td>8 (22.2)</td>
<td>0.58</td>
</tr>
<tr>
<td>Asteraceae, <em>Sonchus asper</em> (L.) Hill (spiny sowthistle)</td>
<td>1 (2.8)</td>
<td>0.25</td>
</tr>
<tr>
<td>Convolvulaceae, <em>Ipomoea</em> spp. (morningglory)</td>
<td>2 (5.6)</td>
<td>2.47</td>
</tr>
<tr>
<td>Euphorbiaceae, <em>Chamaesyce maculata</em> (L.) Small (spotted sandmat)</td>
<td>-</td>
<td>0.04</td>
</tr>
<tr>
<td>Fabaceae, <em>Senna obtusifolia</em> (L.) Irwin &amp; Barneby (Java-bean)</td>
<td>3 (8.3)</td>
<td>2.17</td>
</tr>
<tr>
<td>Geraniaceae, <em>Geranium carolinianum</em> L. (Carolina geranium)</td>
<td>-</td>
<td>2.24</td>
</tr>
<tr>
<td>Malvaceae, <em>Abutilon theophrasti</em> Medik. (velvetleaf)</td>
<td>-</td>
<td>0.0</td>
</tr>
<tr>
<td>Malvaceae, <em>Sida spinosa</em> L. (prickly fanpetals)</td>
<td>-</td>
<td>1.25</td>
</tr>
<tr>
<td>Onagraceae, <em>Oenothera biennis</em> L. (common evening primrose)</td>
<td>-</td>
<td>0.21</td>
</tr>
</tbody>
</table>
Table 1.1 (continued)

<table>
<thead>
<tr>
<th>Plant Family, genus species (common)</th>
<th>2015</th>
<th></th>
<th>2016</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sum (and percentage) of <em>E. servus</em> adults*</td>
<td>Mean percentage weed cover</td>
<td>Sum (and percentage) of <em>E. servus</em> adults*</td>
<td>Mean percentage weed cover</td>
</tr>
<tr>
<td>Onagraceae, <em>Oenothera laciniata</em> Hill (cutleaf evening primrose)</td>
<td>-</td>
<td>1.89</td>
<td>12 (5.3)</td>
<td>1.17</td>
</tr>
<tr>
<td>Poaceae members other than <em>Cynodon dactylon</em> (L.) Pers.</td>
<td>6 (16.7)</td>
<td>13.14</td>
<td>37 (16.4)</td>
<td>13.01</td>
</tr>
<tr>
<td>Poaceae, <em>Cynodon dactylon</em> (L.) Pers. (Bermudagrass)</td>
<td>9 (25.0)</td>
<td>31.69</td>
<td>13 (5.8)</td>
<td>23.16</td>
</tr>
<tr>
<td>Polygonaceae, <em>Polygonum hydropiperoides</em> Michx. (swamp smartweed)</td>
<td>1 (2.8)</td>
<td>0.10</td>
<td>-</td>
<td>0.23</td>
</tr>
<tr>
<td>Rosaceae, <em>Rubus idaeus</em> L. (American red raspberry)</td>
<td>2 (5.6)</td>
<td>1.52</td>
<td>3 (1.3)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Only *E. servus* adults observed on live plants are included in the calculation.
Table 1.2  Seasonal sum (and percentage of total) of *E. servus* nymphs on non-agronomic hosts and the mean seasonal percentage coverage of various non-agronomic host plant species in the weedy field border, North Carolina 2015-2016

<table>
<thead>
<tr>
<th>Plant Family, genus species (common)</th>
<th>Sum (and percentage) of <em>E. servus</em> nymphs*</th>
<th>Mean percentage weed cover</th>
<th>2015</th>
<th>Mean percentage weed cover</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cucurbitaceae, <em>Melothria pendula</em> L. (Guadeloupe cucumber)</td>
<td>-</td>
<td>0.38</td>
<td>1 (4.3)</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Cupressaceae, <em>Juniperus</em> sp. (juniper)</td>
<td>-</td>
<td>0.00</td>
<td>1 (4.3)</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Fabaceae, <em>Senna obtusifolia</em> (L.) Irwin &amp; Barneby (Java-bean)</td>
<td>-</td>
<td>2.17</td>
<td>1 (4.3)</td>
<td>1.65</td>
<td></td>
</tr>
<tr>
<td>Onagraceae, <em>Oenothera biennis</em> L. (common evening primrose)</td>
<td>-</td>
<td>0.21</td>
<td>4 (17.4)</td>
<td>4.22</td>
<td></td>
</tr>
<tr>
<td>Onagraceae, <em>Oenothera laciniata</em> Hill (cutleaf evening primrose)</td>
<td>-</td>
<td>1.89</td>
<td>2 (8.7)</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>Poaceae members other than <em>Cynodon dactylon</em> (L.) Pers.</td>
<td>-</td>
<td>13.14</td>
<td>8 (34.8)</td>
<td>13.01</td>
<td></td>
</tr>
<tr>
<td>Poaceae, <em>Cynodon dactylon</em> (L.) Pers. (Bermudagrass)</td>
<td>4 (100)</td>
<td>31.69</td>
<td>6 (26.1)</td>
<td>23.16</td>
<td></td>
</tr>
</tbody>
</table>

*Only *E. servus* nymphs observed on live plants are included in the calculation.*
Table 1.3  Effect of weed manipulation through mowing and herbicide application on broadleaf and grasses composition and total weed cover on the weed plots in 2015 and 2016

<table>
<thead>
<tr>
<th>Effect</th>
<th>Level</th>
<th>Broadleaf Mean area (± SEM) of plant cover per m²</th>
<th>Grasses Mean area (± SEM) of plant cover per m²</th>
<th>Total cover Mean area (± SEM) of plant cover per m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mowing</td>
<td>Yes</td>
<td>0.244 ± 0.02a</td>
<td>0.413 ± 0.03a</td>
<td>0.668 ± 0.03a</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0.172 ± 0.02b</td>
<td>0.514 ± 0.03a</td>
<td>0.697 ± 0.03a</td>
</tr>
<tr>
<td>Herbicide</td>
<td>Yes</td>
<td>0.100 ± 0.01B</td>
<td>0.532 ± 0.03A</td>
<td>0.643 ± 0.03B</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>0.315 ± 0.02A</td>
<td>0.396 ± 0.03B</td>
<td>0.721 ± 0.02A</td>
</tr>
</tbody>
</table>

Year

<table>
<thead>
<tr>
<th>Effect</th>
<th>Level</th>
<th>Broadleaf Mean area (± SEM) of plant cover per m²</th>
<th>Grasses Mean area (± SEM) of plant cover per m²</th>
<th>Total cover Mean area (± SEM) of plant cover per m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mowing</td>
<td></td>
<td>F = 6.5; df = 1, 22.64; P = 0.0180</td>
<td>F = 4.23; df = 1, 22.08; P = 0.0518</td>
<td>F = 0.99; df = 1, 20.93; P = 0.3313</td>
</tr>
<tr>
<td>Herbicide</td>
<td></td>
<td>F = 58.47; df = 1, 22.64; P &lt; 0.001</td>
<td>F = 7.57; df = 1, 22.08; P = 0.0116</td>
<td>F = 6.78; df = 1, 20.93; P = 0.0166</td>
</tr>
<tr>
<td>Mowing*herbicide</td>
<td></td>
<td>F = 2.32; df = 1, 19.17; P = 0.1443</td>
<td>F = 1.70; df = 1, 18.75; P = 0.2076</td>
<td>F = 0.84; df = 1, 18.06; P = 0.3714</td>
</tr>
</tbody>
</table>

Mean plant cover within a column bearing same lowercase letters are not significantly different between the mowing levels (Tukey’s HSD; α = 0.05). Mean plant cover within a column bearing same uppercase letters are not significantly different between the herbicide levels (Tukey’s HSD; α = 0.05).
Table 1.4  Land area and total adult *E. servus* counts in unmanaged weed plots (source habitat) and seedling corn (sink habitat) during 2015 and 2016

<table>
<thead>
<tr>
<th>Year</th>
<th>Unmanaged weeds*</th>
<th>Corn*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total land area in m²</td>
<td>Mean (± SEM) <em>E. servus</em> adults density/m²</td>
</tr>
<tr>
<td>2015</td>
<td>4,000</td>
<td>0.20 ± 0.04</td>
</tr>
<tr>
<td>2016</td>
<td>4,000</td>
<td>0.70 ± 0.40</td>
</tr>
</tbody>
</table>

*To avoid interference from the weed treatment effects (mowing or herbicide application) in the calculation, only mean *E. servus* adults recovered from the unmanaged weed plots and from the corresponding corn plots were included in the calculation.
Figure 1.1  Mean *E. servus* adult and nymph densities in unmanaged weed plots and associated corn plots during 2016 and 2017.
Figure 1.2  The influence of previous season crops on adult *E. servus* abundance (mean ± SEM) in weedy field borders during 2016 and 2017.
Figure 1.3  The influence of mowing and herbicide field border weed manipulation on *E. servus* adult numbers on corn and weed plots during 2015 and 2016. (A) the mean (± SEM) number of *E. servus* adults in field corn and (B) the mean (± SEM) number of *E. servus* adults in weed plots.
Figure 1.4  The influence of mowing and herbicide field border weed manipulation on stink bug damage on corn ears during 2015 and 2016. (A) the mean (± SEM) number of stink bug damaged kernels per ear and (B) the mean (± SEM) area of aborted kernels in cm$^2$ per ear.
CHAPTER II

BASELINE FLIGHT POTENTIAL OF ADULT BROWN STINK BUG (HEMIPTERA: PENTATOMIDAE) AND ITS IMPLICATIONS ON LOCAL DISPERSAL.
2.1 Abstract

Brown stink bug, *Euschistus servus* (Say), is a damaging pest of multiple crops in the southeastern United States. In addition to the crops, both weedy field borders and the wooded areas of a typical farmscape in this region harbor *E. servus* host plants, many of which are temporally and spatially limiting in availability or nutritional suitability. Therefore, local dispersion of *E. servus* is required so that individuals efficiently track and utilize host resources. This research sought to establish the baseline flight capacity of adult *E. servus* across the season in relation to body weight, sex, overwintering status, nutritional status, and plant host using a computer-monitored flight mill system. Across all the flight sessions, 90.7% of individuals tested flew in a range of 0-1 km, with an individual maximum flight distance of 6.4 km in 22-h. The mean total distance flown, mean flight speed and mean total time spent on actual flight varied across the season. The highest mean flight potential was observed soon after overwintering emergence and a relatively low flight potential was observed during the cropping season. Preflight body weight generally did not influence flight distance, except for a negative linear relationship observed in long distance fliers from stink bugs obtained from corn. Individuals collected from wheat, corn and early season weed hosts lost higher proportion of body weight during flight than individuals from soybean and late season weed hosts. The baseline dispersal potential information generated from this study will help to develop, plan, and implement *E. servus* management programs.
2.2 Introduction

In the southeastern United States, the brown stink bug, *Euschistus servus* (Say), is a common polyphagous pest that feeds and reproduces on multiple crop and non-crop hosts. This pest overwinters as an adult and prefers open weedy field borders as overwintering sites (Jones and Sullivan 1981) with high overwintering success observed under common mullein, *Verbascum thapsus* L., plants (Rolston and Kendrick 1961). A typical farmscape in the southeastern United States is comprised of multiple crops including wheat, *Triticum aestivum* L., corn, *Zea mays* L., tobacco, *Nicotiana tabacum* L., cotton, *Gossypium hirsutum* L., peanut, *Arachis hypogaea* L., and soybean, *Glycine max* L. along with buildings, farm roads, weedy field borders, and wooded areas. In addition to many of the crops that serve as hosts for *E. servus*, both weedy field borders and the wooded areas of a typical farmscape harbor multiple *E. servus* host plants (Woodside 1947, Jones and Sullivan 1982, Bakken et al. 2015), of many of which are temporally and spatially limiting in availability or nutritional suitability. As a result, *E. servus* population dynamics in a farmscape depend on the seasonal succession of multiple crop and non-crop hosts. Host-switching is common in stink bugs in response to the nymph to adult transition (Panizzi 1997), changes in availability of food or food quality, plant senescence (Blinka 2008, Tillman 2011, Reisig et al. 2013), or the availability of a more preferred host (Tillman et al. 2009).

Depending on the timing and availability of host plants of a suitable phenological state in the landscape, the host sequence utilized by the *E. servus* can be regionally specific (Panizzi 1997). However, in general, for the southeastern United States, adult *E. servus* emerges from overwintering locations during late March through April and they will sustain themselves on non-agronomic hosts in the weedy field borders or wooded area (Woodside 1947, Jones and Sullivan 1982) until a suitable cultivated host becomes available. In this region, winter-planted
wheat can serve as the earliest host crop available for *E. servus* infestations (Reay-Jones 2010, Pilkay et al. 2015). On wheat, *E. servus* develops an F₁ population that can later infest surrounding crops including corn, mainly during wheat senescence or soon after the wheat harvest (Reisig et al. 2013). In contrast, when wheat is absent in the landscape, a portion of the post-overwintered *E. servus* population disperses directly from weed hosts to early vegetative stage corn, possibly as a response of adults to newly available food (A. B., unpublished data). During the senescence of corn, the *E. servus* population that colonized and reproduced in corn disperses to subsequent crops including cotton, peanut, or soybean (Tillman 2011). Finally, late-planted and late-maturing soybean is usually the last crop host that supports high populations of *E. servus* (Bundy and McPherson 2000, Gore et al. 2006), and can be the major contributor of overwintering (F₂ generation) *E. servus* in a farmscape (Herbert and Toews 2011, A. B. unpublished data). After the senescence of all the crop hosts in the farmscape, it has been suggested that weed hosts support the *E. servus* until they diapause. Altogether the above information suggests the importance of local dispersion of *E. servus* adults. These individuals need to efficiently track and utilize host resources that can be spatially or temporally overlapping and isolated at variable degrees in a farmscape.

Flight mill studies are one of the common methods used to assess the baseline flight capacity of an insect. Flight mill data can also be used to provide information concerning the influence of insect body weight, sex, age, or mating status, environmental conditions, time of the year, or crop of origin on the flight capacity of tested specimens (Taylor et al. 2010, Lee and Leskey 2015, Wiman et al. 2015). For pentatomid pests, only limited flight mill studies are available. For example, tethered flight mill studies that examined the flight capacity of brown marmorated stink bug, *Halyomorpha halys* (Stål), found that this invasive pest is capable of long
distance flights, partially explaining their relatively rapid spread across the United States after their first appearance (Lee and Leskey 2015, Wiman et al. 2015). For *E. servus* specifically, studies describing the farmscape dispersal pattern or spatial distribution of the pest will provide the preliminary information on their movement potential. For example, one *E. servus* adult marked with egg albumin in a wheat crop was recaptured from an adjacent corn field, 59 m away from the nearest marked location and 3 h after marking (Reisig et al. 2013). Similarly, Tillman et al. (2009) observed a female *E. servus* disperse ≈ 400 m across the farmscape. Other than some casual observations, baseline flight capacity is not established for *E. servus* adults. This research sought to establish the baseline flight capacity of adult *E. servus* across the season in relation to *E. servus* body weight, sex, overwintering status, nutritional status, and plant host of origin using a computer-monitored flight mill system.

2.3 Materials and Methods

2.3.1 Study Site, Collection and Handling of Insects

All adult *E. servus* used in the tethered flight mill study, except those individuals from the wheat crop, were hand-collected from weeds, corn or soybean plots within a large corn-soybean farmscape located at Carteret Co., NC (34.8805, -76.6113). This farmscape is primarily comprised of large corn, and soybean fields bordered with dirt roads, weedy strips, and drainage canals. The entire farmscape is surrounded by a pine (*Pinus taeda* L.) plantation. Weedy field borders in this farmscape serve as an *E. servus* overwintering site and also harbor multiple herbaceous non-agronomic *E. servus* hosts (A. B., unpublished data). During the study year-2017- the nearest wheat field was situated at least 5 km away from the *E. servus* collection sites (CropScape USDA-NASS 2017). The *E. servus* adults from wheat crop for this study were collected using a sweep net (38 cm in diameter), from fields located in Jones Co., NC (35.2237, -
77.4880), approximately 88 km away from the weeds-corn-soybean study site. No insecticides were applied to any of the fields during the collection period.

From the weedy field border, adult *E. servus* were collected from weed hosts during late March to early April, and then from mid-late October, after the last standing crop was harvested in this farmscape (Table 1). The first set of collection dates- henceforth referred to as “weeds early”- coincided with the timing of peak emergence of *E. servus* from the overwintering sites in the weeds surrounding the fields. In contrast, the individuals from the October collection dates- henceforth referred to as “weeds late”- represented the adult *E. servus* population that likely developed from crops (soybean in this location, A.B., unpublished data) but were feeding or beginning to overwinter in the weeds. During the cropping season, *E. servus* were collected from wheat, corn, and soybean fields. These crops represent the top three crops in terms of planted acreage in North Carolina (USDA-NASS 2017). These three crops are also known to serve as sources of *E. servus* infestation for subsequent crops or non-crop hosts (Bundy and McPherson 2000, Blinka 2008, Tillman 2011). Since most *E. servus* dispersal happens during crop senescence or immediately after the crop harvest (Tillman 2011, Reisig et al. 2013), *E. servus* sampling from each of these crops was restricted to the time of crop senescence, with the exception of corn (explained next; Table 1).

Additionally, to study the influence of the phenological and nutritional status of field corn on *E. servus* flight capacity, adult *E. servus* were collected at weekly intervals from a single corn field starting from their first appearance in this crop (≈ V4 stage) until the insects moved out from the corn after full maturity (R6 stage) to other available plant hosts such as soybean. To avoid confusion when referring to the previous two data sets, the first set of flight mill data which constitute the results from adult *E. servus* collected from different crop and non-crop hosts
across the season will be referred to as “whole season data” while the second set of data derived exclusively from adult *E. servus* collected from various corn growth stages (V4-R6) will be referred to as “corn data”. Flight mill data recorded from specimens collected from R4 to R6 corn are shared between the corn data set and the whole season data.

From each host, 25-35 *E. servus* adults per sampling date were collected in an insect cage of 50 X 35 X 25 cm (LBH) dimension with nylon mesh sides. The cage was provisioned with freshly collected food from the same plant host of equal quality. The cages with insects were transported to the laboratory located in Raleigh, NC, and kept shaded outside in shade for 2-3 days as the individuals were removed for the flight mill studies. Before the flight mill trials, male to female sex ratio of adult *E. servus* per collection date was also noted.

### 2.3.2 Flight Mill Sessions

A flight mill system with eight arm-pivots was assembled following Attisano et al. (2015) with modifications on the structural parts, and with metal rods instead of acrylic. The complete instructions required for the operation, data acquisition and data management, along with custom Python scripts (Python Co., Wilmington, DE) to standardize and analyze the voltage signals recorded during each flight session by the data logger and an acquisition software (WINDAQ V 2.95, DATAQ instruments Inc. Akron, OH), can be found in their publication. Four male and four female *E. servus* were randomly selected for a flight mill assay at a single time. The flight sessions were run in the laboratory at room temperature; 24 ± 2 °C, RH of 60 ± 5 % and a photoperiod of 14:10 light and dark cycle. To tether individuals to the flight mill system, insects were cooled in a refrigerator of 4 °C for a maximum of 10 minutes. Insects were then tethered to the flight mill arm using insect pins, with the head of the pin attached to the middle of insect pronotum using a small drop of melted glue on the pin head (Stanley DualMelt®, Stanley Black
& Decker, New Britain, CT) from a low temperature (135 °C) glue gun (Stanley GR25, Stanley Black & Decker, New Britain, CT). The pointed free end of the pin with the insect attached to the other end was inserted to the tip of one end of a flight mill arm. Flight mill sessions were initiated around ≈ 11.00 am of each day and insects were left undisturbed for 22 h. On each sampling date, the first flight session was carried out on the day after collection and the subsequent flight sessions (replications) were repeated with new set of individuals from the cage on the following 1-2 days.

To monitor weight loss after flight, individuals (free of any glue drop or attached pin), were weighed before, and immediately after, the 22 h flight mill assay to the nearest 0.001 g. Based on the abdominal sternal coloration, post-flown individuals were assigned to one of the three overwintering status; ‘overwintering’, ‘transition’, or ‘normal’ color morphs (Fig. 1). The ‘overwintering’ category had reddish-brown sternal coloration which is an indicative of overwintering physiological state, whereas the ‘normal’ category had yellowish green to dark green coloration (Borges et al. 2001). The individuals that exhibited coloration between the overwintering and normal color morphs were categorized as ‘transitional’. Flight performance was evaluated by measuring several variables including total distance flown (m), mean flight speed (m/s), time spent in actual flight (s), percentage time spent in actual flight, time spent on the longest flying bout (s), and the number of discrete flying bouts. All the above parameters were calculated per individual basis for 22 h flight time. Individuals with no flight activity (total distance flown = 0 and flight time = 0), were omitted from all calculations.

2.3.3 Data Analysis

Previous flight mill studies on a stink bug species, H. halys, classified individuals into two groups based on flight potential: long distance fliers which flew more than 5 km, and short
distance fliers which flew ≤ 5 km within 22 or 24 h of flight time (Lee and Leskey 2015, Wiman et al. 2015). Similar to their method, by plotting the frequency distribution graph of all individuals’ flight distance across the season irrespective of host plants, we classified the adult *E. servus* individuals into either long distance fliers or short distance fliers. However, we identified different distance thresholds than *H. halys* studies for this classification to match the observed flight potential of our organism. To develop a regression model to identify the most important variables that predict the total distance flown by individuals, regression models with significant variables were selected from a group of 11 initial variables, using a stepwise regression (PROC GLMSELECT, SAS Institute 2012). In this analysis, a single variable’s explanatory contribution to the model was assessed by Akaike’s information criterion; the Schwarz Bayesian information criterion was specified as the stop criterion. This approach for model selection was performed separately for short and long-distance fliers of both the whole season and the corn data. The initial variables included in the stepwise selection process included: 1. collection date (*date*), 2. plant host of origin (*host*), 3. sex, 4. overwintering state (*OS*), 5. preflight body weight (*PBW*), 6. percentage weight loss after flight (*PWL*), 7. mean flight speed (*FS*), 8. time spent on actual flight (*FT*), 9. number of discrete flying bouts (*NFB*), 10. percentage of time spent on actual flight (*PFT*), and 11. time spent on longest flying bout (*TLFB*). To meet the assumption of normality, total distance flown, number of discrete flying bouts, percentage of time spend in actual flight, and time spend on longest flying bout, were log transformed (log10 + 1) before analysis.

To identify the possible influence of adult *E. servus* preflight body weight on the total distance flown, linear regression analysis (PROC REG, SAS 9.4, SAS Institute 2012, Cary, NC), was performed with log transformed (log10 + 1) flight distance as a dependent variable and
preflight body weight as an independent variable for whole season data and corn data. Two additional regressions were performed to evaluate the relationship of preflight weight to the distance flown by the both short and long-distance fliers of each data set. Influence of sex on the preflight body weight was tested with a general linear analysis of variance model (PROC GLM, SAS Institute 2012). Two separate linear models were performed for the whole season data (at each crop host) as well as the corn data (at each corn phenological stage).

For both the whole season data and the corn data, relevant response variables influencing flight potential of *E. servus* including: 1) mean preflight body weight, 2) mean percentage weight loss after flight, 3) mean flight speed, 4) mean flight distance, 5) mean time spend in actual flight, 6) mean time spent on longest flying bout, and 7) mean number of discrete flying bouts, were compared between the groups of interest (i.e., among plant hosts or corn growth stages). Transformed response variables were analyzed using general linear mixed analysis of variance models (PROC MIXED, SAS Institute 2012). In each model, the only fixed effect was host plant (n = 5; weeds early, wheat, corn, soybean, and weeds late) for the whole season data, and corn growth stage (n = 12; from V4-R6) for the corn data. The only random effect was replication. Before analysis, data from individual insects within each flight mill session was averaged to avoid pseudoreplication. Degrees of freedom in our models were calculated using the Kenward-Roger procedure (Kenward and Roger 1997) to adjust for the estimator of variance of the fixed effects. Means were separated using the Least Significant Difference method at α = 0.05. Untransformed means are presented.
2.4 Results

2.4.1 Classification of Flight Potential

During all the flight sessions, including the whole season and the corn data (Table 1), 33.9% (n = 138) of the total individuals tested had no flight activity (total flight distance = 0). The flight distance frequency histogram indicated that 90.7% (n = 369) of all the individuals tested had flight distances of 0-1 km. Following the method of Wiman et al. (2015), we classified the individual *E. servus* adults into either long distance fliers (LDF) which flew >1 km or short distance fliers (SDF) which flew ≤ 1 km in 22 h flight mill session (Fig. 2). Only five individuals flew more than 5 km; four of them were captured from early season weed hosts (weeds early) and the remaining individual was collected from late season weed hosts (weeds late). The longest distance flown by any individual in 22-h flight mill session was 6.4 km.

2.4.2 Regression Models

For long distance fliers in the whole season data set, the stepwise regression identified two variables, number of discrete flying bouts (*NFB*) and mean flight speed (*FS*), as the most important variables for predicting the total flight distance (*Y*) of an individual in a flight mill session. The corresponding regression equation of the final model (*R^2 = 0.57*) was:

\[
\log_{10} Y = 1.445 + 0.811 \left( \log_{10} NFB \right) + 0.784 \left( FS \right) \tag{2.1}
\]

Similarly, for short distance fliers in the same data set, the stepwise regression identified four variables, 1) number of discrete flying bouts (*NFB*), 2) mean flight speed (*FS*), 3) time spent in longest flying bout (*TLFB*), and 4) percentage of time spent on actual flight (*PFT*), as the most important variables in predicting the flight distance (*Y*) of an individual. The corresponding regression equation of the final model (*R^2 = 0.73*) was:
\[ \log_{10} Y = 0.013 + 1.195 (\log_{10} NFB) + 0.988 (FS) + 0.156 (\log_{10} TLFB) \]
\[ - 0.528 (PFT) \] (2.2)

For long distance fliers in the corn data set, a single variable, preflight body weight \((PBW)\), was sufficient to predict flight distance \((Y)\). The corresponding regression equation of the final model \((R^2 = 0.28)\) was:
\[ \log_{10} Y = 3.693 - 2.673 (PBW) \] (2.3)

Similar to the short distance fliers in the whole season data set (equation 2), the stepwise regression for the corn data set identified the four same variables, 1) number of discrete flying bouts \((NFB)\), 2) mean flight speed \((FS)\), 3) time spent in longest flying bout \((TLFB)\), and 4) percentage of time spend on actual flight \((PFT)\), as the most important variables in predicting the flight distance \((Y)\) of an individual. The final model \((R^2 = 0.74)\) was:
\[ \log_{10} Y = -0.119 + 1.211 (\log_{10} NFB) + 0.906 (FS) + 0.267 (\log_{10} TLFB) \]
\[ - 1.036 (PFT) \] (2.4)

2.4.3 Preflight Body Weight on Distance Flown

There was no significant relationship between preflight body weight of \(E. servus\) adults to the total distance flown on a flight mill for the whole season data when data from both long and short distance fliers were combined \((F = 0.4; \, df = 1, 234; \, P = 0.5275)\). Similarly, there was no significant relationship between the preflight body weight and total distance flown during the 22 h flight mill session using whole season data, either for long distance fliers \((F = 0.16; \, df = 1, 35; \, P = 0.6933)\), or short distance fliers \((F = 0.01; \, df = 1, 197; \, P = 0.9117)\).

There was no significant relationship between preflight body weight to the distance flown by the \(E. servus\) adults in the corn data set \((F = 0.25; \, df = 1, 118; \, P = 0.6185)\), when data from both long and short distance fliers were combined. Separate analyses of long and short distance
fliers revealed a significant linear relationship between the preflight body weight and the
distance flown by *E. servus* adults in long distance flier category (*F* = 4.99; df = 1, 13; *P* = 0.0437), where a negative relationship between log transformed flight distance (*Y*) and preflight body weight (*PBW*) (log$_{10}$ *Y* = 3.692 – 2.673 *PBW*) was observed. However, no such linear relationship observed for short distance fliers (*F* = 0.29; df = 1, 101; *P* = 0.5918).

### 2.4.4 Sex Bias on Preflight Body Weight

On average, *E. servus* adult females collected from early season weed hosts (weeds early) and from soybean were significantly heavier than their male counterparts (weeds early, *F* = 6.99; df = 1, 34; *P* = 0.0123 and soybean, *F* = 4.46; df = 1, 28; *P* = 0.0437; Fig. 3). However, mean preflight body weight was not significantly different between female and male *E. servus* adults collected from wheat, late season weed hosts (weeds late) or corn (wheat, *F* = 4.07; df = 1, 31; *P* = 0.0523; weeds late, *F* = 0.30; df = 1, 17; *P* = 0.5907; corn, *F* = 0.32; df = 1, 116; *P* = 0.5706).

For the corn data set, mean preflight body weight of females and males were significantly different during certain corn growth stages, but not during others (Fig. 4), and no clear trend of sex bias in preflight body weight was observed across the corn growth stages.

### 2.4.5 Influence of Plant Hosts on *E. servus* Flight Parameters and Body Weight

The mean time spent in actual flight, mean flight speed and total distance flown by *E. servus* adults during the flight mill sessions were significantly different among the host plants from which the insects were collected (time spent in actual flight, *F* = 4.58; df = 4, 18.8; *P* = 0.0094; flight speed, *F* = 4.11; df = 4, 18.8; *P* = 0.0147; total flight distance, *F* = 3.51; df = 4, 21.1; *P* = 0.0240; Fig. 5). However, the mean number of discrete flying bouts or the mean time spent in the longest flying bout were not significantly different among the plant hosts (number of
discrete flying bouts, $F = 1.98; \text{df} = 4, 20.7; P = 0.1351$; time spent in the longest flying bout, $F = 2.11; \text{df} = 4, 18.9; P = 0.1192$).

The mean preflight body weight of *E. servus* adults was significantly different among the plant hosts ($F = 4.46; \text{df} = 4, 12.5; P = 0.0184$; Fig. 6). Similarly, the mean percentage weight loss after flight mill sessions was also influenced by the plant hosts that insects were collected from for the study ($F = 12.88; \text{df} = 4, 18; P < 0.0001$). Mean weight loss after flight mill sessions was significantly lower for individuals collected from soybean and late season weed hosts (weeds late), compared with those collected from wheat, corn and early season weed hosts (Fig. 6).

### 2.4.6 Influence of Corn Growth Stages on Flight Parameters

Mean preflight body weight of *E. servus* adults and mean percentage weight loss after the flight mill sessions were influenced by the corn growth stages (preflight body weight, $F = 4.47; \text{df} = 11, 10.1; P = 0.0123$; percentage weight loss, $F = 3.61; \text{df} = 11, 10.9; P = 0.0220$; Fig. 7). However, no significant differences among the corn growth stages were observed on flight parameters including: mean flight speed ($F = 2.49; \text{df} = 11, 10.4; P = 0.0771$), mean total distance flown ($F = 0.70; \text{df} = 11, 10.6; P = 0.7193$), mean total time spend on actual flight ($F = 0.78; \text{df} = 11, 10.8; P = 0.6566$), mean time spend on longest flying bout ($F = 1.49; \text{df} = 11, 10.4; P = 0.2644$), and mean number of discrete flying bouts ($F = 0.44; \text{df} = 11, 11; P = 0.9040$).

### 2.5 Discussion

The results of this study provide information on the baseline flight potential of *E. servus* adults and suggests that a portion of the adult population possesses considerable potential to disperse across the farmscape, especially early in the season just after emerging from overwintering. Among the individuals that exhibited a flight response in the flight mill, adults
collected from the weed hosts early in the season (late March to early April) flew on an average of 1.86 km in 22 h. Moreover, 9.3% of all the adults tested in this study flew further than 1 km (but mostly < 5 km) in 22-h flight period, further indicating their potential to disperse and utilize new host resources that might be spatially disjointed in a farmscape. However, it is important to note that, compared with the invasive stink bug species *H. halys* in the United States, the flight potential of *E. servus* is generally low. For *E. servus* individuals flight distances ≥ 5 km is rare (1.2%) while about 11.1% of *H. halys* population flies ≥ 5 km (Lee and Leskey 2015).

Interestingly, our results also indicate that a majority of (33.9%) individuals exhibited no flight response at all in the flight mill system. Though this could partially be an artifact of factors associated with flight mill system and insect related biological factors including age or mating status (Johnson 1969); however, the observed no flight response of a portion of the population is also partially in agreement with our previous *E. servus* behavioral observations that *E. servus* adults mostly evade the human presence by quickly walking to the less visible part of the plant rather than an immediate flight response (Babu and Reisig 2018). Moreover, their reduced propensity for flight during cropping season is also evident from the observation that a portion of adult *E. servus* remain on soybean (Jones and Sullivan 1982) and wheat (Reisig et al. 2013) even after plant senescence and that movement is usually triggered by the crop harvest.

Along with a change in the host utilization pattern of *E. servus* across the growing season (Blinka 2008, Pilkay et al. 2015), the mean flight potential of adults also varied with the host plants from which the insects were collected (Fig. 5C). The highest mean dispersal potential of *E. servus* adult was observed from the individuals that were collected from early season weed hosts just after their overwintering emergence. The numerical mean flight potential declined gradually as the season progressed with the lowest numerical mean flight distance observed
towards the end of the cropping season from the individuals collected from senescing soybean. Compared with many of the non-agronomic *E. servus* hosts and crops hosts, soybean is regarded as highly suitable food plant for *E. servus* adult survival and nymphal development (Panizzi 1997, Herbert and Toews 2011, Olson et al. 2018). Moreover, it is probable that most of the adult individuals collected from the both the early season and late season weed hosts in this farmscape utilized soybean as food while they were nymphs (A.B., unpublished data, Pilkay et al. 2015).

Consequently, it is unlikely that host quality or nutrition alone is responsible for the differences in flight distance observed among the plant hosts in our study, especially when considering the fact that the lowest flight potential was observed on the soybean-fed population. Previous research suggests that, along with the influence of plant host phenology, *E. servus* has an inherent phenology dependent on the season and photoperiodic changes (Herbert and Toews 2011). It is therefore reasonable to assume that the changes in flight capacity observed among the host plants with collection dates range across the season can be considered as temporal changes with relatively less influence from host crop nutrition.

Temporal changes in the *E. servus* dispersal potential observed in our study could be a reflection of their adaptation to variations in the spatiotemporal distribution or availability of suitable overwintering sites or plant hosts. For example, the maximum numerical mean flight potential observed in our study was for individuals collected from weed hosts during late March to early April. These collection dates coincide with the typical *E. servus* overwintering emergence time reported for the southeastern United States (Jones and Sullivan 1981). Though adult *E. servus* utilize multiple non-agronomic hosts in the weeds along field borders as feeding host for survival at this time (A. B., unpublished data, Jones and Sullivan 1982), host plant distribution is scattered and patchy in the farmscape. Moreover, access to the nearest wheat crop,
one of the earliest crop hosts that becomes attractive to the *E. servus* around early to late-April in this region (Blinka 2008, Reay-Jones 2010), might also require considerable flight. The elevated flight potential experienced at this time of the year, therefore, is most likely a function of the host distribution within the farmscape.

Contrary to the relatively higher mean flight potential (1.86 km per 22-h) exhibited by the *E. servus* adults collected from the early season weeds hosts, the individuals from weed hosts towards the later in the growing season (last week of October), had a lower mean flight potential (0.36 km). Though the exact reason for the difference in flight response by the populations from the early and late season weeds host is unknown, the low mean flight potential exhibited by the individuals collected from the soybean and late season weeds seems to match *E. servus* overwintering location requirements. This insect prefers to overwinter among the weeds in field borders (Jones and Sullivan 1981), which it can access without much flight in most farmscape situations. Interestingly, *H. halys*, which has specific overwintering requirements (Lee et al. 2014), showed the highest flight potential near the end of the season before the diapause (Wiman et al. 2015) further support the above hypothesis.

Similarly, the relatively low flight potential observed during the cropping season also reflects the seasonal abundance of the *E. servus* crop and non-crop host availability in the farmscape. Our results agree with the previous research exploring stink bug movement in the farmscape, suggesting that during the cropping season *E. servus* exhibits field edge mediated dispersion between crop and non-crop host habitat (Tillman et al. 2009, Reisig et al. 2013, Tillman et al. 2014), and most of the *E. servus* movements within and among host habitats are of relatively short range distances when the food sources are abundant and closer (Huang 2012). Comparable results which indicate a propensity for short distance movement by stink bugs
during cropping season were also observed for redbanded stink bug, *Piezodorus guildinii* (Westwood), in soybeans (Bastola and Davis 2018).

Both the mean flight speed and total time spent on actual flight during the flight mill session varied across the season (Fig 5B and 5A). The trends in temporal changes of mean flight speed, followed the same trend of air temperature changes across the season (National Weather Service 2018). The flight speed increased as the temperature rose across the season while the opposite relationship was observed with the air temperature for mean total time spend on actual flight. The fastest mean flight speed and lowest numerical mean total time spent on actual flight, were recorded for the individuals collected from the late reproductive stage corn (R4-R6 stage) during mid-July to early August. This period of flight mill session corresponded with the hottest period of the year. Although flight mill sessions were operated in a laboratory maintained at room temperature with minimal temperature fluctuations, the possible behavioral adaptation of the insects to cope with the field temperature and minimize the water loss associated with the flight exercise could be a reason for observed change in flight parameters (distance, duration and speed) across the season. Moreover, the diurnal flight pattern of *E. servus* adults detected using pheromone-baited traps also suggests that there is less movement during late morning or mid-afternoon than during the early morning and evening hours (Ni et al. 2016). This could indicate a reduction in propensity to fly during the hot period of the day. However, we recognize that temperature in this study is collinear with many factors including age, generation, reproductive status, food availability, nutrition, light regimen, and humidity can influence flight parameters (Dingle 1968, Evenden et al. 2014); therefore, the exact reason behind the seasonal changes in flight parameters needs further investigation.
The preflight body weight of the insects is known to positively influence flight performance of some insects (Evenden et al. 2014). However, results from our whole season data set suggest that there is not a linear relationship between preflight body weight and mean total flight distance flown by either long or short distance fliers. Similarly, no linear relationship was observed for the short distance fliers using the corn data set. Interestingly, a negative linear relationship was observed between preflight body weight and the mean total flight distance flown by long distance fliers of corn data. This seemingly inconsistent result can be explained by the relationship of *E. servus* adult body weight to insect age during this period of time (8 May to 2 August). Depending on the time of the year, *E. servus* populations in corn can be composed of a mixture of overwintered and F1 generation individuals (Blinka 2008, Herbert and Toews 2011). For timely planted corn in the North Carolina, the *E. servus* adult population in early vegetative stage corn is composed almost entirely of the overwintered population. By contrast, in late vegetative and reproductive-stage corn, the population can be composed mostly of the F1 generation, depending on the degree of reproduction in corn and dispersal from nearby crops (Blinka 2008). Furthermore, Blinka (2008) observed a heavy die off of overwintered *E. servus* adults feeding in wheat during late May to early June as the small F1 nymphs developed on the wheat crop. This time period corresponded to the V9-V14 corn growth stage in our experiment. Accordingly, the average preflight body weight of adult *E. servus* declined around these corn growth stage in our study (Fig. 4), which probably reflected the gradual die-off of the relatively heavier overwintered generation adults and a gradual shift towards the younger, lighter F1 generation adults. Hence, the influence of adult age is probably the biological reason behind the negative linear relationship observed between the preflight body weight and the mean total flight distance flown by long distance fliers in the corn data set.
Mean percentage weight loss after the flight mill sessions was significantly lower for individuals collected from soybean and late season weed hosts (weeds late), compared with those collected from wheat, corn and early season weed hosts. Stink bug generation, nutritional status and physiological state, especially fat reserves, all play a major role in weight loss pattern (Wiman et al. 2015). The *E. servus* adults collected from early season weed host were the overwintered generation with a depleted fat reserve, while the adult individuals collected from the wheat and corn crops were a mixture of mostly F\textsubscript{1} generation and overwintered adults (Blinka 2008). In contrast the F\textsubscript{2} generation that developed in soybean and weeds, were the overwintering generation (Pilkay et al. 2015) that later moved to weeds hosts (weeds late) for overwintering. These individuals had considerable fat reserves and other physiological adaptations to survive in the winter months during diapause. Studies on the mountain pine beetle, *Dendroctonus ponderosae* Hopkins, suggested that the beetle uses body lipids for flight activities (Evenden et al. 2014). Among possible flight energy sources, lipid is a more concentrated source of energy per unit weight than stored glycogen and lipid metabolism generates more metabolic water than carbohydrates (Beenakkers et al. 1981). Consequently, the presence of fat bodies as an energy source and other potential physiological adaptations for overwintering could explain the lower percentage weight loss observed for the individuals collected from soybean and late season weed hosts experienced after flight mill sessions. Similar seasonal trends in weight loss after flight mill sessions were also observed for *H. halys* populations (Lee and Leskey 2015, Wiman et al. 2015).

Despite the potential limitations normally associated with a flight mill studies (Taylor et al. 2010), this study provides baseline flight potential information of adult *E. servus* across the season. For *E. servus*, the highest mean dispersal potential was observed soon after the
overwintering emergence in the spring. Our results agree with the previous observations that, during the cropping season, *E. servus* exhibits a relatively low flight potential and most flights are of a relatively short range distance. Since most long distance movement occurs early in the season, scouting for stink bugs in an early colonized crop like wheat or early vegetative stage corn could help forecast the landscape-level *E. servus* risk, whereas the reduced dispersal potential during the mid to late cropping season could help growers to focus *E. servus* management measures at the farmscape level.
2.6 Acknowledgments

Dan Mott and Dannyel Akira Nelson provided the technical assistance. We thank Antonio Cinti Luciani and Jonathan Peppers of Open Ground Farms, Beaufort, NC, for allowing the access to fields for sample collection. We thank Robert L. Blinn (North Carolina State Insect Museum, Raleigh, NC.) for assisting in stink bug species identification. Partial funding of this project was provided by NC Agricultural and Life Sciences Research Foundation and the Corn Growers Association of North Carolina.
2.7 References Cited


Huang, T.-I. 2012. Local dispersal of stink bugs (hemiptera: Pentatomidae) in mixed agricultural landscapes of the coastal plain. PhD Dissertation, University of Georgia Athens, GA.


2.8 Tables and Figures

Table 2.1  *E. servus* sampling date range and the corresponding host growth stage. Flight mill data collected from these specimens constituted the ‘whole season’ data set.

<table>
<thead>
<tr>
<th>Host</th>
<th>Sampling date range</th>
<th>Host growth stage range^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weeds early</td>
<td>28 March - 4 April</td>
<td>__^b</td>
</tr>
<tr>
<td>Wheat</td>
<td>12 - 30 May</td>
<td>83 - 92</td>
</tr>
<tr>
<td>Corn^c</td>
<td>19 July - 2 August</td>
<td>R4 - R6</td>
</tr>
<tr>
<td>Soybean</td>
<td>25 September - October</td>
<td>R8</td>
</tr>
<tr>
<td>Weeds late</td>
<td>17 - 26 October</td>
<td>__^b</td>
</tr>
</tbody>
</table>

^a Host growth stages were identified based on prior published works containing growth stage descriptions; wheat (Zadoks et al. 1974), corn (Ritchie et al. 1993) and soybean (Fehr et al. 1971).

^b Adult *E. servus* were collected from multiple non-agronomic hosts in weedy field borders, and, therefore, no specific plant growth stages are provided.

^c Additional weekly samples of *E. servus* adults were collected from corn from 8 May to 11 July representing *E. servus* from V4-R4 corn growth stages. Flight mill data recorded from specimens collected from R4 to R6 corn of the corn data set are shared between the whole season data set.
Figure 2.1  Adult *E. servus* color morphs. Images 1-3 show overwintering coloration; image 4 represents the transitional coloration between the overwintering and normal morph; and images 5-9 show variation in normal color morphs. The bright yellow or light greenish yellow color (image 5 and 6) is usually associated with the male sex.
Figure 2.2  Frequency histogram of the total flight distance of all *E. servus* adults (n = 407). Based on flight potential, individuals are classified into long distance fliers that flew >1 km or short distance fliers that flew 0 to ≤ 1 km during 22-h flight mill session.
Figure 2.3  Mean (± SEM) preflight body weight of male (M) and female (F) *E. servus* adults during 2017. Mean preflight weight of male and female *E. servus* adults within a host with different letters are significantly different (LSD; α = 0.05).
Figure 2.4  Mean (± SEM) preflight body weight of male (M) and female (F) *E. servus* adults in corn during 2017. Mean preflight weight of male and female *E. servus* adults within a corn growth stage with different letters are significantly different (LSD; \( \alpha = 0.05 \)).
Figure 2.5  Mean (± SEM) time spend in actual flight (A), mean (± SEM) flight speed (B), and mean (± SEM) distance flown (C) by *E. servus* adults after 22-h flight mill sessions during 2017. Individuals with no flight activity (total distance flown, and time spend on actual flight = 0) were omitted from the analyses.
Figure 2.6  Mean (± SEM) preflight body weight (A) and mean (± SEM) loss of body weight expressed as the percentage of preflight weight (B) of *E. servus* adults after 22-h flight mill sessions during 2017. Individuals with no flight activity (total distance flown, and time spend on actual flight = 0), were omitted from the analyses.
Figure 2.7  Mean (± SEM) preflight body weight (A) and mean (± SEM) weight loss after flight expressed as the percentage of preflight weight (B), of *E. servus* adults after 22-h flight mill sessions at various corn growth stages during 2017. Individuals with no flight activity (total distance flown, and time spend on actual flight = 0), were omitted from the analyses.
CHAPTER III

WITHIN-PLANT DISTRIBUTION OF ADULT BROWN STINK BUG (HEMIPTERA: PENTATOMIDAE) IN CORN AND ITS IMPLICATIONS ON STINK BUG SAMPLING AND MANAGEMENT IN CORN

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3.1 Abstract

Brown stink bug, *Euschistus servus* (Say), has emerged as a significant pest of corn, *Zea mays* L., in the southeastern United States. A 2-year study was conducted to quantify the within-plant vertical distribution of adult *E. servus* in field corn, to examine potential plant phenological characteristics associated with their observed distribution, and to select an efficient partial plant sampling method for adult *E. servus* population estimation. Within-plant distribution of adult *E. servus* was influenced by corn phenology. On V4 and V6-stage corn, most of the individuals were found at the base of the plant. Mean relative vertical position of adult *E. servus* population in corn plants trended upward between the V6 and V14 growth stages. During the reproductive corn growth stages (R1, R2 and R4), a majority of the adult *E. servus* were concentrated around developing ears. Based on the multiple selection criteria, during V4-V6 corn growth stages, either the corn stalk below the lowest green leaf or basal stratum method could be employed for efficient *E. servus* sampling. Similarly, on reproductive corn growth stages (R1-R4), the plant parts between two leaves above and three leaves below the primary ear leaf were found to be areas to provide the most precise and cost-efficient sampling method. The results from our study successfully demonstrate that in the early vegetative and reproductive stages of corn, scouts can replace the current labor intensive whole-plant search method with a more efficient, specific partial plant sampling method for *E. servus* population estimation.
3.2 Introduction

Stink bugs have emerged as a significant pest of crops in southeastern United States (Greene and Turnipseed 1996, Smith et al. 2009). The elevated pest status of this insect in cotton, *Gossypium hirsutum* (L.), has been attributed in part to the widespread adoption of Bt crops and subsequent reduction of broad spectrum insecticide use (Bundy and McPherson 2000). For brown stink bug, *Euschistus servus* (Say), wheat, *Triticum aestivum* (L.) is the most important spring crop host in southeastern United States (Jones and Sullivan 1982), and *E. servus* can complete the F1 generation entirely in this crop (Blinka 2008). In this region, wheat is planted during the fall of the previous year and harvested during the spring of the following year. As a result, wheat often supports high populations that subsequently infest nearby crops, including corn, *Zea mays* (L.), mostly during or after wheat harvest (Reisig et al. 2013). Similarly, soybean, *Glycine max* (L.) Merrill, serves as an important fall crop host for stink bugs in the southeastern United States (Jones and Sullivan 1982) where they develop the F2 overwintering generation. Though historically *E. servus* was not considered to be a significant pest of corn (Bergman 1999), significant damage from this pest is occasionally reported in literature (Townsend and Sedlacek 1986, Sedlacek and Townsend 1988) and in recent years, growers and scouts across southeastern United States have reported elevated *E. servus* damage to their corn crop.

In North Carolina, corn is the first row-crop host available to *E. servus* that is planted during the spring (Todd and Herzog 1980). *Euschistus servus* can infest and damage corn during both the vegetative and the reproductive growth stages. In young corn plants, *E. servus* feeding can result in seedling death, induce tiller production, and stunted growth (Townsend and Sedlacek 1986, Apriyanto et al. 1989a). No information is available relating to *E. servus* damage potential to the pretasseling corn growth stages; however, a related pest, the southern
green stink bug, *Nezara viridula* (L.), can result in a significant reduction in ear length and weight when feeding on V15-stage corn (Negrón and Riley 1987). Moreover, *E. servus* feeding at the VT or R1 corn growth stages can result in significant reduction of kernel weight (Ni et al. 2010).

Knowing the within-plant distribution of *E. servus* is critical to understanding how crop damage occurs, to develop a reliable and efficient sampling plan, and to implement effective integrated pest management tactics. The within-plant feeding location of a pest on a crop often influences its damage potential. For example, in seedling corn, *E. servus* damage symptoms, such as tiller production and reduced extended leaf height, are observed only when *E. servus* feed from the base of a seedling corn plant (Townsend and Sedlacek 1986). Yield reduction resulting from *N. viridula* (L.) induced damage at the V15 growth stage is attributed to the destruction or abortion of the developing ear (Negrón and Riley 1987); this further supports the importance of the within-plant feeding location of stink bugs since damage to the corn ear was associated with yield reduction. Similarly, during the tasseling and post tasseling corn growth stages, economic damage from *E. servus* feeding is restricted to corn ears and is attributed to reduced kernel weight or kernel abortion (Ni et al. 2010). Despite this possible association of within-plant distribution of *E. servus* to its plant damage potential, little is known about the within-plant *E. servus* distribution in field corn.

Many scientific studies describing the within-plant distribution of insects have demonstrated that sampling can be restricted to the specific plant part where a portion of individuals are consistently found throughout the intended sampling period (Wilson et al. 1982, Arx et al. 1984, Naranjo and Flint 1994, Arno et al. 2006). Specifically for pentatomids, the within-plant spatial distribution of individuals or their associated damage has been reported.
from major row crops like soybean and corn, and underlying distribution is found to be nonrandom (Russin et al. 1987, Blinka 2008, Owens et al. 2013). For instance, at low infestation levels, stink bug damage to soybean seeds is concentrated at the upper half of the plant (Russin et al. 1987). Similarly, in corn, *E. servus* are frequently found feeding at the base of V2 corn plants (Townsend and Sdlacek 1986). On reproductive corn (R1-R3), Blinka (2008) found a significantly higher number of adult *E. servus* from the ‘corn ear zone’, compared with the plant zones above and below the ear zone, and suggested that, during reproductive stages, stink bug sampling efforts should focus on the ear zone. This partial plant sampling method is clearly efficient over the current whole-plant sampling method in terms of sampling cost (Blinka 2008). However, an arbitrarily defined partial plant sampling method may not represent the most efficient sampling method.

The default stink bug sampling procedure in corn is whole-plant searches. Since this sampling protocol involves examination of the top of corn plants - often above the sampler’s eye level- stink bug sampling is tiresome, laborious and costly especially when the plants are tall (Blinka 2008). Conversely, developing a partial plant sampling method based on within-plant *E. servus* distribution data could potentially enhance the sampling efficiency. The objectives of this research were, therefore: 1) to quantify the within-plant distribution of adult *E. servus* on corn plants of selected plant growth stages; 2) to examine the plant factors that influence *E. servus* distribution in field corn; and 3) to propose partial plant sampling method(s) of *E. servus* in field corn based on their observed within-plant distribution.
3.3 Materials and Methods

3.3.1 Within-Plant Distribution of Adult *E. servus* on Field Corn

During 2016 and 2017, adult *E. servus* within-plant vertical distribution data were collected from corn fields in a commercial production farm located at Carteret Co., NC (34.894083, -76.566750). During each year, four random corn fields were selected for *E. servus* sampling. Overall, three corn varieties were used in this experiment with seeding rate ranging from 69,000 to 75,000 seeds per hectare (Table 1). Planting date varied from 12 to 19 April in 2016 and from 3 to 5 April in 2017. Seeds were treated with either clothianidin (1.25 mg A.I. per seed) or thiamethoxam (0.25 mg A.I. per seed) + chlorantraniliprole (0.25 mg A.I. per seed) combination. No foliar insecticides were applied to the test plots during this study. Corn plots were planted in blocks of size ranging from 2.4 to 9.9 ha and plots were typically bordered on two to three sides by other corn fields and at least one side by a dirt road edged by pine forest. In southeastern United States, harvesting winter-planted wheat in the spring often results in large scale movement of the *E. servus* to neighboring corn fields (Blinka 2008, Reisig et al. 2013). Such large-scale movement of *E. servus* from an external host could temporarily confound the natural *E. servus* with-in plant distribution in corn. To avoid interference from the stink bug population from wheat fields, the study location was chosen such that the nearest wheat field was located at least 5 km away from the experimental plots during both years (CropScape USDA-NASS 2017).

From each field, random corn plants were sampled for adult *E. servus* at three fixed sampling points marked using a single transect at 5, 10 and 15 m into the field. At each sampling point, 100 plants were randomly selected and adult *E. servus* were counted using whole-plant visual inspection. During sampling, severely stunned plants (lagging behind the average corn growth stage of the sampling field) encountered were replaced by sampling.
another random plant. Fields were considered a replication and each sampling point within a field was considered to be a subsample. Data from the three sampling points within a field were pooled together before analysis. During each year, observations were repeated at V2, V4, V8, V12, V14, R1 (silking), R2 (bister), and R4 (dough) corn growth stages (Ritchie et al. 1993). In 2017, an additional growth stage, V6 was also included in the study.

Adult *E. servus* spatial position on corn plants was recorded as the vertical height from the soil surface. When *E. servus* adults were observed on corn plants, plant heights were also noted and this information was utilized to convert the within plant spatial position of *E. servus* into percent plant height. To relate the observed within-plant distribution of adult *E. servus* to corn morphology at each corn growth stage, selected morphological characteristics including the vertical position of topmost brace roots, the position of leaf collars, the position of the primary ear leaf collar (on reproductive stages), and the plant height were recorded from the soil level of five random corn plants per field. Plant height varied greatly across the growth stage and year. Consequently, the vertical position of *E. servus* distribution on plants, as well as the position of selected plant morphological features (brace roots, leaf collar, primary ear leaf collar), was converted to percent plant height before graphing to facilitate a direct comparison of results across the growth stages and years. The *E. servus* vertical position frequency distribution graph was plotted across corn growth stage and sampling year using GraphPad Prism 6 software (GraphPad Software Inc. San Diego, CA). Each graph was paired with a box-and-whisker plot representing the variation in average position of brace roots (if present), and leaf collars of the typical corn plants of experimental plots of same growth stage. The within-plant adult *E. servus* frequency distributions for a specific corn growth stage between the
sampling years were compared using the Kolmogorov-Smirnov two-sample test (GraphPad Software Inc.).

3.3.2 Plant Structures Associated with Adult *E. servus* Activity

To identify the plant structures associated with adult *E. servus* activity, *E. servus* locations on corn plants were categorized into one of five exclusive plant structure categories. Since plant morphology varied greatly among vegetative and reproductive corn growth stages, a separate set of categories was defined for both vegetative and reproductive stages. During the vegetative corn growth stages, adult *E. servus* locations on a plant were grouped into brace roots, stem, leaf, whorl, or sucker plants. The stem category comprised the corn stalk and leaf sheath covering the stalk. *Euschistus servus* adults found inside the leaf collar close to the main stem (<2 cm from the stalk) were also categorized as stem. The leaf category comprised the leaf blade and collar area (>2 cm from the stalk) of fully developed leaf with a visible collar. All the plant parts above the topmost visible leaf collar were categorized as whorl. During our experiment, those corn plants that produced suckers did so around V6 or later and only *E. servus* adults found on a relatively small sucker plant (<10 cm height) associated with a main plant were included in sucker plant category. No *E. servus* observations were taken from larger sucker plants as our observations were directed to plants around a specific corn growth stages. Plant structure categories defined for reproductive corn growth stages included brace roots, stem, leaf, ear, and tassel. The ear category comprised the primary and secondary ears. *Euschistus servus* adults found on the leaf sheath covering the ear were also classified into the ear category. Data from vegetative and reproductive stages were analyzed separately for each year and were subjected to a generalized linear mixed model with a Gaussian distribution (PROC GLIMMIX, SAS Institute 2011). The response variable was the relative frequency of *E. servus*. 

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E. servus on a plant part of a specific corn growth stage. The fixed effects were corn growth stage (n = variable) and plant structure (n = 5); field (n = 4) was classified as a random effect. Significant two-way interactions, when present, were partitioned using the SLICE statement for simple effect comparison. Means were separated using Tukey’s HSD. The mean proportion of individuals in each plant structure (mean ± SEM) were plotted using GraphPad Software.

### 3.3.3 Plant Factors That Influence Stink Bug Distribution

Preliminary observations suggested that during the early vegetative stages (up to the V6 stage), adult *E. servus* were mainly found feeding from the base of the corn plant near the soil surface, often upside down directing the head toward the very base of the plant. Damage from stink bugs at these corn growth stages is often attributed to mechanical or chemical damage to the plant growing point (Sedlacek and Townsend 1988). To relate the proximity of the plant growing point to the stink bug feeding zone in early vegetative stage corn, 10 random plants each from four random corn fields were sampled at V2 to V6 corn growth stages in 2016 growing season. Plants were carefully dug out after marking the soil level on plant stalks using a permanent marker. Plants were then transported to the lab and stalks were dissected longitudinally to reveal the growing point inside. Position of the growing point in relation to the soil level was measured. Data were analyzed as completely randomized design using a linear mixed model ANOVA (PROC MIXED, SAS Institute 2011). Heteroskedasticity was adjusted by adding GROUP = growth stage option in the repeated statement, and degrees of freedom for the unequal variance were adjusted by using the SATTERTHWAITE method for degree of freedom approximation. The response variable using this analysis was the position of growing point in a plant in relation to soil level. The fixed effect was corn growth stage (n = 5) and
random effects were plant nested in field, field, and replication within a corn growth stage. Means were separated using Tukey’s HSD.

Blinka (2008) suggested that adult *E. servus* has a clear preference for the ear zone during corn reproductive stages. While taking observations on within-plant vertical distribution of adult *E. servus* during reproductive corn growth stages (R1, R2, R4), *E. servus* location was noted in relation to the primary ear leaf collar by counting the stink bug location as the number of leaf collars above or below the primary ear leaf. Percentage of *E. servus* in a leaf collar within a corn growth stage and year was averaged across fields (PROC MEANS, SAS Institute 2011) and plotted.

### 3.3.4 Developing a Partial Plant Sampling Plan for Adult *E. servus*

From the vertical within-plant distribution data, changes in mean relative vertical position of adult *E. servus* across corn growth stages were analyzed by fitting an appropriate regression model (GraphPad Software Inc.). The mean relative vertical position of *E. servus* adult in a corn growth stage (dependent variable) was regressed against mean number of fully developed leaf with a visible collar (independent variable), a proxy for corn growth stages. During the vegetative corn stages, the growth stage itself represented the mean number of fully developed leaf (eg. V14 = 14 fully developed leaf). During reproductive stages of corn (R1, R2, R4), the mean number of fully developed leaves was calculated from five random plants per growth stage (n = 3) and field (n = 4) combination. Between sampling years, the extra-sum-of squares *F* test suggests no significant difference between the regression curves parameters (*F* = 0.4974; df = 3, 9; *P* = 0.6932), and data from both years were combined and fitted with a single regression curve.
To identify the possible adult *E. servus* preference for a particular plant stratum within a corn growth stage, within-plant vertical locations of adult *E. servus* were categorized into one of three vertical plant strata; basal one-third, middle one-third and upper one-third height; henceforth referred to as the basal, middle, and upper stratum. *Euschistus servus* vertical within-plant location and plant height information were utilized to designate the corresponding plant stratum. The *E. servus* number in each plant stratum was calculated for each corn growth stage and field combination. Arcsine transformed data were subjected to a generalized linear mixed model with a multinomial distribution and GLOGIT as link function (PROC GLIMMIX, SAS Institute 2011). The probability of *E. servus* occurrence in a plant stratum was predicted using the upper stratum as a reference category. Means were separated using Tukey’s HSD.

Based on the regression model of mean relative vertical position of *E. servus* adults across various corn growth stages, we identified and grouped corn growth stages with similar *E. servus* distribution patterns (V4+V6 and R1+R2+R4). The predicted probability distribution of *E. servus* abundance on a given plant stratum helped us to identify the potential plant stratum for designing partial plant sampling method (basal stratum for V4 and V6 and middle stratum for R1, R2 and R4). Nearly all the *E. servus* adults during V4 and most of the individuals during V6 were found on the stalk below the lowest green leaf. Similarly, for reproductive corn growth stages (R1, R2, R4), a majority of *E. servus* adults were concentrated around the primary ear.

Based on the above data for early vegetative corn growth stages (V4 and V6), we identified two potential partial plant sampling methods for comparison and evaluation. These included the basal stratum (all the plant parts up to 33.3% of plant height from the ground level) and the corn stalk below the lowest green leaf (all the corn stalk area below the lowest green leaf not including the lowest green leaf and leaf collar). Similarly, for reproductive corn growth stages
(R1, R2, and R4), seven partial plant sampling methods were selected for evaluation. These included: 1) middle stratum; 2) ‘Z’ (the entire plant between one leaf above and one leaf below the primary ear leaf); 3) ‘ZP1’ (the entire plant between two leaves above and one leaf below the primary ear leaf); 4) ‘ZM1’ (the entire plant between one leaf above and two leaves below the primary ear leaf); 5) ‘ZP1M1’ (the entire plant between two leaves above and two leaves below the primary ear leaf); 6) ‘ZM1M2’ (the entire plant between one leaf above and three leaves below the primary ear leaf); and 7) ‘ZP1M1M2’ (the entire plant between two leaves above and three leaves below the primary ear leaf).

Various criteria can be used to select the most efficient sampling technique, including pest abundance, low sample variability (Naranjo and Flint 1994, Arno et al. 2006), high fidelity of population estimates by partial plant sampling methods to the absolute estimate across a range of population densities (Pedigo et al. 1972), and high sampling efficacy (Pedigo et al. 1972, Buntin 1994). Population density estimates from an absolute sampling technique are needed to assess the accuracy and fidelity of the density estimate from a relative sampling method. The whole sampling method described in our research is a population intensity estimate that can readily converted to an absolute estimate by converting the values into a unit land area basis (Buntin 1994). Since in our study, the whole-plant estimates and corresponding partial plant estimates, were derived from the same sample units covering the same land area, direct comparison between the whole-plant versus partial plant sampling method was followed.

The accuracy of partial plant sampling can be evaluated by comparing the means estimate of each technique to an absolute estimate (Buntin 1994). In our study, to compare sampling accuracy, mean *E. servus* adult counts from partial plant sampling methods of both vegetative and reproductive stages were expressed as a percentage of whole-plant counts. Mean
percentage *E. servus* counts from the selected partial plant sampling methods for early vegetative corn growth stages (V4 and V6) were compared using paired two-tailed *t*-tests at the *α* < 0.05 significance level (GraphPad Software Inc.). For reproductive stages, mean percentage *E. servus* counts from selected partial plant sampling methods were analyzed using a generalized linear mixed model with a Gamma distribution (PROC GLIMMIX, SAS Institute 2011). The response variable was the mean percentage of adult *E. servus* per field. The fixed effects were sampling year (*n* = 2), partial plant sampling method (*n* = 7), corn growth stage (*n* = 3) and their interactions. The random effect was field nested in year. Denominator degrees of freedom were calculated using Kenward-Roger’s degree of freedom approximation. Significant two-way interactions were partitioned using the SLICE statement to achieve simple effect comparisons. Simple effects were compared using Tukey-Kramer’s mean separation at the *α* < 0.05 significance level.

The sampling precision of the partial plant sampling methods was assessed by comparing the relative sample variation (RV). Relative variation is a measure of sample variability and, thereby, sampling precision. Low RV indicates high sampling precision. Relative variation can be calculated using the formula (Buntin 1994):

\[
RV = \left( \frac{SE}{\bar{x}} \right) \times 100
\]

(3.1)

where SE is the sample standard error of the mean, and \( \bar{x} \) is the sample mean. For developing an intensive sampling plan, an RV value of 10 or below is regarded as the adequate level of sampling precision (Pedigo et al. 1972). The RV value of a sampling method in each growth stage was calculated. The RV value (precision) from selected partial plant sampling methods for early vegetative growth stages (V4 and V6) were compared using paired two-tailed *t*-tests at the *α* < 0.05 significance level (GraphPad Software Inc.). The RV value of seven partial plant
sampling methods for reproductive stages (R1, R2 and R4) were compared using a generalized linear mixed model with a Gaussian distribution (PROC GLIMMIX, SAS Institute 2011). The response variable was RV during a growth stage within a year. The fixed effects were sampling year (n = 2), partial plant sampling method (n = 7) and their interactions. The random effect was growth stage. Denominator degrees of freedom were calculated using Kenward-Roger’s degrees of freedom approximation.

High fidelity of population estimates, by comparing a relative sampling technique to the absolute estimate across a range of population densities, indicate the suitability of a specific relative sampling method. We used linear regression analysis to compare adult E. servus numbers from partial plant sampling method (dependent variable) to whole-plant E. servus numbers (independent variable) using GraphPad Software. High R² values of regression analysis suggest a high fidelity between the means (Pedigo et al. 1972).

Finally, a sampling technique with a high relative net precision (RNP) indicates a high sampling precision for a given cost. RNP can be calculated using the formula (Southwood 1966):

\[ \text{RNP} = \frac{1}{(\text{RV}_m \times \text{Cu})} \times 100 \]  

(3.2)

where RV\(_m\) is mean relative variation of several data set, and Cu is the sampling cost measured in human-minutes required to collect and process a single sample unit. Higher RNP suggests better sampling efficiency (Buntin 1994). Since the RNP value is derived from the mean RV, no statistical analysis of RNP is possible (Buntin 1994).

No sampling cost information was collected for the basal stratum of early vegetative stage corn (V4 and V6). Other than that, the time required to estimate adult E. servus density was measured for all other partial plant sampling methods, as well as the whole-plant sampling
method. Each sample unit consisted of 20 consecutive corn plants and sampling time was measured from eight replicated sampling units separated 64 meters apart. A stop watch was used to measure sampling time, which included both the time to sample an individual sampling unit as well as the time required to walk between the two adjacent sampling units. Mean sampling cost (in seconds) was calculated for each sampling method and mean time gained for each partial plant sampling method over the whole-plant method was also calculated. Data on the sampling cost of the corn stalk below the lowest green leaf sampling method and of the whole-plant sampling method for early vegetative corn growth stages (V4 and V6) were compared using paired two-tailed t-tests at the $\alpha < 0.05$ significance level (GraphPad Software Inc.). Data on the sampling cost of the whole-plant sampling method and partial plant sampling methods for reproductive growth stages (R1, R2, and R4) were analyzed as a randomized complete block design using a linear mixed model ANOVA (PROC MIXED, SAS Institute 2011). The response variable was sampling cost. The fixed effect was sampling method (n=8). The random effect was replication (n=8). Denominator degrees of freedom were calculated using Kenward-Roger’s degrees of freedom approximation.

3.4 Results

3.4.1 Within-Plant Vertical Distribution of *E. servus* Adults

In total, across all corn growth stages, fields, and years tested, the within-plant distribution of 1,492 adult *E. servus* was documented from 20,400 corn plants, of which a majority of individuals (n = 1204, 80.7%) were sampled during the 2016 field season. Out of the 1,200 corn plants searched during each year, in the V2 corn growth stage, only a single adult *E. servus* was observed in 2016 and none in 2017 (data not shown). During the V4 corn growth stage, most of the individuals (93.8% during 2016 and 99.6% during 2017) were found at the
base of the corn plant (Fig 1), on the stalk area below the collar of the lowest intact leaf, which corresponds to approximately 10% of the plant height. Similarly, during the V6 growth stage, most of the individuals (88.6% in 2017) were found at the base of the corn plant on the stalk below the collar of the lowest intact leaf, which again corresponds to approximately 10% of the plant height. By the V8 corn growth stage, however, part of the adult *E. servus* population moved vertically up the plant. The *E. servus* adults at this corn growth stage were mostly concentrated at the base of the corn plant within 10% of the plant height from soil (71.6% in 2016 and 50% in 2017) and the rest of the population was mostly on the stem area located between 30 and 80% of the plant height from the soil (Fig. 1). At the V12 corn growth stage, *E. servus* adults were found vertically throughout most of the plant except at the topmost 10 and 30% of the plant height from the soil level in 2016 and 2017, respectively (Fig. 2). At the V14 stage, during both years, adult *E. servus* were concentrated around 20-60% of the plant height from the soil. This effect was more pronounced in reproductive corn growth stages as the individuals concentrated around the developing ears (Fig. 3). Within a specific corn growth stage, there was a difference in *E. servus* distribution during all the vegetative growth stages tested between years (V4: $ks = 0.4092, P = 0.0004$; V8: $ks = 0.2769, P = 0.0341$; V12: $ks = 0.3848, P = 0.0002$; V14: $ks = 0.3123, P = 0.0114$). However, no difference was observed for any of the reproductive stages tested between years (R1: $ks = 0.2048, P = 0.0899$; R2: $ks = 0.1699, P = 0.2377$; R4: $ks = 0.2787, P = 0.1880$).

### 3.4.2 Location of Adult *E. servus* on Corn Plant Structures

During the vegetative growth stages, *E. servus* adults were located on a variety of plant structures including brace roots, stalks, leaves, whorls and sucker plants. In general, during the vegetative growth stages, most *E. servus* adults were located on the plant stalk (Fig 4). During
the V4 and V6 growth stages, more than 90% of *E. servus* adults were found on the plant stalk (V4-2016: 94.06% ± 3.06%; V4-2017: 100% ± 0.00%; V6 2017: 92.19% ± 5.92%; Fig. 4), mostly near base of the corn plants (Fig. 1). During 2017, between the V4 and V6 stages, no significant differences (*t* = 0.28; df = 6; *P* = 0.7883) were observed on the proportion of adult *E. servus* on plant stalk. During 2016 and 2017, sucker plants, possibly triggered by *E. servus* damage, were observed around the V6 growth stage and brace roots grew beginning at the V8 stage or later. During 2017 a higher proportion of individuals were found on the brace roots of corn plant at the V12 and V14 growth stages compared with 2016 (V12, *t* = 3.25; df = 6; *P* = 0.0174 and V14, *t* = 4.64; df = 6; *P* = 0.0035) (Fig. 4).

There were significant interactions between corn growth stages and plant structures at the reproductive corn growth stages during both years (2016, *F* = 8.4; df = 8, 45; *P* < 0.0001 and 2017, *F* = 3.86; df = 2, 45; *P* = 0.0016). Except at the R1 growth stage (silking), most adult *E. servus* were found on the ear (Fig. 5). In contrast, at R1, a high percentage of adult *E. servus* were found both on the ear (2016, 33.97% ± 8.05% and 2017, 42.36% ± 3.47%) as well as on the stem (2016, 55.06% ± 9.33%; and 2017, 44.45% ± 9.35%). No differences were observed in the proportion of adult *E. servus* present on leaves across growth stages in either year (2016, *F* = 0.58; df = 2, 45; *P* = 0.5632 and 2017, *F* = 2.55; df = 2, 45; *P* = 0.089). Throughout the reproductive growth stages, adult *E. servus* were nearly absent from both the brace roots and the tassels (Fig. 5).

### 3.4.3 Plant Factors that Influence Within-plant *E. servus* Adult Distribution

In early vegetative stage corn (V2-V6), the position of the growing point in relation to the soil surface was associated with the corn growth stage (*F* = 17.79; df = 4, 12; *P* < 0.0001). The mean location of the growing point remained below the soil surface until the V5 growth
stage (Fig. 6; V5, -0.098 ± 0.10 cm from the soil surface). At the V6 growth stage, the growing point of the plants moved rapidly above the soil surface (1.11 ± 0.14 cm from the soil surface). However, the relative position of the growing points observed within a single growth stage was relatively variable. Furthermore, a portion of the plants had growing points above the soil surface from the V3 growth stage onwards.

Quantification of the mean relative frequency of *E. servus* adults in relation to the location of the primary ear leaf collar suggests a preference for adult *E. servus* to reside near the ear zone during the reproductive growth stages (Fig. 7). In 2016 during R1 (silking), a relatively high proportion of individuals were aggregated around the primary (‘Z’, 31.45% ± 6.75%) and secondary ear leaf collar (M1, 33.10% ± 4.77%). Similarly, in 2017, at R1, a relatively high proportion of individuals were aggregated around the primary ear leaf collar (37.50% ± 4.17%). In contrast to the previous year, during 2017 a relatively high proportion of individuals were found on the plant area between one and two leaf collars above the primary ear (P1: 20.83 ± 12.50), rather than the secondary ear leaf collar (M1, 9.72% ± 6.56%). Across both years of this study, during the R2 (blister) and R4 (dough) growth stages, a majority of adult *E. servus* (ranging from 65.83 to 79.30%) were observed around the primary ear leaf collar (Fig. 7).

### 3.4.4 Developing Partial Plant Sampling Plan for *E. servus* Adults

Changes in the mean relative vertical position of *E. servus* adults across the growth stages were analyzed by fitting a quadratic regression model (Fig. 8). During the early vegetative growth stages (V4 and V6), the mean relative vertical position of the *E. servus* adults remained in the lower 10% of the plant height. However, as the plants grew, the mean relative vertical position of *E. servus* adults moved rapidly up in the plant before stabilizing to around the 40th percentage of total plant height. In 2016, the mean position of *E. servus* adults reached
40.52% ± 1.23% of the total plant height by the V12 growth stage. In contrast, during 2017, the within-plant mean position of E. servus approached 35.29% ± 4.64% of the total plant height by only the V14 growth stage and stabilized around 40% later, during the R1 growth stage. Since the mean relative vertical position of adult E. servus changed as the plants matured, selecting a single partial plant sampling method relevant to all corn growth stages was not practically possible. Based on the regression model of mean relative vertical position of E. servus adults across various growth stages, and by grouping growth stages with similar E. servus susceptibility (Annan and Bergman 1988, Ni et al. 2010, Koch et al. 2017), we identified and grouped corn growth stages with a similar E. servus distribution pattern (V4 and V6; and R1, R2 and R4).

The predicted mean probabilities of E. servus adult within-plant distribution in each of three plant strata during various corn growth stages are displayed in Figure 9. Plant strata suitable for designing a partial plant sampling method were selected by identifying areas with relatively high proportion of E. servus on a stratum coupled with a low sample variability (RV). Overall predicted probabilities of E. servus adults located on the upper plant strata were much lower than the two other strata. The highest probability for finding E. servus adults on any single growth stage on the upper stratum was 0.14 ± 0.05 at the R2 stage in 2017. The upper stratum of the corn plant is the most difficult plant region to accurately sample stink bugs using whole-plant searches since the sampling region is often above the sampler’s eye level. Furthermore, analysis of the relative variation indicated that the upper stratum had a higher mean RV, suggesting higher sample variability (Table 2). Therefore, we identified the upper stratum as the least suitable region for reliable adult E. servus sampling in corn plants and discarded it from further consideration.
During the V4 and V6 growth stages, *E. servus* adults were mainly found on the basal stratum (Fig. 9). Moreover, the mean RV of basal stratum was much lower than the other plant strata in both 2016 and 2017 (Table 2). Because there were relatively high *E. servus* numbers in this area and low sample variability in the basal stratum during V4 and V6 growth stages, the basal stratum was chosen for designing partial plant sampling method. In contrast, during the R1, R2 and R4 growth stages (reproductive growth stages) in both years, a majority of adult *E. servus* were found on the middle stratum with a lower RV than the rest of the plant strata. Hence, we chose this stratum for *E. servus* sampling during the R1, R2 and R4 growth stages.

The suitability of two partial plant sampling methods (stalk below the lowest green leaf and basal stratum) for early vegetative corn growth stages (V4 and V6), were compared (Table 3). The pest population expressed as percentage of whole-plant *E. servus* number captured by the two partial plant sampling methods showed no significant difference between the methods (*t* = 1.33; df = 11; *P* = 0.2112). Population estimates by both partial plant sampling methods had a very high fidelity to the whole-plant counts, across a range of population densities (R² = 0.99 for both methods; Tables 3 and 4). Moreover, both partial plant sampling methods had a similar mean relative variation (*t* = 1.652; df = 2; *P* = 0.2403; Table 3). Though both partial plant sampling methods were equally desirable in terms of accuracy, precision (RV value), and fidelity, sampling the corn stalk below the lowest green leaf was chosen to compare to whole-plant sampling at the V4 and V6 growth stages, since it was more specific than sampling the basal stratum. Moreover, field-level approximation of the stratum without measuring individual plant height might lead to a sampling bias. Averaged across both growth stages (V4 and V6), the mean time required to count *E. servus* adults from 20 corn plants by ‘corn stalk below the lowest green leaf sampling method’ was estimated at 64.3 ± 0.9 s. At the same growth stages,
mean sampling time for whole-plant sampling was estimated at 74.9 ± 0.6 s, requiring more
time to sample than the corn stalk below the lowest green leaf method ($t = 10.27; \text{df} = 15; P < 0.0001$).

The suitability of seven different partial plant sampling methods for R1, R2 and R4
growth stages (reproductive growth stages) were compared (Table 5). When the mean partial
plant *E. servus* counts from a corn growth stage, expressed as percentage of mean whole-plant
counts (sampling accuracy), were compared across partial plant sampling methods, there was a
significant interaction between the partial plant sampling method and plant growth stage ($F = 2.86; \text{df} = 12, 134; P = 0.0015$). Upon further simple effect analysis, the sampling accuracy of
*E. servus* adults significantly varied among the growth stages using multiple partial plant
sampling methods (‘Z’, $F = 28.37; \text{df} = 2, 134; P < 0.0001$; ‘ZP1’, $F = 16.48; \text{df} = 2, 134; P < 0.0001$; ‘ZM1’, $F = 7.23; \text{df} = 2, 134; P = 0.001$; ‘ZP1M1’, $F = 3.13; \text{df} = 2, 134; P = 0.0469$;
‘ZM1M2’, $F = 4.45; \text{df} = 2, 134; P = 0.0135$), except for the ‘ZP1M1M2’ and ‘middle stratum’
sampling methods (‘ZP1M1M2’, $F = 1.73; \text{df} = 2, 134; P = 0.1805$; ‘middle stratum’, $F = 0.31$;
$\text{df} = 2, 134; P = 0.7356$). The partial plant sampling method ZP1M1M2 had the numerically
lowest sample variability and was significantly lower than methods Z and ZP1 (Table 5). The
ZM1M2 method had the highest fidelity ($R^2 = 0.99$) with whole-plant estimates, followed by the
ZP1M1M2 ($R^2 = 0.98$) and middle strata methods (also $R^2 = 0.98$; Table 5 and 6). Sampling cost
varied among the partial sampling methods ($F = 58.5; \text{df} = 7, 49; P < 0.0001$). Mean sampling
cost for the whole-plant sampling method was estimated at 263.4 s ± 7 s and was significantly
more time-consuming than all other partial plant sampling techniques, except the ‘middle
stratum’ method (Table 5). Furthermore, the RNP was highest for the ZP1M1M2 method,
suggesting a high precision of *E. servus* estimate per unit sampling cost.
3.5 Discussion

Studies on the within-plant spatiotemporal distribution of stink bugs and other insect pests generally find that the vertical distribution of these insects on plants is not random (Arx et al. 1984, Blinka 2008, Walzer et al. 2009); often, the distribution is closely associated with the plant phenology (Wilson et al. 1983, Ramalho et al. 1984). In our study, *E. servus* adult within-plant distribution on field corn was nonrandom and the observed distribution was associated with plant phenology. During early vegetative corn growth stage (V4, V6) most *E. servus* adults were found at the base of the plant; sometimes they were resting on plant stalk or soil surface, but usually they were directing their head down, relative to the soil, and inwards, relative to the plant, on the base of the corn plant. This distribution and observed insect behavior of adult *E. servus* on early vegetative stage corn is consistent with previous field observations (Townsend and Sedlacek 1986, Sedlacek and Townsend 1988). The within-plant distribution preference of the insect could reflect an attempt to attain the optimum nutritional requirements or to shelter from predators or extreme weather conditions. During early vegetative growth stages, roots and apical meristematic region (growing point) are a major sink for photosynthate assimilate (Hofstra and Nelson 1969), and feeding close to these structures, which are below the soil surface, might help the stink bugs access a higher concentration of nutrients. During early corn growth stages, stink bug infestation and damage are more frequently observed in no-till fields with heavy cover (Townsend and Sedlacek 1986, Koch et al. 2017) or in fields with excessive weed growth (Clower 1958). This suggests their preference for a sheltered feeding location or for thigmotaxis, neither of which is exclusive. Moreover, the brownish grayish dorsal coloration of *E. servus* adults blends with the color of surrounding soil and dried plant debris, which could provide camouflage while they feed from the base of the plants, while adequately fulfilling their nutritional requirements.
Sedlacek and Townsend (1988) suggested that stink bug feeding damage in VE-V4 corn results from mechanical and chemical damage to the growing point. Mechanical damage can result from stylet insertion, while chemical damage can result from digestive enzymes produced by salivary glands (Hori 2000, Depieri and Panizzi 2011). However, it is unlikely that the *E. servus* stylet can physically reach the growing point of majority of corn plants during these stages by feeding at the soil level, because the growing point was below the soil until the V5 growth stage. The maximum theoretical stylet penetration depth of *E. servus* adult is estimated at 0.301 cm (range: 0.104-0.301 cm; Esquivel 2015). Hence, until the V5 growth stage, growing point of majority of corn plants remained below the soil surface below the range of *E. servus* stylet penetration depth. Despite this, nonuniform seed furrow closure is common in no-till fields (Annan and Bergman 1988) and excessive soil erosion can potentially expose the below ground growing point of early vegetative stage corn for *E. servus* feeding access. Moreover, digestive enzymes in the saliva can spread beyond the stylet penetration depth and damage the tissue (Nuorteva and Reinius 1953). Finally, many damage symptoms, including tiller production, resulting from stink bug feeding in early vegetative stage corn, may not be a direct result of feeding injury to the growing point (Apriyanto et al. 1989a, b).

After the V6 growth stage, mean relative within-plant vertical position of adult *E. servus* rapidly moved upward until the location stabilized at around approximately 40% of the plant height from the soil level. This vertically upward position shift of adult *E. servus* as plant matures may have been influenced by a change in physical and nutritional qualities in the basal part of the plant from where the *E. servus* primarily feed until V6 stage. A majority of internode elongation occurs from V6 onwards and up to this growth stage any above-ground portion of stalk is almost entirely covered with layers of leaf sheaths (Abendroth et al. 2011).
Simultaneously, adult *E. servus* began to move upward after V6 likely in an attempt to optimize feeding locations as the basal part of the plants exterior became more fibrous over time. In our study, almost all the individuals found on the plant base from the V8 growth stage onwards were either on the fleshy brace roots or on the base of small sucker plants.

We were surprised to find *E. servus* feeding on brace roots and, to our knowledge, are the first to report this. Feeding on brace roots might create uncertainty in sampling predictability. For example, in our study, it is likely that the presence of a relatively high proportion of the adult *E. servus* in brace roots during 2017 delayed the stabilization of relative mean vertical position of *E. servus* at around 40% of plant height until the R1 growth stage; in contrast this stabilization occurred at the V12 growth stage during 2016, when there was little brace root feeding. Though this result indicates a possibility variable within-plant distribution of *E. servus* in corn plants (depending on the brace root quality as a feeding substrate), the year to year variability observed in our study might have little implication on *E. servus* management. This is because the possibility for an economic damage by *E. servus* between V6 and V14 corn is limited (Negrón and Riley 1987, Annan and Bergman 1988) or at least poorly understood (Reisig 2011).

During reproductive stages of corn most of the *E. servus* adults were concentrated around the developing primary and secondary ear. This observed distribution is in agreement with results from Blinka (2008) studies, where relatively high numbers of adult *E. servus* were found on the ‘ear zone’ defined as the plant parts between a single leaf above and below the primary ear. Reproductive corn is nutritionally adequate for *E. servus* nymphal development and adult survival (Tillman 2010) and, with the exception of the R1 growth stage, *E. servus* adults were found mostly on ear tissue or on the leaf sheath covering the ear in reproductive-
stage corn. During the R1 growth stage, however, when the developing ears on the plants were relative small, they were generally located on ear tissue and the stalk around the primary and secondary ear. Therefore, the *E. servus* adult distribution is likely a reflection of the insect’s attempt to optimize its nutritional requirements, especially considering the fact that the ear represents only a small portion of total available area for feeding and other plant parts could serve as shelter from predators or potentially negative environmental conditions.

The within-plant *E. servus* distribution in corn plants could also impact the efficiency of insecticide management. Insecticide applications targeting *E. servus* during the V4 and V6 corn growth stages should target the base of corn plants and are not needed above 10% of the plant height from the soil surface. Insecticide applications during the V12 and V14 growth stages, if needed, can be particularly challenging. For example, depending on the proportion of *E. servus* inhabiting the brace roots, the spray might need to reach the base of the plants for maximum effectiveness. Finally, insecticide applications targeting *E. servus* adults during R1-R4 should target the ear zone for maximum efficacy.

The current whole-plant stink bug sampling method in corn is laborious and costly (Blinka 2008). Based on our findings of within-plant adult *E. servus* distribution, we developed efficient partial plant sampling methods to estimate *E. servus* population densities in corn that can potentially replace the current whole-plant sampling method. A fixed partial plant sampling method applicable to all corn growth stages, based on the specific part of the plant or a constant proportion of plant height (eg, basal one-third of plant height), can greatly simplify the sampling process. However, the quadratic relationship observed between the mean number of fully developed leaves and mean vertical position of the adult *E. servus* distribution in plants suggests a non-linear relationship between growth stage on *E. servus* distribution. Such a relationship
precludes the possibility for developing a single fixed partial plant sampling method applicable for all the corn growth stages and, thus, separate partial plant sampling methods have been developed for early vegetative stages (V4-V6) and the reproductive stages (R1-R4).

For certain pests, sampling a constant proportion of the plant height is suggested as an efficient alternative to whole-plant sampling (Arno et al. 2006). Our results suggest that during the reproductive corn growth stages (R1-R4), among the three plant strata compared, the middle stratum, which has relatively high pest abundance and low sample variability, was most suitable for *E. servus* sampling. However, further comparison with selected partial plant methods suggests that the middle stratum method is less precise for a given sampling cost. Moreover, from a scouting perspective, this method may further complicate the sampling procedure, since individual plant height information is needed for accurately delineating the specific plant stratum. However, due to the small size of V4 and V6 growth stage corn plants, approximation of the basal stratum (0-33% of plant height) in the early vegetative corn growth stages is relatively effortless and such approximation should not result in a highly biased estimation since there are relatively few *E. servus* adults above this zone.

Defining a sampling unit is the first step toward the development of a practical field monitoring program (Wilson et al. 1983). For early vegetative stage corn (V2-V6), both partial plant sampling methods selected (sampling only the stalk area below the lowest green leaf and sampling only the basal stratum), were equally accurate and precise for *E. servus* adult sampling. Emerging corn (VE growth stage) is more susceptible to *Euschistus variolarius* (Palisot de Beauvios) infestation than older-stage seedlings at the V2 or V4 growth stages and plants infested with stink bugs at the V6 growth stage can outgrow stink bug injury (Annan and Bergman 1988). In this study, for early vegetative stage corn, V6 was conservatively considered
as the upper boundary for *E. servus* susceptibility. Contrary to the frequent reports of stink bug detection from V2 and younger seedling corn in earlier literatures (Clower 1958, Seldacek and Townsend 1988), in our study only a single *E. servus* was detected out of 2400 V2 stage corn plants. This apparent near absence of *E. servus* from V2 corn in our observations could be due the changes in corn management practices, such as the ubiquitous adoption of neonicotinoid seed treatments, availability of more preferred alternate feeding hosts on the weedy field borders or simply a mismatch of corn planting time and overwintered *E. servus* activity.

During the reproductive corn growth stages (R1-R4), sampling all the plant parts between two leaves above and three leaves below the primary ear leaf (ZP1M1M2) consistently had a relatively high sampling accuracy, higher sampling precision, lower sampling cost, and a high sampling precision per unit cost compared with whole-plant sampling for reproductive-stage corn. This method, on an average, can save 24% of the sampling time compared with whole-plant sampling. Furthermore, this sampling procedure is less laborious than whole-plant sampling since the sampling area is concentrated at 40% of the plant height, which is close to eye level for most humans. Additionally, since the sampling area is concentrated around the primary ear, scouts and growers can accurately identify the targeted area without much effort. Finally, we observed that *E. servus* adults tend to evade sampling by quickly shifting to the less visible plant regions or dropping to the ground. Adopting this particular partial plant sampling method should minimize the disturbance created during the sampling process by focusing on the plant region with the highest *E. servus* density, minimizing the lack of precision often associated with the direct field counts (Ni et al. 2017).

Dispersal of pests from the nearby host plants can temporarily disrupt the typical within-plant distribution of pests observed in a crop of specific growth stage till the dispersed
individuals settled have for the favorable site within the plant (Barron and Margolies 1991). In North Carolina, large scale dispersion of stink bug from wheat fields to the neighboring corn fields is often observed at or around the wheat harvest. Though it is not directly documented in this study, casual field observations suggest that such large-scale movement may temporarily disrupt the natural within-plant distribution observed in this study. In such situations, partial plant sampling suggested in this study should follow with caution.

In conclusion, our study identified the within-plant vertical distribution of *E. servus* adults on various corn growth stages and related the observed distribution to plant architecture. Furthermore, we explored the potential plant factors that potentially influence within-plant distribution of *E. servus* adults. Based on the observed distribution, we identified partial plant sampling methods for *E. servus* sampling for both early vegetative and reproductive stage corn. Results reported here will be useful for scouts and growers to efficiently carry out stink bug sampling in field corn, with impact on management decisions. Future work could incorporate these sampling techniques to develop a sampling plan with consideration to the within-field spatial distribution of stink bugs in field corn.
3.6 Acknowledgments

Allen Scott, Dan Mott and Dannyel Akira Nelson provided the technical assistance. We thank Antonio Cinti Luciani and Jonathan Peppers of Open Ground Farms, Beaufort, NC, for their collaboration and support in establishing and maintaining the experimental plots. Partial funding of this project was provided by NC Agricultural and Life Sciences Research Foundation and the Corn Growers Association of North Carolina.
3.7 References Cited


### 3.8 Tables and Figures

Table 3.1  Corn hybrid, planting date, seeding rate and insecticidal seed treatment

<table>
<thead>
<tr>
<th>Year</th>
<th>Field no.</th>
<th>Variety</th>
<th>Planting date</th>
<th>Seeds/ha</th>
<th>Seed treatment (mg a.i./kernel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2016</td>
<td>1</td>
<td>P1197</td>
<td>19 April</td>
<td>69,000</td>
<td>1.25 clothianidin</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>P1197</td>
<td>15 April</td>
<td>75,000</td>
<td>1.25 clothianidin</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>DKC 57-75</td>
<td>15 April</td>
<td>69,000</td>
<td>1.25 clothianidin</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>P1197</td>
<td>12 April</td>
<td>75,000</td>
<td>0.25 thiamethoxam and 0.25 chlorantraniliprole</td>
</tr>
<tr>
<td>2017</td>
<td>1</td>
<td>P2160</td>
<td>3 April</td>
<td>74,000</td>
<td>0.25 thiamethoxam and 0.25 chlorantraniliprole</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>P2160</td>
<td>3 April</td>
<td>74,000</td>
<td>0.25 thiamethoxam and 0.25 chlorantraniliprole</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>P2160</td>
<td>3 April</td>
<td>74,000</td>
<td>0.25 thiamethoxam and 0.25 chlorantraniliprole</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>P2160</td>
<td>5 April</td>
<td>74,000</td>
<td>0.25 thiamethoxam and 0.25 chlorantraniliprole</td>
</tr>
</tbody>
</table>
Table 3.2  Mean relative variation \((RV= (SE/\bar{x}) \times 100)\) of *E. servus* adults on corn plant stratum

<table>
<thead>
<tr>
<th>Plant stratum</th>
<th>Corn growth stage</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V4 + V6</td>
<td>R1 + R2 + R4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>3.32 ± 1.86</td>
<td>48.47 ± 10.83</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>75.72 ± 0.00</td>
<td>10.58 ± 2.72</td>
<td></td>
</tr>
<tr>
<td>Upper</td>
<td>77.39 ± 0.00</td>
<td>69.05 ± 9.39</td>
<td></td>
</tr>
</tbody>
</table>

Basal = 1/3 lower part of plant; middle = 1/3 middle part of the plant; upper = 1/3 upper part of the plant.
Table 3.3  Selection of the optimum partial plant sampling method for *E. servus* adult on early vegetative corn (V4 and V6 growth stages) based on selection criteria

<table>
<thead>
<tr>
<th>Partial plant sample selection criteria</th>
<th>Cornstalk below the lowest green leaf</th>
<th>Basal stratum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (± SEM) <em>E. servus</em> adults (%)</td>
<td>94.03 ± 2.41a</td>
<td>95.94 ± 2.27a</td>
</tr>
<tr>
<td>Mean relative variation (RV)</td>
<td>3.18 ± 1.82A</td>
<td>3.32 ± 1.86A</td>
</tr>
<tr>
<td>Fidelity (R²)</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>Relative net precision (RNP)</td>
<td>29.33</td>
<td>-</td>
</tr>
</tbody>
</table>

Basal stratum = 1/3 lower part of the plant. Means followed by the same letters within a row are not significantly different. Relative Net Precision (RNP) was not calculated for the basal stratum as no sampling cost information was collected for this method.
Table 3.4  Linear regression equations relating the *E. servus* adult counts from partial plant sampling methods (independent variable) to whole-plant search method (dependent variable) and the corresponding economic threshold (ET) calculations for early vegetative stage corn

<table>
<thead>
<tr>
<th>Sampling method</th>
<th>Intercept ± SEM</th>
<th>Slope ± SEM</th>
<th>F</th>
<th>P</th>
<th>R²</th>
<th>n</th>
<th>ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn stalk below the lowest green leaf</td>
<td>-0.467 ± 0.429</td>
<td>0.983 ± 0.029</td>
<td>1184</td>
<td>&lt;0.0001</td>
<td>0.99</td>
<td>12</td>
<td>1.5</td>
</tr>
<tr>
<td>Basal stratum</td>
<td>-0.281 ± 0.436</td>
<td>0.982 ± 0.029</td>
<td>1142</td>
<td>&lt;0.0001</td>
<td>0.99</td>
<td>12</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Basal stratum = 1/3 lower part of the plant. In this study, an individual sampling unit constituted 300 plants. However, the ET is calculated for a 20 plant sample. The current economic threshold for whole-plant searches during early vegetative corn growth stage is two stink bugs per 20 plants (Koch et al. 2017).
Table 3.5 Selection of optimum partial plant sampling method for *E. servus* adult on reproductive growth stage corn (R1, R2 and R4) based on the selection criteria

<table>
<thead>
<tr>
<th>Partial plant sample selection criteria</th>
<th>Z</th>
<th>ZM1</th>
<th>ZP1</th>
<th>ZM1M2</th>
<th>ZP1M1</th>
<th>ZP1M1M2</th>
<th>Middle stratum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (± SEM) <em>E. servus</em> adults (%)</td>
<td>60.72 ± 5.35c</td>
<td>73.03 ± 3.97ab</td>
<td>66.78 ± 5.15bc</td>
<td>80.99 ± 3.65a</td>
<td>79.09 ± 3.00ab</td>
<td>87.06 ± 2.40a</td>
<td>80.58 ± 3.65a</td>
</tr>
<tr>
<td>Mean relative variation (RV)</td>
<td>15.64 ± 2.10a</td>
<td>9.54 ± 1.86ab</td>
<td>15.59 ± 2.09a</td>
<td>7.79 ± 2.08b</td>
<td>7.69 ± 0.93b</td>
<td>5.47 ± 0.69b</td>
<td>10.58 ± 2.72ab</td>
</tr>
<tr>
<td>Fidelity (R²)</td>
<td>0.80</td>
<td>0.96</td>
<td>0.78</td>
<td>0.99</td>
<td>0.95</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Relative net precision (RNP)</td>
<td>2.60</td>
<td>4.19</td>
<td>2.38</td>
<td>4.96</td>
<td>3.94</td>
<td>5.46</td>
<td>2.31</td>
</tr>
<tr>
<td>Mean (± SEM) sampling cost in seconds</td>
<td>148 ± 2C</td>
<td>150 ± 3C</td>
<td>161 ± 3C</td>
<td>155 ± 4C</td>
<td>198 ± 6B</td>
<td>201 ± 5B</td>
<td>246 ± 14A</td>
</tr>
</tbody>
</table>

Z= leaf collar with primary ear; M1= leaf collar below primary ear; M2= two leaf collar below primary ear; P1= leaf collar above primary ear; middle stratum=1/3 middle part of the plant. Means followed by the same letters within a row are not significantly different.
Table 3.6  Linear regression equations relating the *E. servus* adult counts from partial plant sampling methods (independent variable) to the whole-plant search method (independent variable) for corn reproductive stages (R1, R2 and R4) and the corresponding economic threshold (ET) calculations

<table>
<thead>
<tr>
<th>Sampling method</th>
<th>Intercept ± SEM</th>
<th>Slope ± SEM</th>
<th>F</th>
<th>P</th>
<th>R²</th>
<th>n</th>
<th>ET</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>0.571 ± 2.522</td>
<td>0.575 ± 0.062</td>
<td>87.3</td>
<td>&lt;0.0001</td>
<td>0.80</td>
<td>24</td>
<td>6.3</td>
</tr>
<tr>
<td>ZP1</td>
<td>0.876 ± 2.744</td>
<td>0.596 ± 0.067</td>
<td>79.3</td>
<td>&lt;0.0001</td>
<td>0.78</td>
<td>24</td>
<td>6.8</td>
</tr>
<tr>
<td>ZM1</td>
<td>0.048 ± 1.339</td>
<td>0.752 ± 0.033</td>
<td>530.2</td>
<td>&lt;0.0001</td>
<td>0.96</td>
<td>24</td>
<td>7.6</td>
</tr>
<tr>
<td>ZP1M1</td>
<td>0.352 ± 1.527</td>
<td>0.773 ± 0.037</td>
<td>431.1</td>
<td>&lt;0.0001</td>
<td>0.95</td>
<td>24</td>
<td>8.1</td>
</tr>
<tr>
<td>ZM1M2</td>
<td>-0.735 ± 0.852</td>
<td>0.873 ± 0.021</td>
<td>1762</td>
<td>&lt;0.0001</td>
<td>0.99</td>
<td>24</td>
<td>8.0</td>
</tr>
<tr>
<td>ZP1M1M2</td>
<td>-0.431 ± 1.003</td>
<td>0.894 ± 0.024</td>
<td>1336</td>
<td>&lt;0.0001</td>
<td>0.98</td>
<td>24</td>
<td>8.5</td>
</tr>
<tr>
<td>Middle stratum</td>
<td>-0.342 ± 1.079</td>
<td>0.831 ± 0.026</td>
<td>997</td>
<td>&lt;0.0001</td>
<td>0.98</td>
<td>24</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Z= leaf collar with primary ear; M1= leaf collar below primary ear; M2= two leaf collar below primary ear; P1= leaf collar above primary ear; middle stratum=1/3 middle part of the plant. In this study, an individual sampling unit constituted 300 plants. However, the ET is calculated for a 20 plant sample. The current economic threshold for whole-plant searches during early vegetative stage corn is two stink bugs per 20 plants (Koch et al. 2017).
Figure 3.1  Frequency distribution of within-plant vertical position of *E. servus* adults and corresponding mean leaf collar positions of model corn plants of corn growth stages V4, V6 and V8 during 2016 and 2017. Both the vertical position of *E. servus* adults and the leaf collar position on a plant is expressed as percentage of plant height. *E. servus* adult frequency data is from 1200 plants/growth stage/year. Leaf collar position information is from five corn plants each from four fields. The V6 growth stage was only sampled during 2017.
Figure 3.2  Frequency distribution of within-plant vertical position of *E. servus* adults and corresponding mean leaf collar positions from model corn plants of corn growth stages V12, and V14 during 2016 and 2017. Both the vertical position of *E. servus* adults and leaf collar positions on the corn plant are expressed as percent of plant height. *E. servus* adult frequency data is from 1200 plants/growth stage/year. Leaf collar position information is from five corn plants each from four different fields.
Figure 3.3  Frequency distribution of within-plant vertical position of *E. servus* adults and corresponding mean leaf collar positions from model corn plants of reproductive corn growth stages R1, R2, and R4 during 2016 and 2017. Both the vertical position of *E. servus* adults and leaf collar positions on the corn plant are expressed as percent of plant height. *E. servus* adult frequency data is from 1200 plants/growth stage/year. Leaf collar position information is from five corn plants each from four different fields. Leaf collar marked with a downward arrow sign indicates position of the primary ear leaf collar.
Figure 3.4  Percentage of *E. servus* adults (mean ± SEM) on various plant structures of vegetative growth stages during 2016 and 2017. *V6 only sampled during 2017.*
Figure 3.5  Percentage of *E. servus* adults (mean ± SEM) on various plant structures of reproductive growth stages during 2016 and 2017.
Figure 3.6  Box and whisker plots depicting the relative location of the growing point in relation to the soil level at early vegetative growth stages during 2016. Zero values in the y-axis and corresponding horizontal dotted lines represent the soil level. Positive values represent the above ground location and negative values represent the below ground location. Growth stages followed by the same letters are not significantly different (Tukey’s HSD, P < 0.05).
Figure 3.7  Mean relative frequency of *E. servus* adults (± SEM) in relation to the primary ear leaf collar at the R1, R2, and R4 growth stages during 2016 and 2017. Z= leaf collar with primary ear; M1= leaf collar below primary ear; M2= two leaf collar below primary ear etc.; P1= leaf collar above primary ear, P2= two leaf collar above primary ear etc. A small percentage of *E. servus* adults (0.42 ± 0.42% at the R1 stage during 2016, and 2.5 ± 2.5% at the R2 stage during 2017) were outside the graph’s y-axis range and not included.
Figure 3.8  Quadratic regression model of mean (± SEM) relative vertical position of *E. servus* adults on various corn growth stages. Vertical *E. servus* adult location on plants (y-axis) is expressed as the percentage of plant height. X-axis denotes the mean number of fully developed leaves within a visible leaf collar. For reproductive growth stages (R1, R2 and R4) the mean number of fully developed leaves was estimated from 20 plants per growth stage per year.
Figure 3.9 Predicted mean (± SEM) probability of *E. servus* adult distribution on a corn plant stratum. V6 growth stage was only sampled during 2017.
CHAPTER IV

DEVELOPING A SAMPLING PLAN FOR BROWN STINK BUG
(HEMIPTERA: PENTATOMIDAE) IN FIELD CORN

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4.1 Abstract

Brown stink bug, *Euschistus servus* (Say), is a damaging pest of corn, *Zea mays* (L.), in the southeastern United States. Developing a reliable and practical sampling plan for population monitoring of this pest is essential for implementing integrated pest management measures. *Euschistus servus* was sampled from commercial corn fields (n=14) in North Carolina in 2016 and 2017. Both the adults and nymphs had a predominantly aggregated spatial distribution, estimated using the variance to mean ratio and Taylor’s power law constant (*b*). Using the Taylor’s power law constants, the optimum sample size required to estimate population density with a given level of reliability was calculated. For early vegetative stage corn (V4-V6), using whole plant visual sampling and an economic threshold density of 2 adult stink bugs per 20 plants, 27 sample units were required to estimate population density within 30% of the mean. At the same growth stage, using partial plant sampling and an economic threshold density of 1.73 adult stink bugs per 20 plants, 28 sample units were required to estimate population density with the same level of reliability. Reproductive stage corn (R1-R4) required eight sample units for whole plant sampling and nine sample units for partial plant sampling (*Dx*=0.3). For *E. servus* adults, the partial plant sampling method was equally or more cost-reliable than the whole-plant sampling method for pest management in all corn growth stages tested.
4.2 Introduction

Stink bugs (Hemiptera: Pentatomidae) have recently emerged as a significant pest of corn, *Zea mays* (L.), in the United States. In the midwestern United States, this increased significance of Pentatomidae as pests of corn is primarily attributed to the appearance and spread of the invasive stink bug species, *Halyomorpha halys* (Stål), along with increased abundance of native stink bug species (Koch and Pahs 2015, Koch et al. 2017). For the midsouth and southeastern United States, the elevated pest status is largely attributed to the widespread adoption of Bt crops, especially Bt cotton, eradication of boll weevil, *Anthonomus grandis grandis* Boheman, and reduction in insecticide usage in crops targeting primary pests (Bundy and McPherson 2000). The potential for economic damage from this pest to field corn necessitates the development of a practical and reliable sampling plan for population estimation and to aid in pest management.

In the southeastern United States, corn is the first spring planted row-crop host available to *E. servus* infestation (Todd and Herzog 1980). This pest is the predominant stink bug species present in corn (94-98%), while multiple other species are present in relatively low population densities (Blinka 2008, Reisig 2011). Corn plants are primarily susceptible to stink bug damage during the early vegetative stage (Annan and Bergman 1988, Apriyanto et al. 1989a), during the pretasseling stage through the initiation of pollination (Negrón and Riley 1987, Koch et al. 2017), and during pollination through the early dough stage (Ni et al. 2010, Hunt et al. 2014). During the early vegetative stage, *E. servus* feeding can result in seedling death, tiller production, crinkled leaves with oblong holes, stunted growth (Townsend and Sedlacek 1986, Apriyanto et al. 1989a), and yield reduction (Apriyanto et al. 1989a). The economic threshold at these corn growth stages (VE-V6) is 10% infested plants or one stink bug per 10 plants (Koch et al. 2017, Reisig and Babu 2017). Only limited information is available on stink bug damage potential in
pretasselling stage corn. Although the available results are inconclusive for the extent of stink bug damage potential in various pretasseling growth stages, stink bugs clearly can cause yield loss during at least some of these stages. Compared to control plants, two adult *Nezara viridula* (L.), per plant at V15 stage corn (top ear 1.5 - 2.0 cm) for six days results in significantly lower ear weight and length (Negrón and Riley 1987). However, significant yield impact was not observed with V18 corn. Moreover, three adult *E. servus* confined on a primary ear for nine days at the VT or R1 growth stage resulted in significant reduction in kernel weight (Ni et al. 2010).

The economic threshold recommended from the late vegetative stage (V14) through the initiation of pollination (early R1) is one stink bug per four plants or 25% infested plants (Reisig and Roberson 2011, Hunt et al. 2014). Finally, the threshold after pollination (mid-late R1) through early dough stage (R4) is two stink bugs per four plants or 50% infested plants (Hunt et al. 2014).

Whole plant visual inspection is a recommended sampling method for stink bugs in corn. However, this sampling method is laborious and costly to implement in the field (Blinka 2008). In contrast, sampling that focuses on specific plant parts where the stink bugs are most likely to be found can be efficient and relatively less labor intensive (Blinka 2008). For example, during the V4-V6 growth stages, sampling the corn stalk below the lowest green leaf is an efficient sampling method compared to whole plant sampling for stink bugs in corn (Babu and Reisig 2018). Studies by Blinka (2008) in reproductive stage corn (R1-R4), suggested that *E. servus* sampling can be restricted to the corn ear zone, defined as one leaf above and one leaf below the primary ear leaf and all the plant part in between. However, these methods should be validated for their practical applicability.
Developing a reliable and practical sampling plan for *E. servus* in field corn is essential for population estimation and for informed pest management decisions. The spatial distribution data supporting such a sampling plan should ideally consider both the within-plant and the within-field spatiotemporal distribution of the targeted pest. Knowledge on the within-plant distribution will facilitate the design of an efficient and reliable sampling technique, while the within-field distribution data can be used to develop sampling plans for population estimation (parameter estimation) and for commercial monitoring purposes (decision estimation). The population estimation required for sampling in research emphasizes the reliability of the parameter estimate, often requiring a large number of sample units and considerable sampling time investment (Wilson et al. 1989). For pest management, however, a highly reliable population estimate is not required, especially when mean densities far exceed or fall well below the economic threshold. The primary aim of such a sampling plan is to classify the pest population level above or below the action or economic threshold (Wilson 1985). Accordingly, a sequential sampling protocol, which classifies the pest above or below the threshold, requires considerably fewer sampling units for pest management decisions and often results in a 40-60% reduction in the sampling cost than other sampling procedures of comparable error rate (Wilson et al. 1989). Currently, no comprehensive sampling plan has been developed for population estimation or pest management purposes for *E. servus* in field corn. The objectives of this research are to: 1) determine the minimum sample size required to assess the population mean estimate of *E. servus* nymphs and adults in field corn with a given reliability; 2) to propose and validate a sequential sampling plan for *E. servus* in field corn; and 3) to compare the relative cost efficiency of the whole plant *E. servus* sampling method to newly developed partial plant sampling methods in population estimation and pest management decisions.
4.3 Materials and Methods

4.3.1 Sampling Procedure

Over the 2-yr study, *E. servus* nymphs and adults were sampled from 14 commercial corn fields (four in 2016 and 10 fields in 2017) located in North Carolina. Across the sampling years, field size ranged from 2.2 to 9.3 ha with an average area of 5.5 ha. From each field, *E. servus* were sampled from a nonrandom sampling grid consisting of a fixed sampling point for every 0.404 ha, with adjacent sampling points separated at 63.7 m. Both the *E. servus* nymphs and adults were sampled separately at V2, V4, V6, V8, V12, V14, R1, R2, and R4 corn growth stages. Since the V8 and V12 corn growth stages fall outside the range of a defined *E. servus* vulnerability window, observations from these growth stages were not included in the final analysis.

In 2016, two sampling methods, whole plant visual search and partial plant visual search were employed. In 2017, an additional sampling method was added to the study. The method was infested plant counts, defined as the number of *E. servus* infested plants encountered during whole plant visual sampling. Unique sampling observations were made and analyzed separately for *E. servus* adults and nymphs during both 2016 and 2017. In 2017, from each sampling point, observations using the whole plant, partial plant, and infested plant sampling methods were concurrently noted from the same sampling unit of 20 consecutive plants located within the same row. In 2016 however, from each sampling point, *E. servus* counts using the whole plant, and partial plant sampling method were taken from 20 consecutive corn plants separately from two adjacent parallel corn rows. For the whole plant sampling method, all the above-ground parts of the corn were examined for *E. servus*. The specific partial plant method relied on previous research on *E. servus* within-plant vertical distribution data (Babu and Reisig 2018, Blinka 2008) and parts of the plant searched varied across growth stage. During the early vegetative stages
(V2-V6), *E. servus* were sampled from the corn stalk below the lowest green leaf. During the pretasseling stage, from the V14 growth stage until the VT growth stage, sampling was focused on the developing ear zone. During the V14 growth stage, the V11 and V13 leaf and all the plant parts in between were sampled. After the V14 growth stage until the VT growth stage, the sampling area included one leaf above and below the potential primary ear leaf collar, typically V13 (Abendroth et al. 2011), and all the plant parts in-between. During the reproductive growth stages (R1, R2, R4), the ear zone was sampled, which included the area one leaf above and below the primary ear leaf and all the plant parts in-between (Blinka 2008). For the infested plant count method, the number of plants with *E. servus* (separately for adult and nymphs) present was noted from the 20 consecutive corn plants in a sampling unit during the whole plant visual inspection. From the selected fields, both the time taken to carry out the *E. servus* sampling and the time required to walk between the two adjacent sampling units, for whole plant and specific plant sampling methods, were noted to later infer sampling cost information.

4.3.2  **Spatial Distribution**

Corn growth stages were grouped into three different stink bug susceptibility windows (V2-V6, V14-R1 and R1-R4). Within an individual susceptibility window, corn plants exhibit similar degrees of stink bug vulnerability (Negrón and Riley 1987, Apriyanto et al. 1989b, Ni et al. 2010), sharing the same economic threshold for stink bug management (Reisig and Roberson 2011, Koch et al. 2017). Mean and variance of both the *E. servus* counts, and number of infested plants, were calculated for each field at each sampling date. Spatial distribution of nymphs and adults within a stink bug vulnerability window were described using Taylor’s power law (Taylor 1961). Taylor’s power law relates the population variance to population mean counts by a power law relationship:
\[ s^2 = ax^b \]  

(4.1)

Where \( s^2 \) is the sample variance, \( x \) is the sample mean and \( a \) and \( b \) are Taylor’s coefficients.

Log transformed means and log variances were fit to log linearized Taylor’s power law equations 
(\( \log s^2 = \log a + b \times \log x \)) using linear least squares regression in GraphPad Prism 6 software 
(GraphPad Software Inc. San Diego, CA). Significant departure of the \( b \) coefficient from a value 
of 1 was tested using the \( t \)-test; \( t = [\text{slope} \times 1] / [\text{SE of slope}] \), at \( \alpha = 0.05 \) and with \( n - 2 \) degrees 
of freedom (Zar 1999). The variance to mean ratio was calculated from the raw data for each 
field at each corn growth stage. Significant departure of the variance to mean ratio from the 
value of 1 within a stink bug susceptibility window was tested by calculating the index of 
dispersion \( (I_D) \). 
\[ I_D = (n - 1)s^2 / x, \] 
where \( n \) is the number of samples, \( s^2 \) is the sample variance 
and \( x \) is the sample mean. A significant departure of the variance to mean ratio from the value of 
1 is established when the corresponding \( I_D \) values fall outside the \( \chi^2 \) confidence interval of 0.95 
and 0.05 probability levels with \( n - 1 \) degree of freedom (Davis 1994). The Taylor’s index of 
aggregation, the \( b \) coefficient, along with the variance to mean ratio, calculated for a specific 
corn stink bug susceptibility window, were used to describe the \( E. \ servus \) spatial distribution in 
field corn.

4.3.3 Sample Size Estimation

Number of sample units required to estimate the mean population with a given reliability 
is estimated by the equation proposed by the Karandinos (1976), and independently modified by 
Ruesink (1980) and Wilson and Room (1982):
\[ n = t_{\alpha/2} D_x^{-2} ax^{b-2} \]  

(4.2)
Where, \( n \) is the number of sample units required to estimate the mean population with a given reliability, \( t_{\alpha/2} \) is the standard normal variate for a two-tailed confidence interval, \( Dx \) is the proportion defined as the ratio of half the desired confidence interval to the mean. The parameter \( x \) is the mean \( E. \ servus \) density and the parameters \( a \) and \( b \) are Taylor’s coefficient from the Taylor’s power law regression. In this study, the sample size required (\( n \)), for a mean population estimation within 10, 20, or 30\% of mean (\( Dx = 0.1, 0.2, \) or 0.3.), with 90\% confidence (\( \alpha = 0.1 \)), were estimated.

### 4.3.4 Sequential Sampling Plan

To aid decisions for \( E. \ servus \) management in field corn, a sequential sampling plan was developed for the whole plant, partial plant, and infested plant sampling methods. Sequential stop lines were calculated by using the equation (Wilson 1985):

\[
  n = t_{\alpha}^2 \times or \beta |x - T|^{-2} ax^b
\]

(4.3)

Where, \( n \) is the sample size, \( t \) is the standard normal variate for a one-tailed confidence interval. \( \alpha \) and \( \beta \) are the type I and type II error rates, \( x \) is the mean population density, \( T \) is the economic threshold, and \( a \) and \( b \) are Taylor’s power law coefficients. Sequential sampling stop lines were prepared for \( \alpha \) and \( \beta \) error rates of 10 and 20\% (\( \alpha = \beta = 0.1 \) and \( \alpha = \beta = 0.2 \)).

For the whole plant sampling method, the stink bug economic threshold (\( T \)) used in the calculations was one stink bug per 10 plants, from VE (corn emergence) until the V6 or V8 (<61cm) corn growth stages (Koch et al. 2017, Reisig and Babu 2017). Prior to pollination (\( \approx \) early R1), the whole plant economic threshold was set at one stink bug per four plants. The lower limit for this susceptible window is vaguely defined and, therefore, difficult to attribute to a specific corn growth stage. For example, V15 growth stage corn is highly susceptible to \( N. \ viridula \) damage (Negrón and Riley 1987) but no relevant information is available for the V12 to
V14 growth stages. As a result, corn plants were dissected at both the V12 and V14 growth stages (five plants in each of four fields, during each growth stage and year). Position of the primary ear was determined (Abendroth et al. 2011) and then we speculated the potential for *E. servus* to injure this ear based on the stylet penetration length (Esquivel 2015), the primary ear size, and the distance of the primary ear to the outer leaf sheaths that the stink bug would have to feed through. From this information, we conservatively chose the V14 growth stage as the lower boundary for the *E. servus* susceptibility window. After pollination (≈ mid R1) until early dough stage (early R4), the whole plant threshold is set at one stink bug per 10 plants (Reisig and Roberson 2011, Hunt et al. 2014). Since thresholds do not distinguish among nymphs and adults (Koch et al. 2017), *E. servus* nymph thresholds were the same as those for adults. To relate whole plant thresholds to partial plant and infested plant sampling methods, linear regressions (for both adult and nymphs) were performed (GraphPad Software Inc. San Diego, CA) relating whole plant *E. servus* counts to partial plant *E. servus* counts or infested plant counts.

Individual sequential sampling plans on a fixed error rate consisted of two decision lines, which translated the cumulative *E. servus* or infested plant counts after each successive sample unit inspection into one of three decision rules: ‘stop sampling no treatment needed’, ‘continue sampling’ or ‘stop sampling treatment needed’. Cumulative counts of *E. servus* or infested plants from a minimum of four sampling units (20 plants each) are recommended before making the first management decision. This arbitrarily defined minimum sample units requirement is to reduce the chances for a biased estimate arising from personal bias or environmental heterogeneity, and has been recommended by similar sequential sampling plans for stink bugs on rice (Espino et al. 2008) and cotton (Reay-Jones et al. 2009).
Relative cost-reliability of partial plant sampling methods were compared to whole plant sampling methods for both population estimation (equation 4) and for pest management decisions (equation 5); they were calculated using the following equations (Wilson et al. 1989):

\[
\frac{C_p}{C_w} = \frac{a_p(A+Bx)^{(b_p-2)}(\theta_p + \varphi_p)}{(a_w x^{(b_w-2)})(\theta_w + \varphi_w)} \quad (4.4)
\]

\[
\frac{C_p'}{C_w'} = \frac{B^{-2} a_p(A+Bx)^{b_p}(\theta_p + \varphi_p)}{(a_w x^{b_w})(\theta_w + \varphi_w)} \quad (4.5)
\]

Where, \( \frac{C_p}{C_w} \) and \( \frac{C_p'}{C_w'} \) are the relative cost-reliability of partial plant sampling methods with respect to the whole plant sampling method for population estimation and pest management decisions, respectively, \( a_w \) and \( b_w \) are the Taylor’s power law constants for the whole plant sampling method, \( a_p \) and \( b_p \) are Taylor’s power law constants for the partial plant methods, \( A \) and \( B \) are the intercept and slope of the linear equation relating the whole plant sampling method to the partial plant sampling method, \( \theta_w \) and \( \theta_p \) are the average time required to sample a single sample unit of 20 plants using the whole plant sampling method and using the partial plant sampling method, respectively, and \( \varphi_w \) and \( \varphi_p \) are the time required to walk between two adjacent sampling units separated 63.7 m apart. Since, in this study, the specific sampling methods do not influence the average time required to walk between the two sampling units in the field, time requirement to move between sampling units was considered equal for both the whole plant and partial plant sampling methods (\( \varphi_w = \varphi_p \)). When the ratio of \( \frac{C_p}{C_w} \) or \( \frac{C_p'}{C_w'} \) is greater than one, the partial plant sampling method costs more than the whole plant sampling method, for obtaining a population estimate (equation 4) or to reach a control decision (equation 5) within a given reliability level (Wilson 1985).
4.3.6 Comparison of a Fixed Sampling Plan to a Sequential Sampling Plan

During the 2017 field season, *E. servus* were sampled from an additional 33 randomly selected commercial corn fields located in northeastern North Carolina. In each field, we followed a fixed *E. servus* sampling plan, which consisted of 10 sample units, with 20 corn plants each per sample unit. The samples were drawn from sampling units separated ≈64 m apart. During the sampling process, the number of *E. servus* nymphs and adults were noted using the whole plant and partial plant sampling methods, as well as the number of adult and nymphs infested corn plants for infested plant count method. The average corn growth stage of a field dictated the specific partial plant sampling techniques employed in that field (stalk below the lowest green leaf, ear zone sampling etc.). The *E. servus* data obtained from the fixed sampling plan were then compared to the corresponding sequential sampling plans developed by this study. Mean sample numbers required to make a control decision through fixed and sequential sampling plans were compared using the paired *t*-test (GraphPad Prism). Mean percentage reduction in sample size when following the sequential sampling plan over the fixed sampling plan is reported.

4.4 Results

Across both years and the corn growth stages sampled during this study, 2154 *E. servus* adults and 434 nymphs were observed with the whole plant sampling method. Across all corn growth stages, the mean *E. servus* counts per 20 plants with the whole plant sampling method were 2.08 ± 0.099 (mean ± SEM; range 0-33) for adults and 0.420 ± 0.042 (range 0-14) for nymphs. Across both years, no *E. servus* nymphs or adults were sampled from V2 corn and, therefore, V2 data were not included in the analysis. The current *E. servus* economic thresholds for stink bugs are defined using the whole plant sampling method. The corresponding economic
thresholds at various corn growth stages for the partial plant sampling method and infested plant
count method were calculated and reported in Tables 1 and 2.

When dividing the corn growth stages into three stink bug susceptibility windows, for
V4-V6 corn, across all the sampling points, the number of *E. servus* adults or adult *E. servus*
infested plants per 20 plants averaged $1.36 \pm 0.117$ (mean $\pm$ SEM; range 0-13), for the whole
plant method, $1.23 \pm 0.122$ (range 0-20) for the partial plant method, and $1.13 \pm 0.095$ (range 0-7)
for the infested plant sampling method. The nymph densities per 20 plants at these corn
growth stages were extremely low and averaged $0.044 \pm 0.018$ (range 0-4) for the whole plant
method, $0.041 \pm 0.017$ (range 0-4) for the partial plant method, and $0.049 \pm 0.021$ (range 0-4) for
the infested plant sampling method. Consequently, although we reported Taylor’s power law
constants for *E. servus* nymphs in V4-V6 corn across the whole plant, partial plant, and infested
plant sampling methods, no associated sampling plans were defined. For the stink bug
susceptibility window from V14-R1, adult *E. servus* counts or infested plant counts per 20 plants
averaged $2.60 \pm 0.20$ (range 0-33) for the whole plant, $1.69 \pm 0.141$ (range 0-17) for partial plant,
and $2.20 \pm 0.142$ (range 0-11) for infested plant sampling method. Similarly, nymph counts per
20 plants averaged $0.918 \pm 0.120$ (range 0-14) for whole plant, $0.44 \pm 0.056$ (range 0-10) for
partial plant and $0.91 \pm 0.117$ (range 0-10) for infested plant sampling method. Finally, for the
stink bug susceptibility window from R1-R4, adult *E. servus* number per 20 plants averaged $2.26$
$\pm 0.141$ (range 0-26), $1.77 \pm 0.116$ (range 0-25) and $2.23 \pm 0.123$ (range 0-16) respectively for
the whole plant, partial plant, and infested plant sampling method. Nymph counts per 20 plants
averaged $0.36 \pm 0.042$ (range 0-9), $0.28 \pm 0.035$ (range 0-11), and $0.34 \pm 0.042$ (range 0-6),
respectively, for the whole plant, partial plant, and infested plant sampling method.
4.4.1 Spatial Distribution of *E. servus*

The within-field spatial distributions of *E. servus* adults and nymphs at various stink bug susceptibility windows were described using Taylor’s power law and variance to mean ratio calculations (Table 3). Slopes of Taylor’s power law regression, the *b* coefficients, were significantly different from a value of 1 in most cases, except for adults sampled by the infested plant sampling method during all corn growth stages and for adults sampled by the partial plant sampling method during the V4-V6 growth stages. Similarly, for both adults and nymphs, overall *I_D* values were different from a value of 1 for all sampling methods tested and during all stink bug susceptibility windows. However, within a field, *I_D* values varied among the sampling dates and significant departures of *I_D* values from 1 were more often observed at higher insect densities (data not shown). The value of the Taylor’s index of aggregation (*b*), as well as the sample variance to mean ratio for both *E. servus* adults and nymphs on all stink bug susceptibility windows in field corn, were >1, indicating a predominantly spatially aggregated *E. servus* distribution for both adults and nymphs at higher field densities.

4.4.2 Sample Size Estimation and Sequential Sampling Plan

The number of sample units required to estimate the mean *E. servus* adult and nymph populations with a 10, 20 and 30% of precision were calculated for the whole plant sampling method (Fig. 1), the partial plant sampling (Fig. 2), and the infested plant sampling method (Fig. 3). For early vegetative stage corn (V4-V6), the whole plant sampling method for adult *E. servus* at an economic threshold density of two stink bugs per 20 plants required 247, 62, and 27 sample units to estimate the population within 10, 20, and 30% of mean respectively. At a corresponding economic threshold density of 1.73 stink bugs per 20 plants, the partial plant sampling method also required a similar number of sample units (248, 62, 28) to estimate the population within.
same precision levels. However, for the infested plant sampling method at an economic density of 1.65 infested plants per 20 plants, the number of sample units required to estimate the population within 10, 20 and 30% of mean was the lowest (209, 52, 23 respectively) among the sampling methods tested.

At the V14-R1 growth stages, for adult *E. servus* population estimation at corresponding economic threshold densities with a 10, 20, and 30% precision level, the infested plant sampling method consistently required the lowest sample unit numbers (102, 26, 11), followed by the whole plant sampling method (135, 32, 15) and the partial plant method (190, 47, 21). However, for a population estimation of *E. servus* nymphs at these growth stages, the whole plant sampling method required the lowest number of sample units (172, 43, 19), followed by the infested plant sampling method (183, 46, 20) and the partial plant sampling method (294, 73, 33). For adult *E. servus* at the R1-R4 growth stages, both the whole plant and the infested plant sampling method required a similar number of sampling units (68, 17, 8) to estimate the population with a 10, 20 and 30% precision level. For nymphs, however, the infested plant sampling method required the lowest sample unit number (68, 17, 8), compared to the whole plant sampling method (91, 23, 10) or the partial plant sampling method (138, 34, 15). To simplify management decisions for *E. servus* in field corn, sequential sampling plans for *E. servus* adults and nymphs were developed at an $\alpha = \beta$ error rates of 10, and 20% on specific stink bug susceptible corn growth windows, for each sampling method (Figs. 4-6).

### 4.4.3 Cost-Reliability Calculations

The average time required, in seconds, to take the whole plant *E. servus* sample from 20 corn plants was $34.1 \pm 1.2$ ($\pm$ SEM), $146.0 \pm 7.7$ and $188.2 \pm 6.2$ at the V4-V6, V14-R1 and R1-R4 growth stages, respectively. For the partial plant sampling method, the average sampling
time, in seconds, from 20 corn plants was 24.1 ± 1.0, 91.9 ± 3.5 and 120.8 ± 4.0 at the V4-V6, V14-R1 and R1-R4 growth stages, respectively. The average time investment in seconds to walk between samples was 40.1 ± 0.5, 46.3 ± 0.5 and 50.0 ± 0.8 at the V4-V6, V14-R1 and R1-R4 growth stages, respectively, for both sampling methods.

The relative cost-reliability of a partial plant method, with respect to the whole plant sampling method for population estimation is illustrated in Fig. 7. At a relative cost-reliability of one, the partial plant and whole plant sampling method have the same cost-reliability. When the relative cost-reliability is less than one, the specific partial plant sampling method is costlier than whole plant method for population estimation or pest management decisions with a given reliability. For a relative cost-reliability value less than one, the opposite is true.

When sampling adult *E. servus* in the R1-R4 growth stages, for a population estimation at a given reliability, the partial plant method was more cost effective than the whole plant sampling method for all the *E. servus* densities tested. However, for the V4-V6 and V14-R1 growth stages, the partial plant sampling method became more cost-effective only when the *E. servus* densities were above 0.5 and 5.7 stink bugs per 20 plants, respectively (Fig. 7A). For nymphs in the V14-R1 and R1-R4 growth stages, other than at densities below one *E. servus* per 20 plants at the R1-R4 growth stages, the whole plant method was more cost reliable than the partial plant sampling method for all the insect densities tested. For adult *E. servus* management in field corn, specific partial plant sampling methods were more cost reliable than the whole plant sampling method at the V4-V6, V14-R1 and R1-R4 growth stages, while the opposite was true for nymph management at the V14-R1 and R1-R4 growth stages (Fig 7B).
4.4.4 Comparison of a Fixed Sampling Plan to a Sequential Sampling Plan

The average number of sample units required to reach *E. servus* adult and nymph management decisions in field corn using a fixed sampling plan versus the sequential sampling plan for the whole plant, partial plant, and infested plant sampling methods at 10% error rate ($\alpha = \beta = 0.1$) are described in Table 4. Compared to fixed sampling plan of 10 sample units per field, corresponding sequential sampling plans for the whole plant, partial plant, and infested plant sampling methods during *E. servus* susceptibility windows significantly reduced the sample units required to reach a management decision for both adult and nymphs (Table 4). For whole plant sampling methods, the sample unit requirement of sequential sampling plans ranged from $4.0 \pm 0.0$ to $4.3 \pm 0.3$ sample units per field, for both adult and nymphs (Table 4). In comparison to a fixed sampling plan, this corresponds to a 60 to 57.5% reduction in the sampling unit requirement. Similarly, for the partial plant sampling method, a sequential sampling plan reduced the sample unit requirement to $4.0 \pm 0.0$ to $4.6 \pm 0.3$ sample units per field, which correspond to a 60 and 53.8% reduction in the sample unit requirement, respectively, for both adults and nymphs. The sequential sampling plan with an infested plant sampling method also reduced the sample unit requirement to $4.0 \pm 0.0$ and $4.5 \pm 0.3$ samples per field for adult and nymph *E. servus*, respectively. A similar result was observed for sequential sampling plans for the whole plant, partial plant, and infested plant sampling methods at a 20% error rate ($\alpha = \beta = 0.2$) (result not shown).

4.5 Discussion

Our results suggest a predominantly aggregated spatial distribution of *E. servus* adults and nymphs in the field corn (Table 3). Similar aggregated distributions are reported for *E. servus* and other pentatomid pests in multiple crops, including corn. For example, studies
conducted in the southeastern United States suggest a predominantly aggregated spatial
distribution of *E. servus* adults and nymphs in soybean, corn, cotton, and wheat (Reay-Jones et
are aggregated in United States soybeans (Pilkay et al. 2015), and in Japanese rice (Hokyo and
Kiritani 1962, Nakasuji et al. 1965). The spatial distribution of a species is mostly determined by
its behavior (Davis 1994), and the intensity of aggregation within a species can differ between
the sex and life stages of conspecific individuals. The aggregated distribution of *N. viridula*
males observed in Japanese rice fields has been attributed to the attraction of males towards
females (Nakasuji et al. 1965). The female behavior of laying egg in egg masses has been
credited to the clumped distribution pattern in nymphs up to the third instar (Hokyo and kiritani
1962). Similarly, the observed intra-field aggregation pattern of *E. servus* adults in our results is
likely mediated by the male-specific volatile, methyl *(E,Z)-2,4-decadienoate*, an aggregation
pheromone that attracts male, female, and nymphs (Aldrich et al. 1991). The female behavior of
laying eggs in masses, and the limited mobility of early instar nymphs, could be one the possible
explanatory reasons behind the aggregated distribution of *E servus* nymphs. Since multiple
exogenous and endogenous factors can result in a similar spatial pattern (Vinatier et al. 2011),
such a hypothesis demands validation with empirical evidence.

Currently, the stink bug economic threshold for field corn is defined for the whole plant
sampling method, and specific thresholds values are available for each of the three corn stink bug
susceptibility windows (Koch et al. 2017), which we modified for the VE-V6, V14-early R1, and
mid R1-early R4 growth stages. Our newly calculated economic thresholds for the partial plant
and infested plant sampling methods, during each of the above corn susceptibility windows, will
provide growers and scouts with new choices of sampling methods that are often more efficient
than the current whole plant sampling method (Fig. 7). Furthermore, stink bug whole plant count data are often converted to percentage infestation data, although empirical data are not often available to justify this conversion. For example, an economic threshold of one stink bug per two plants in the mid R1 to early R4 growth stages (post pollination to early dough), is often equated to a 50% infestation (Hunt et al. 2014). Contrary to the current threshold recommendation for a constant ratio between count and percentage data, our results suggest that there should be distinct thresholds for both the whole plant sampling method and infested plant sampling method (Table 2).

Regardless of the specific corn stink bug susceptibility window or E. servus life stage, we observed strong positive linear relationships between whole plant counts to infested plant counts under a range of E. servus densities we encountered. However, we do not assume that the relationship remains linear throughout all possible E. servus densities experienced in the field. At very high insect densities, an asymptotic relationship is expected between the insect density and infestation counts (Davis 1994). This is because at a saturation insect density, every plant in a sample unit will have at least one E. servus, and any further increases in the population density will have no effect on the counts of infested plants. The occurrence of such high population levels in corn is unlikely for E. servus in most field situations. Moreover, sequential sampling plans developed for infested plant sampling methods would still be useful, even under high populations, as the economic thresholds are well below the possible saturation density for 20 plants sample units. Consequently, when using a sequential sampling plan, the cumulative infested plant counts from fields with high E. servus density will probably always fall within the “stop sampling control needed” region and will trigger an E. servus management intervention.
The characterization of a population’s spatial pattern can be influenced by the sampling method and sample unit size (Wilson 1985, Serra and Trumper 2006). In our study, partial plant sampling methods consistently required higher number of sample units to estimate the population mean with a given reliability compared to whole plant and infested plant sampling methods. This is because a reduction in the sampling unit size using the partial plant sampling method, by restricting the sampling area to a specific part of the plant, generally reduces the catch efficiency (Wilson 1982); in turn, this can increase the variance, resulting in an increased number of sample units requirements for the population estimation within a given reliability. A partial plant sampling method is usually designed to attain a significant reduction in cost per sample unit with minimum loss in sampling accuracy or precision. Therefore, an increase in sample unit requirements is often, but not always, compensated for by a significant saving in the total sampling time in a well-designed partial plant sampling method. In such instances, the overall time required to walk between the sample units, which increases with the increase in the number of sample unit requirement, as well as time required to examine an individual sample unit, which depends on the sampling method, influences the overall efficiency of specific sampling method. Relative cost-reliability calculations have been utilized to compare the overall efficiency of different sampling procedures in such situations (Wilson and Room 1982, Espino et al. 2008, Reay-Jones et al. 2009).

The relative cost-reliability calculation depends on the sampling costs, variance and insect density (Reay-Jones et al. 2009). When sampling adult *E. servus* in R1-R4 corn for population estimation, the partial plant method was more cost effective than the whole plant sampling method for all the insect densities tested. However, for the V4-V6 and V14-R1 growth stages, the partial plant sampling method became more cost-effective only when the *E. servus*
densities were above 0.5 and 5.7 stink bugs per 20 plants, respectively. For adult *E. servus* management in field corn, specific partial plant methods were more cost-reliable than the whole plant sampling method, while opposite was true for the nymphs. Since optimal population estimation is often a goal of research, and efficient scouting for optimal pest management is a priority for growers, these results are useful to help different practitioners choose an appropriate sampling method for their predetermined goal. Additionally, the selection of a sampling method should be based on the corn growth stage, approximate insect density in the field, and life stages being sampled.

Compared to a fixed sampling plan, the corresponding sequential sampling plan consistently reduced the sample unit requirement to reach a management decision, irrespective of the sampling method. The reduction in sample unit size ranged from 53.8 to 60% to that of the fixed sampling plan. A sequential sampling plan can save considerable time when the mean densities of a targeted species far exceeds or falls well below the economic threshold. Multiple studies in the southern United States indicate that the stink bug densities are generally low in corn (Tillman 2010, Reisig 2011, Pilkay et al. 2015), and often well below the economic threshold. Moreover, the peak densities are usually associated with the corn reproductive stages (Tillman 2010), when economic threshold values are relatively high. Moreover, *E. servus* sampling from random corn fields (n = 33) during this study suggested that the mean densities of *E. servus* adults and nymphs in most fields were well below the economic threshold. Hence, compared to a fixed sampling plan of comparable average error rates, the adoption of a sequential sampling plan could considerable save sampling time in majority of the fields.

In conclusion, our study suggests an aggregated spatiotemporal distribution of *E. servus* adults and nymphs in field corn. The economic threshold defined for the partial plant and
infested plant sampling method for various stink bug susceptibility windows in field corn will enable the adoption of these efficient sampling methods over the more labor intensive whole plant method. Furthermore, the sampling plans reported here will assist choices for appropriate sample sizes, efficient sampling methods, and proper sampling plans to calculate *E. servus* densities or to make pest management decisions with an acceptable precision level. Altogether, these results will, hopefully, encourage frequent sampling for *E. servus* in field corn with positive economic benefits.
4.6 Acknowledgments

Allen Scott and Dannyel Nelson provided the technical assistance. Francis Reay-Jones (Clemson University) helped with data analysis. We thank Antonio Cinti Luciani, Jarman Sullivan, Jay Sullivan, Jonathan Peppers, and multiple other growers across the northeastern North Carolina for allowing access to their corn fields for stink bug sampling. Partial funding of this project was provided by NC Agricultural and Life Sciences Research Foundation and the Corn Growers Association of North Carolina.
4.7 References Cited


### 4.8 Tables and Figures

Table 4.1  Linear regression equations relating *E. servus* counts from the whole plant sampling method (independent variable) to the partial plant sampling method (dependent variable), and the corresponding economic threshold for partial plant sampling method

<table>
<thead>
<tr>
<th><em>E. servus</em> GS</th>
<th>Corn GS</th>
<th>Intercept ± SEM</th>
<th>Slope ± SEM</th>
<th>$F$</th>
<th>$P$</th>
<th>$R^2$</th>
<th>$n$</th>
<th>Economic threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>V4 - V6</td>
<td>-0.017 ± 0.042</td>
<td>0.874 ± 0.018</td>
<td>2264</td>
<td>&lt;0.0001</td>
<td>0.90</td>
<td>246</td>
<td>1.73</td>
</tr>
<tr>
<td></td>
<td>V14 – VT</td>
<td>-0.047 ± 0.099</td>
<td>0.522 ± 0.028</td>
<td>351.9</td>
<td>&lt;0.0001</td>
<td>0.64</td>
<td>203</td>
<td>2.56</td>
</tr>
<tr>
<td></td>
<td>R1 - R4</td>
<td>0.013 ± 0.064</td>
<td>0.769 ± 0.015</td>
<td>2634</td>
<td>&lt;0.0001</td>
<td>0.88</td>
<td>360</td>
<td>7.70</td>
</tr>
<tr>
<td>Nymph</td>
<td>V4 - V6</td>
<td>-0.002 ± 0.004</td>
<td>0.964 ± 0.012</td>
<td>6203</td>
<td>&lt;0.0001</td>
<td>0.96</td>
<td>246</td>
<td>1.93</td>
</tr>
<tr>
<td></td>
<td>V14 – VT</td>
<td>0.006 ± 0.044</td>
<td>0.399 ± 0.017</td>
<td>530.9</td>
<td>&lt;0.0001</td>
<td>0.72</td>
<td>203</td>
<td>2.00</td>
</tr>
<tr>
<td></td>
<td>R1 - R4</td>
<td>0.055 ± 0.017</td>
<td>0.620 ± 0.016</td>
<td>1575</td>
<td>&lt;0.0001</td>
<td>0.81</td>
<td>360</td>
<td>6.25</td>
</tr>
</tbody>
</table>

GS = growth stage, SEM = standard error mean, $n$ = number of sampling units.

An individual sampling unit consisted of 20 corn plants and for adult and nymphs; the current economic threshold for 20 plants by the whole plant sampling method at VE-V6 is two stink bugs, while at V14-early R1 it is five stink bugs, and at mid R1-early R4 it is 10 stink bugs.
Table 4.2  Linear regression equations relating the *E. servus* counts from the whole plant sampling method (independent variable) to the number of *E. servus* infested corn plants (dependent variable), and the corresponding economic threshold for the infested plant sampling method

<table>
<thead>
<tr>
<th><em>E. servus</em> GS</th>
<th>Corn GS</th>
<th>Intercept ± SEM</th>
<th>Slope ± SEM</th>
<th>$F$</th>
<th>$P$</th>
<th>$R^2$</th>
<th>$n$</th>
<th>ET (n)</th>
<th>Current ET (%)</th>
<th>Revised ET (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>V4 - V6</td>
<td>0.120 ± 0.029</td>
<td>0.772 ± 0.013</td>
<td>3799</td>
<td>&lt;0.0001</td>
<td>0.94</td>
<td>246</td>
<td>1.65</td>
<td>10</td>
<td>8.25</td>
</tr>
<tr>
<td></td>
<td>V14 – VT</td>
<td>0.208 ± 0.046</td>
<td>0.781 ± 0.013</td>
<td>3658</td>
<td>&lt;0.0001</td>
<td>0.95</td>
<td>203</td>
<td>4.11</td>
<td>25</td>
<td>20.55</td>
</tr>
<tr>
<td></td>
<td>R1 - R4</td>
<td>0.456 ± 0.050</td>
<td>0.664 ± 0.012</td>
<td>3144</td>
<td>&lt;0.0001</td>
<td>0.90</td>
<td>360</td>
<td>7.10</td>
<td>50</td>
<td>35.50</td>
</tr>
<tr>
<td>Nymph</td>
<td>V4 - V6</td>
<td>-0.002 ± 0.004</td>
<td>0.964 ± 0.012</td>
<td>6203</td>
<td>&lt;0.0001</td>
<td>0.96</td>
<td>246</td>
<td>1.93</td>
<td>10</td>
<td>9.65</td>
</tr>
<tr>
<td></td>
<td>V14 – VT</td>
<td>0.084 ± 0.029</td>
<td>0.744 ± 0.012</td>
<td>4103</td>
<td>&lt;0.0001</td>
<td>0.95</td>
<td>203</td>
<td>3.80</td>
<td>25</td>
<td>19.00</td>
</tr>
<tr>
<td></td>
<td>R1 - R4</td>
<td>0.048 ± 0.009</td>
<td>0.768 ± 0.009</td>
<td>8109</td>
<td>&lt;0.0001</td>
<td>0.96</td>
<td>360</td>
<td>7.72</td>
<td>50</td>
<td>38.60</td>
</tr>
</tbody>
</table>

GS = growth stage, SEM = standard error mean, $n$ = number of sampling units.

An individual sampling unit consisted of 20 corn plants and for adult and nymphs; the current economic threshold for 20 plants by the whole plant sampling method at VE-V6 is two stink bugs, at V14-early R1 it is five stink bugs, and at mid R1-early R4 it is 10 stink bugs. ET (n) denotes the corresponding economic threshold for the infested plant sampling method expressed as number of *E. servus* infested plants per 20 corn plants. Revised ET (%) is the ET (n) expressed as percentage of infested plant per sampling unit. The current ET (%) is the current ET recommendations expressed as percent infested plants in a sample unit for the infested plant sampling method, which is a direct percentage conversion from the current whole plant ET.
Table 4.3  Taylor’s power law constants and variance to mean ratio for *E. servus* nymphs and adults in field corn sampled with three different sampling methods during 2016-2017

<table>
<thead>
<tr>
<th>Sampling method</th>
<th><em>E. servus</em> GS</th>
<th>Corn GS</th>
<th>$a$</th>
<th>$b$</th>
<th>$R^2$</th>
<th>$n$</th>
<th>$t$ value for $b = 1$</th>
<th>Var/mean</th>
<th>$I_D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole plant Adult</td>
<td>V4-V6</td>
<td>1.403</td>
<td>1.261</td>
<td>0.85</td>
<td>23</td>
<td>2.25$^a$</td>
<td>1.63</td>
<td>867.17$^b$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V14-R1</td>
<td>1.493</td>
<td>1.272</td>
<td>0.94</td>
<td>26</td>
<td>4.23$^a$</td>
<td>1.99</td>
<td>1931.89$^b$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R1-R4</td>
<td>1.491</td>
<td>1.205</td>
<td>0.88</td>
<td>41</td>
<td>2.82$^a$</td>
<td>1.97</td>
<td>2815.12$^b$</td>
<td></td>
</tr>
<tr>
<td>Nymph</td>
<td>V4-V6*</td>
<td>3.687</td>
<td>1.612</td>
<td>0.89</td>
<td>23</td>
<td>2.12$^a$</td>
<td>1.55</td>
<td>597.53$^b$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V14-R1</td>
<td>1.884</td>
<td>1.278</td>
<td>0.95</td>
<td>26</td>
<td>3.85$^a$</td>
<td>1.88</td>
<td>1642.25$^b$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R1-R4</td>
<td>1.683</td>
<td>1.280</td>
<td>0.89</td>
<td>41</td>
<td>3.12$^a$</td>
<td>1.47</td>
<td>1590.40$^b$</td>
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<tr>
<td>Partial plant Adult</td>
<td>V4-V6</td>
<td>1.329</td>
<td>1.171</td>
<td>0.86</td>
<td>23</td>
<td>1.53$^{NS}$</td>
<td>1.63</td>
<td>1043.67$^b$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V14-R1</td>
<td>1.394</td>
<td>1.192</td>
<td>0.97</td>
<td>26</td>
<td>4.53$^a$</td>
<td>1.47</td>
<td>1475.35$^b$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R1-R4</td>
<td>1.403</td>
<td>1.236</td>
<td>0.85</td>
<td>41</td>
<td>2.70$^a$</td>
<td>1.85</td>
<td>2442.97$^b$</td>
<td></td>
</tr>
<tr>
<td>Nymph</td>
<td>V4-V6*</td>
<td>0.897</td>
<td>3.161</td>
<td>0.82</td>
<td>23</td>
<td>6.58$^a$</td>
<td>1.63</td>
<td>625.08$^b$</td>
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<tr>
<td></td>
<td>V14-R1</td>
<td>1.691</td>
<td>1.256</td>
<td>0.94</td>
<td>26</td>
<td>3.44$^a$</td>
<td>1.41</td>
<td>914.47$^b$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R1-R4</td>
<td>1.776</td>
<td>1.294</td>
<td>0.79</td>
<td>41</td>
<td>2.27$^a$</td>
<td>1.55</td>
<td>1387.52$^b$</td>
<td></td>
</tr>
<tr>
<td>Sampling method</td>
<td>E. servus GS</td>
<td>Corn GS</td>
<td>$a$</td>
<td>$b$</td>
<td>$R^2$</td>
<td>$n$</td>
<td>$t$ value for $b = 1$</td>
<td>Var/mean</td>
<td>$I_D$</td>
</tr>
<tr>
<td>-----------------</td>
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<td>-----</td>
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<td>-------</td>
<td>----</td>
<td>-----------------------</td>
<td>----------</td>
<td>------</td>
</tr>
<tr>
<td>Infested plant</td>
<td>Adult</td>
<td>V4-V6</td>
<td>1.105</td>
<td>1.092</td>
<td>0.80</td>
<td>20</td>
<td>0.71$^{NS}$</td>
<td>1.22</td>
<td>485.66$^b$</td>
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<tr>
<td></td>
<td></td>
<td>V14-R1</td>
<td>1.236</td>
<td>1.090</td>
<td>0.88</td>
<td>19</td>
<td>0.91$^{NS}$</td>
<td>1.37</td>
<td>506.49$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R1-R4</td>
<td>1.289</td>
<td>1.131</td>
<td>0.62</td>
<td>29</td>
<td>0.77$^{NS}$</td>
<td>1.61</td>
<td>875.19$^b$</td>
</tr>
<tr>
<td>Nymph</td>
<td></td>
<td>V4-V6*</td>
<td>4.235</td>
<td>1.729</td>
<td>0.88</td>
<td>20</td>
<td>1.93$^a$</td>
<td>1.66</td>
<td>521.08$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V14-R1</td>
<td>1.765</td>
<td>1.208</td>
<td>0.93</td>
<td>19</td>
<td>2.28$^a$</td>
<td>1.75</td>
<td>829.30$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R1-R4</td>
<td>1.323</td>
<td>1.159</td>
<td>0.90</td>
<td>29</td>
<td>1.72$^a$</td>
<td>1.18</td>
<td>677.34$^b$</td>
</tr>
</tbody>
</table>

GS = growth stage, SEM = standard error mean, $n$ = number of sampling units, $I_D$ = Overall index of dispersion
* The mean E. servus nymph densities at the V4-V6 corn was extremely low.
NS not significant.

$a$ t-test with $n - 2$ degrees of freedom and $\alpha = 0.05$ indicate a significant departure of $b$ coefficient from a value of 1.

$^b$ $\chi^2$ test indicate a significant departure of $I_D$ value from 1.
Table 4.4  Comparison of mean number of sample units required to reach the *E. servus* adult and nymph management decisions in field corn using the fixed sampling plan versus the sequential sampling plan for whole plant, partial plant and infested plant count methods at 10% error rate ($\alpha = \beta = 0.1$) in commercial fields of North Carolina during 2017

<table>
<thead>
<tr>
<th>Sampling method</th>
<th>Stage</th>
<th>Corn growth phase</th>
<th>Mean sample units (± SEM)</th>
<th>% reduction</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fixed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No intervention</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Adult</td>
<td>V4-V6</td>
<td>4.0 ± 0.0</td>
<td>60.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V14-R1</td>
<td>4.3 ± 0.3</td>
<td>57.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R1-R4</td>
<td>4.0 ± 0.0</td>
<td>60.0</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Nymph</td>
<td>V14-R1</td>
<td>4.0 ± 0.0</td>
<td>60.0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R1-R4</td>
<td>4.0 ± 0.0</td>
<td>60.0</td>
<td>15</td>
</tr>
<tr>
<td>Partial plant</td>
<td>Adult</td>
<td>V4-V6</td>
<td>4.0 ± 0.0</td>
<td>60.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V14-R1</td>
<td>4.6 ± 0.3</td>
<td>53.8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R1-R4</td>
<td>4.0 ± 0.0</td>
<td>60.0</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Nymph</td>
<td>V14-R1</td>
<td>4.0 ± 0.0</td>
<td>60.0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R1-R4</td>
<td>4.0 ± 0.0</td>
<td>60.0</td>
<td>15</td>
</tr>
<tr>
<td>Infested plant count</td>
<td>Adult</td>
<td>V4-V6</td>
<td>4.0 ± 0.0</td>
<td>60.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V14-R1</td>
<td>4.5 ± 0.3</td>
<td>55.0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R1-R4</td>
<td>4.0 ± 0.0</td>
<td>60.0</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Nymph</td>
<td>V14-R1</td>
<td>4.0 ± 0.0</td>
<td>60.0</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R1-R4</td>
<td>4.0 ± 0.0</td>
<td>60.0</td>
<td>15</td>
</tr>
</tbody>
</table>
Figure 4.1  Optimum sample size required to estimate the *E. servus* population mean with a reliability of 10, 20, and 30% of the mean for the whole plant sampling method. Individual sampling units consisted of 20 consecutive corn plants. The vertical dotted line indicates the economic threshold for the whole plant sampling method.
Figure 4.2  Optimum sample size required to estimate the *E. servus* population mean with a reliability of 10, 20, and 30% of the mean for the partial plant sampling method. Individual sampling units consisted of 20 consecutive corn plants. The vertical dotted line indicates the economic threshold for the partial plant sampling method.
Figure 4.3  Optimum sample size required to estimate the *E. servus* population mean with a reliability of 10, 20, and 30% of the mean for the infested plant sampling method. Individual sampling units consisted of 20 consecutive corn plants. The vertical dotted line indicates the economic threshold for the infested plant count method.
Figure 4.4  Sequential sampling plan for *E. servus* adults and nymphs in field corn when using the whole plant sampling method. Individual sampling units consisted of 20 consecutive corn plants. Sequential stop lines were calculated at \( \alpha \) and \( \beta \) error rates of 10 and 20 % (\( \alpha = \beta = 0.1 \) and \( \alpha = \beta = 0.2 \)).
Sequential sampling plan for *E. servus* adults and nymphs in field corn when using the partial plant sampling method. Individual sampling units consisted of 20 consecutive corn plants. Sequential stop lines were calculated at $\alpha$ and $\beta$ error rates of 10 and 20 % ($\alpha = \beta = 0.1$ and $\alpha = \beta = 0.2$).
Figure 4.6  Sequential sampling plan for *E. servus* adults and nymphs in field corn when using the infested plant sampling method. Individual sampling units consisted of 20 consecutive corn plants. Sequential stop lines were calculated at $\alpha$ and $\beta$ error rates of 10 and 20 % ($\alpha = \beta = 0.1$ and $\alpha = \beta = 0.2$).
Figure 4.7  Relative cost-reliability of the partial plant sampling method over the whole plant sampling method for (A) population estimation and (B) pest management of *E. servus* in field corn. Mean population density is expressed as the number of *E. servus* per 20 corn plants each. A relative cost-reliability of one, where the partial plant sampling method and whole plant sampling method have the same cost-reliability, is indicated by the thick dark line parallel to the x-axis. Above this line, the partial plant sampling method is costlier than the whole plant sampling method for a given reliability.