8-2016

An Ecological Study of the Kudzu Bug in East Tennessee: Life History, Seasonality, and Phenology

Kadie Elizabeth Britt
University of Tennessee, Knoxville, kbritt5@vols.utk.edu

To the Graduate Council:

I am submitting herewith a thesis written by Kadie Elizabeth Britt entitled "An Ecological Study of the Kudzu Bug in East Tennessee: Life History, Seasonality, and Phenology." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

Jerome F. Grant, Major Professor

We have read this thesis and recommend its acceptance:

Scott D. Stewart, Juan-Luis Jurat Fuentes, Gregory J. Wiggins

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

An Ecological Study of the Kudzu Bug in East Tennessee: Life History, Seasonality, and Phenology

A Thesis Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Kadie Elizabeth Britt
August 2016

DEDICATION

*To **Ked** – thank you for loving me, supporting me always, especially throughout this entire process, and for always being the optimistic spirit I need. I could not have gotten through this without you.*

*To **my parents** – Thank you for always loving and supporting me and for always doing everything you can to help me reach my dreams, no matter how crazy they may be. It never gets old hearing you say you are proud of me.*

ACKNOWLEDGEMENTS

I have so many people to thank for their assistance during this process. I absolutely could not have gotten through this on my own. I owe thanks to Renee Follum and Elizabeth Benton for being so hospitable to me during my initial visit to campus. I also thank Renee and Elizabeth for helping me learn my way around campus and the Knoxville area in general. This project could not have happened without Renee! All kudzu sites were found because of her assistance.

I owe a very special thank you to Elizabeth Benton for serving as a mentor to me during my time at UT. She helped me many times with writing, editing, practicing presentations, and countless other things.

I have a great deal of gratitude for all of the lab personnel who were so eager to help with the field portion of my project. I extend a great thank you to Phil Hensley, Bryson Scruggs, John Glafenhein, Derrick Turman, Julia Ferguson, Chelsea Standish, Tammy Stackhouse, Michael Pelle, Forest Palmer, and Amy Michael. I was truly lucky to get to work with such good-spirited folks. I owe a special thanks to Julia Ferguson for helping me find kudzu bugs infected with *Beauveria bassiana*! I would also like to thank Lucas Hietala for field assistance and help with collection of kudzu and kudzu bugs, as well as the many times we were able to bounce ideas off of one another.

I wish to thank all of the fellow graduate students that helped me to remain sane for two years. I have made friendships at UT that will last for a lifetime.

My committee (Dr. Jerome Grant, Dr. Scott Stewart, Dr. Juan Luis Jurat-Fuentes, and Dr. Greg Wiggins) was such a vital component of completing this project. I appreciate all of their help, shared ideas, and support that I received along the way. This experience has truly been a great one and I could not have accomplished it without their help. I also wish to thank Dr. Arnold Saxton for his helpful advice and patience while helping me understand my data analysis. Drs. Grant and Wiggins deserve all of the thanks in the world for helping me edit this thesis over and over! I am so grateful.

I owe my parents a special thank you for all the love and support that they always give me. And lastly, I wish to thank Ked Byrd. Your love and support means more to me than you will ever know. Also, thank you for saving my thesis the time I thought I deleted it. If I had deleted it, it would have taken me months longer to write this sentence.

ABSTRACT

The kudzu bug, *Megacopta cribraria* (Hemiptera: Plataspidae), an invasive species from Asia, was first discovered in the United States in 2009 in Georgia. It has since spread to other states, including Tennessee, where it has spread rapidly to numerous counties in four years. Its common name, kudzu bug, implies a potential benefit to management of the invasive species kudzu; unfortunately, the kudzu bug has shown little impact on reducing growth of kudzu. The kudzu bug causes agricultural, urban, and health-related concerns in the United States. Soybean losses by kudzu bug have exceeded 20% in some areas of the southeastern United States.

Primary research goals of this project are to: 1) assess ecology of kudzu bug in Tennessee; 2) determine behavioral responses of kudzu bug to host substrates; and 3) examine the relationship between kudzu bug and natural enemies present in Tennessee. In 2014 and 2015, studies were conducted to better understand population dynamics of kudzu bug in Tennessee. Kudzu bugs were present on kudzu in Knox County until early November in 2014 and mid-May to late November in 2015, with mid- and late-season peaks both years. Kudzu bugs were present at sites in other counties throughout the same time. Kudzu bugs exhibited different population trends in all counties, possibly due to differences in latitude, agricultural practices, and topography.

In laboratory studies, kudzu bugs were more active on kudzu than alternate host plants (soybean, bush honeysuckle, and ragweed). Ragweed was the least attractive plant species. In no-choice tests, activity was similar across kudzu, soybean, and bush honeysuckle. In choice tests, kudzu had the highest percent of active insects.

An unexpected natural enemy was found at all regularly sampled sites in 2015. *Beauveria bassiana*, an entomopathogenic fungus, was discovered infecting kudzu bug and impacting population densities. Mortality of immature kudzu bugs reached 100%. The kudzu bug egg parasitoid, *Paratelenomus saccharalis*, was not recovered.

The outcome of this research project will provide essential information on ecology of kudzu bug in Tennessee. This information will help to enhance development and implementation of efficient and effective management tools.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I
INTRODUCTION / LITERATURE REVIEW

Invasive species have caused great destruction in many different areas of the world for centuries. Humans have intentionally and unintentionally assisted with the transportation of these alien species for millennia (Hulme, 2009). Invasive species have the potential to cause major losses in many important areas of the world's economy, particularly in the agricultural industry (Pimentel et al., 2000). It was estimated in 2005 that in the United States alone, invasive species caused economic losses greater than $140 billion annually (Pimentel et al., 2005). Invasions by exotic pests have a negative impact on the ecological integrity of a habitat or even an entire ecosystem (Pimentel et al., 2000). With the current Era of Globalization (Hulme, 2009), the continuous increase of human populations, and the growing rate of international travel and trade, the spread of alien species has been accelerated throughout the world (Pimentel et al., 2000). While ease of access to foreign lands provides wonderful opportunities for humans, it is a major pathway for the accidental spread of invasive species. According to the Definitions Subcommittee of the Invasive Species Advisory Committee, an invasive species is defined as a plant, fungus, or animal species that is not native to a specific location (an introduced species), and which has a tendency to spread to a degree believed to cause damage to the environment, human economy, or human health (Committee, 2006).

When alien species invade new areas, native species are at risk. Native species are forced to compete with each other and contend with natural enemies, while invasive pest species often have a competitive advantage and few predators to maintain their populations at low or manageable levels (Pimentel et al., 2000). This ecological discrepancy allows for a tremendous increase in the size of the population of the invasive species. The negative result of invasive species on invaded communities and ecosystems is evident in many systems (Gurevitch & Padilla, 2004). The resulting economic and ecological impact both in losses and costs of control affects every person.

The involvement of homeowners and the general public in monitoring for invasive species is important (Fulton, 2010). The public is an essential component to reporting infestations or the presence of alien invaders in new areas. Outreach is a key element, especially when observing for invasive species (Fulton, 2010). Public awareness may help mitigate problems before they worsen. If invasive species are detected and interventions are made early enough, more cost-effective solutions can sometimes be developed to help manage the presence of invasive species. If not, the species can better establish itself and become extremely difficult to manage (Simberloff, 2008).

Eradication and control requires consistent and thorough efforts to succeed, but these efforts may not always be economically feasible (Simberloff, 2008). In many cases, however, eradication is not realistic or possible. Kudzu, *Pueraria montana* variety *lobata* (Willd.) Maesen and S. Almeida (Fabales: Fabaceae), and the kudzu bug, *Megacopta cribraria* (Fabricius) (Hemiptera: Heteroptera: Plataspidae), are two examples of invasive species that threaten native species and the agricultural industry in the United States.

Kudzu

Kudzu is one of the most problematic and well-known invasive weeds in the United States. This plant species was originally brought to the United States to assist with erosion control, particularly in the southeastern United States. It was first introduced in 1876 at the

Centennial Exposition in Philadelphia and then again in 1883 at the New Orleans Exposition (Frye et al., 2007). Kudzu is a perfect posterchild of an invasive species – it is a plant species that can spread at a highly rapid rate, outcompete native plant species for light, and climb trees, power poles, machinery, or any tall structures and overtake them completely. These growth characteristics lead to high costs associated with routine maintenance in areas adjacent to its presence, not to mention the high ecological costs associated with its capability of killing trees and other plants (Frye et al., 2007).

In 1998, the USDA listed kudzu as a federal noxious weed (Imai et al., 2010). Kudzu is established in the United States, but is most often found in the southeastern region of the country (Fig. 1). Eradication of this nuisance plant has not been successful, and if accomplished on a localized basis, takes time and patience. To effectively kill the plant, one must first kill the extensive root system (Frye et al., 2007). Kudzu has robust, tuberous roots that can extend several feet deep into the soil (Mitich, 2000) and become almost impossible to remove. However, plants in a kudzu patch can be killed if the patch is completely defoliated over many growing seasons (Frye et al., 2007). It has been estimated that one hectare of kudzu left unattended for 100 years would expand to 5,250 hectares (Mitich, 2000). Thus, management of kudzu is vital to conserve, preserve, and protect native habitats and ecosystems.

In addition to the multitude of already-existing ecological and economic problems caused by kudzu, it can also contribute to decreased air quality. Kudzu can emit isoprene and nitrogen oxides, and these chemicals together can increase tropospheric levels of ozone

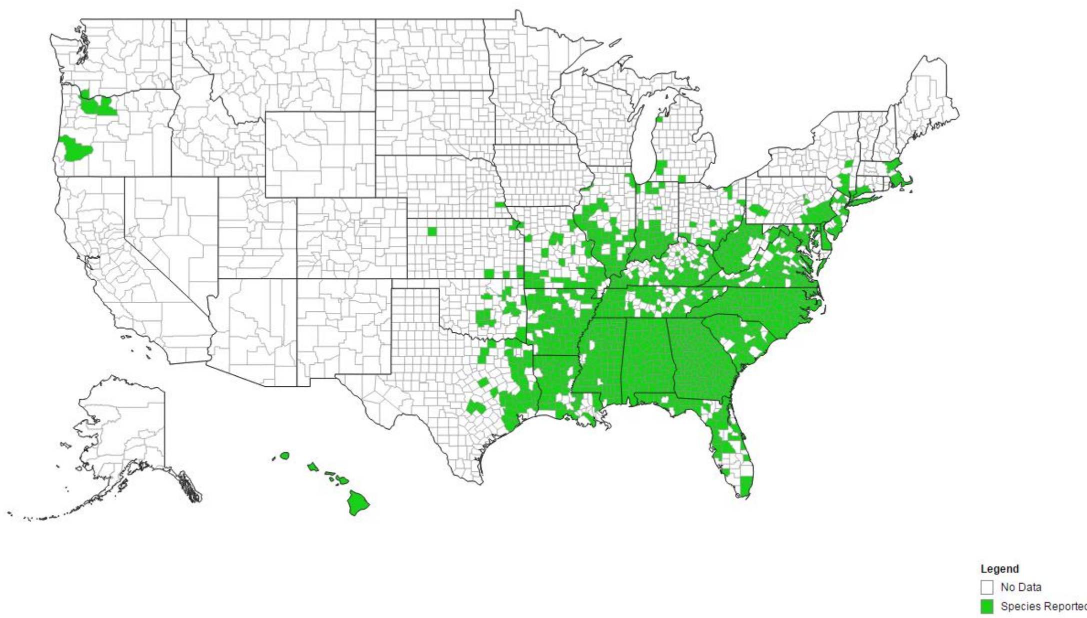

Figure 1. Distribution map of kudzu in the United States (www.eddmaps.org), 2016.

(Eviner et al., 2012). Abiotic environmental factors, such as light intensity, drought stress, and temperature, play a role in the levels at which these chemicals are emitted from kudzu into the atmosphere (Sharkey & Loreto, 1993). The effects on air quality by kudzu will be worse in areas where ozone formation is decreased, typically in more rural areas (Eviner et al., 2012).

Kudzu Bug

The kudzu bug is native to the Eastern Hemisphere and was discovered in the United States in nine northeastern counties in Georgia in mid-October 2009 (Jenkins et al., 2010). This report was the first documentation of this species in the Western Hemisphere – the kudzu bug was previously only known in Australia, China, India, Japan, Korea, Malaysia, and Pakistan (Suiter et al., 2010) (Fig. 2). The kudzu bug has since been found in 13 states and the District of

Figure 2. Distribution map of kudzu bug in the Old World, 2015. Arrow points to Australia.

Columbia (Fig. 3), including Tennessee since 2012 (Fig. 4) (Gardner et al., 2013b) (kudzubug.org).

The kudzu bug, also known as the bean plataspid, lablab bug, and globular stink bug, is an invasive pest in North America because of its tendency to invade homes, other structures, and feed in soybean (*Glycine max* (L.) Merrill; Fabales: Fabaceae) and other cultivated legume crops in large numbers (Gardner et al., 2013b; Suiter et al., 2010). Kudzu bugs are attracted to legumes, especially kudzu and soybeans, where they feed by sucking sap from stems, petioles, and leaves (Halbert & Eger, 2010; Zhang et al., 2012). Kudzu bugs can cause great losses to soybeans if not treated in a timely manner (Seiter et al., 2013). *M. cribraria* was found on homes, structures, landscape plants, and kudzu when first discovered in Georgia (Gardner et al., 2013b). How the bug arrived in the United States and its exact origin are uncertain (Zhang et al., 2012), but it was first reported in the area of Atlanta, Georgia.

No known host-specific natural enemies of the kudzu bug are established in the United States. This lack of host-specific natural enemies is a large contributing factor to its rapid dispersal and population growth since it was first found seven years ago in North America. The kudzu bug is an extremely strong flier and has great dispersal capabilities (Gardner et al., 2013b). Adults have been observed on plants in rooftop gardens as high as 32 stories above ground level in Atlanta, GA (Gardner et al., 2013b). Adults have been observed on windows 20 stories above ground in a high-rise apartment building in Knoxville, TN (personal observations). Kudzu bugs are frequently observed on clothing and vehicles, including air planes, suggesting that they can easily hitchhike from place to place (Halbert & Eger, 2010).

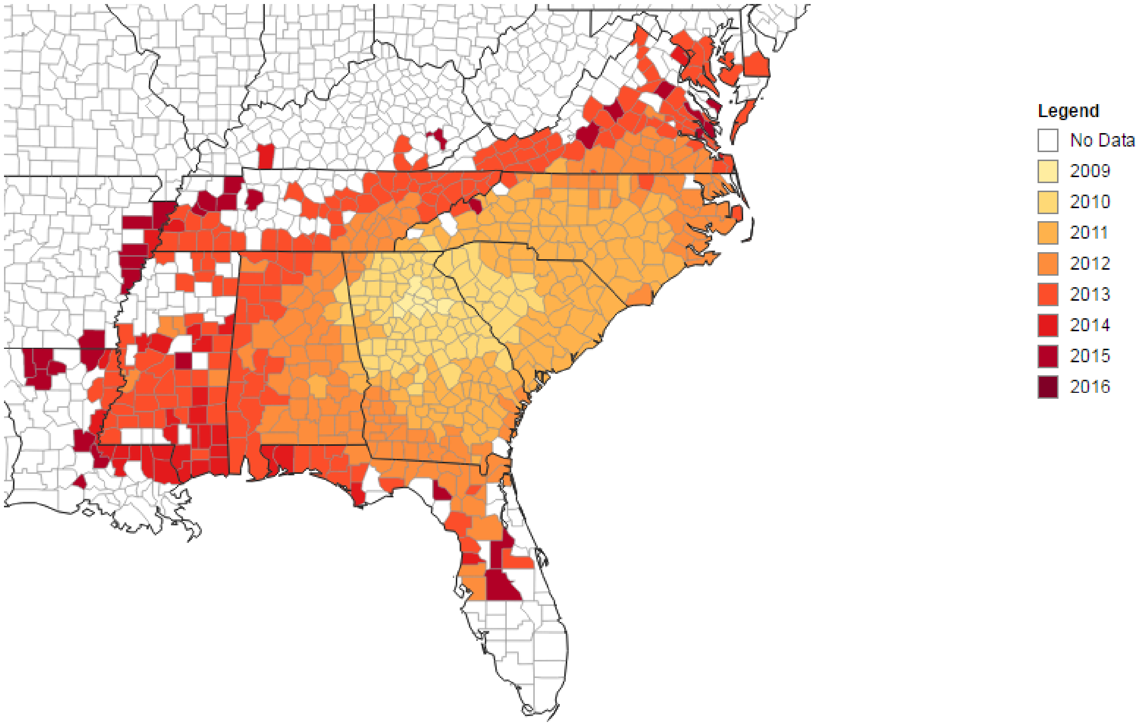

Figure 3. Spread of kudzu bug in the United States from 2009 to 2016 (www.kudzubug.org).

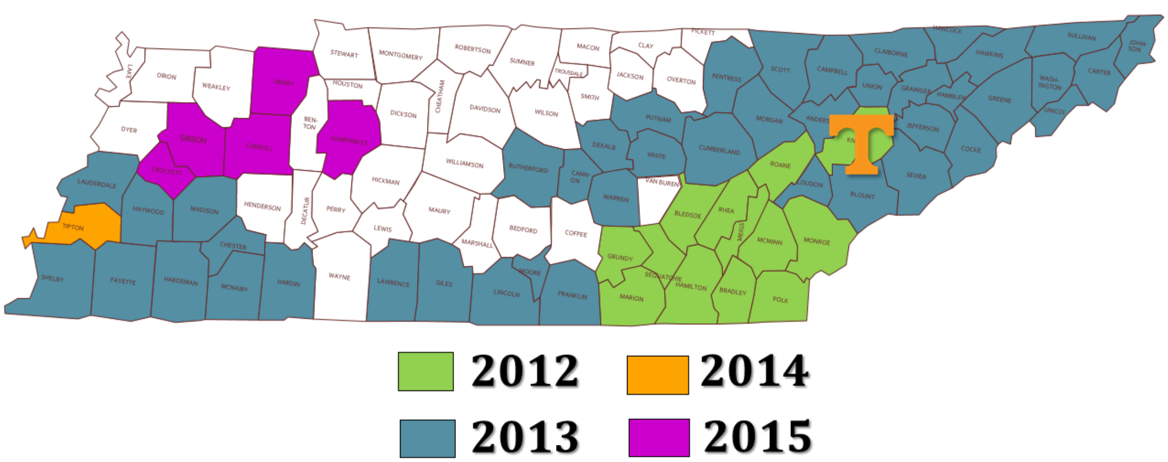

Figure 4. Distribution of kudzu bug in Tennessee, through December 2015.

Adult kudzu bugs, which are brown to olive green with darker-colored pits, are hemispherical in shape with their posterior ends flattened (Halbert & Eger, 2010). Adult *M. cribraria* are 4-6 mm long with greenish brown forewings (Suiter et al., 2010). *M. cribraria* adults and nymphs have piercing-sucking mouthparts, allowing them to feed on vascular tissue of plants (Gardner et al., 2013b). Kudzu bugs undergo hemimetabolous development, molting through five instars before becoming adults. Immature kudzu bugs are fuzzy in texture and anywhere from light brown to light green/yellow in color (Gardner et al., 2013b), which helps them to blend in extremely well when on kudzu vines.

Kudzu bugs are currently the only known species of the family Plataspidae in the continental United States. The only other known occurrence of a plataspid in the United States was the discovery in 1965 of *Coptosoma xanthogramma* (White), commonly called the black stink bug, in the Hawaiian islands (Beardsley & Fluker, 1967), where it is still present. Plataspids are distinct and can be separated easily from other insect families because the scutellum of its members completely covers the abdomen (Halbert & Eger, 2010). When disturbed, the kudzu bug emits a strong, distinct odor, which is similar to stink bugs (Hemiptera: Pentatomidae) (Seiter et al., 2013).

Many molecular studies to trace the origin of entry of the kudzu bug into the United States have been conducted (Jenkins & Eaton, 2011). Unfortunately, the origin of kudzu bug in the United States remains unclear, as well as confusion about the taxonomy of *M. cribraria*. A recent study in Japan strongly suggests that the invasive American populations of *M. cribraria* are derived from a population of the closely-related *Megacopta punctatissima* (Montandon) in the Kyushu region of Japan (Hosakawa et al., 2014). Populations of *M.*

punctatissima are restricted to East Asia where the two *Megacopta* species show different geographical distributions (Hosakawa et al., 2014). Reports indicate that these are two morphologically distinct species; for example, *M. punctatissima* is larger in size and darker in color with a larger indentation on its dorsal cuticle than *M. cribraria* (Hosakawa et al., 2014). However, some taxonomists consider these two species synonymous and suggest that neither *M. punctatissima* nor *M. cribraria* constitute their own distinct species but rather are representatives of local populations of the same species, with a wide range of genetic and phenotypic diversity (Hosakawa et al., 2014). The relationship status between these organisms will ultimately be determined by expert taxonomists.

An interesting characteristic of the kudzu bug is that it relies upon a bacterial endosymbiont, *Candidatus* Ishikawaella capsulate, for normal growth and development (Jenkins et al., 2010; Brown et al., 2014). Prior to laying eggs, the female kudzu bug deposits a small, bacteria-filled, nutritional capsule on which the nymphs will feed immediately after eclosion (Jenkins et al., 2010). Normal growth and development, increased fecundity, and pest status have all been linked to the presence of this endosymbiont in the guts of plataspid true bugs (Jenkins et al., 2010). Research has shown that these endosymbiotic bacterial capsules laid with kudzu bug eggs allow it to thrive on soybean and become a pest (Brown et al., 2014).

Many molecular studies have shown that the genotype of *Candidatus* Ishikawaella capsulate ultimately determines the soybean pest status of *M. cribraria* and *M. punctatissima* (Hosokawa et al., 2007). In Asia, the size and color of *Megacopta* is an indicator of which endosymbiont is present in these insects (Brown et al., 2014). Non-pest *Megacopta* appear smaller and paler than pest *Megacopta*, and insects that never obtain access to the endosymbiotic capsules after eclosion appear even smaller and more pale (Brown et al., 2014). The invasive "pest" insect *M. cribraria* in the United States genetically and morphologically resembles the "non-pest" *M. cribraria* in Asia (Brown et al., 2014). However, in Japan, *M. punctatissima* is the major pest species and has better fitness on soybean (Brown et al., 2014). Pest status of *Megacopta* could also be a result of hybridization of *M. cribraria* and *M. punctatissima* species (Brown et al., 2014). Since *M. cribraria* and the endosymbiont have been present in the United States, the genotype of the endosymbiont has not changed (Brown et al., 2014). The *Ishikawaella* genome occurring in *M. cribraria* (non-pest species in Asia) in the United States is identical to the *Ishikawaella* genome that is found in *M. punctatissima* (pest species in Asia) in Japan (Brown et al., 2014).

Female kudzu bugs can overwinter with viable sperm present in the spermatheca that can successfully fertilize eggs after females emerge from overwintering (Golec & Hu, 2015). Male and female kudzu bugs do not experience a deep winter reproductive dormancy, but rather a weak reproductive dormancy that can be overcome with a period of moderately high temperatures and/or a period of lengthened photoperiod (Golec & Hu, 2015). Overwintered sperm in male testes was as active as sperm found in female spermathecae once spring activation (consistent warm temperatures and longer photoperiod) occurred (Golec & Hu, 2015). This overwintering activity increases the potential for damage by kudzu bug. If a gravid female hitchhikes to a new location, a founder's effect will take place. This hypothetical scenario is potentially similar to the situation that occurred when kudzu bug was first discovered in Georgia in 2009. If the vast spatial distance between the native range of kudzu bug and the introduced area of the United States is considered, it seems that the founding invasive insects were transported due to human activity, although the exact geographic origin of the founders is still unknown (Hosakawa et al., 2014).

Impact of Kudzu Bug

The potential pest status of the kudzu bug in North America is of serious concern. Kudzu bugs have the potential to cause great economic losses in the southeastern United States, due to their feeding on legume plants, especially soybeans (Seiter et al., 2013). The establishment of *M. cribraria* in the southeastern United States adds another species to the long list of established soybean pests (Seiter et al., 2013). *M. cribraria* feeds on vascular fluid primarily from plant stems; therefore, any yield losses will likely be indirect and result from plant stress (Seiter et al., 2013). When kudzu bugs cause damage to plants, purple to brown-colored lesions are present, typically on stems (Blount et al., 2016). In its native countries of China, India, and Japan, the kudzu bug is also a pest of soybeans. In these countries, soybean growth can be reduced with yield losses up to 50% when 80 to 100 adults are found per plant (Seiter et al., 2013). Studies have been conducted in the southeastern United States to determine yield losses due to kudzu bug infestation. At the highest density of infestation observed (ca. 183 adult and nymph kudzu bugs per soybean plant), soybean yield was reduced by as much as 59.6% (Seiter et al., 2013).

Other than causing concerns as an agricultural pest, kudzu bugs can also be serious pests of residences. In the fall, large numbers of adults congregate on the exterior of buildings where they seek a secure place to overwinter (Halbert & Eger, 2010). Adult insects are extremely attracted to white and tan colors, so buildings with these types of exteriors are susceptible to infestation. A 2011 study in Athens (Georgia) showed that when given choices of different trap colors, white was the most attractive to kudzu bugs (Horn & Hanula, 2011). It is helpful for homeowners to secure openings around the home to ensure that bugs do not enter through unattended cracks and crevices (Britt et al., 2016). Homeowners should be extremely attentive to the presence of kudzu bugs during early spring when adult kudzu bugs emerge (typically during April in Tennessee) and search for locations to stay before kudzu is ready to support large populations of bugs. Another problematic time is late September onward since this period likely coincides with freezing temperatures, thus causing kudzu bugs to search for overwintering locations.

A less likely, although probable, concern is the presence of a yellow to orange colored stain on light colored surfaces or human skin in response to crushing kudzu bugs. When crushed, or even handled in large numbers, kudzu bugs emit a substance that is caustic to human skin if emitted in large amounts. Some humans can have a resulting allergic reaction. While an allergic reaction to kudzu bugs is not a typical response, it has been known to happen.

Management of Kudzu Bug

Different methods of control exist to reduce kudzu bug populations. Chemical insecticide applications on soybean plants are a popular and effective control method. A biological control agent of the kudzu bug, the tiny parasitic wasp *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygastridae) originating from its native range, is now present in certain locations of the United States (Gardner et al., 2013b). It was first observed in natural field conditions in Georgia on 30 May 2013. Joni Blount (University of Georgia) discovered dark-colored eggs in the wild (on kudzu vines on the Griffin campus) and brought them into the laboratory for further study. Several small hymenopterans emerged from these eggs – these parasitoids were later confirmed to be *P. saccharalis* (Gardner et al., 2013b). Since this discovery, the wasp has also been observed in the wild in Auburn, AL and Vicksburg, MS (Gardner et al., 2013b). Although finding adequate populations of *P. saccharalis* in 2013 seemed rather promising, wasps were not as readily observed in the wild in 2014 and 2015. It has since been noted that the only known hosts of *P. saccharalis* are restricted to the family Plataspidae (Gardner et al., 2013b). Most reports have shown that *P. saccharalis* begins to parasitize its hosts in late May and early June and continues as long as *M. cribraria* continues to oviposit (personal communication, Richard Evans, USDA- ARS, Stoneville, MS).

Earlier reports indicated that other natural enemies would also attack adult kudzu bugs. These include the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae), and two native dipteran species, *Strongygaster triangulifera* (Loew) and *Phasia robertsonii* (Townsend) (Diptera: Tachinidae). The fungus infects immature and adult kudzu bugs while *S. triangulifera* and *P. robertsonii* have only occasionally been observed to parasitize adult kudzu bugs.

Although the two dipteran species have been found to parasitize adult kudzu bugs (Golec et al., 2013; Ruberson et al., 2012), these species are both generalists and must be studied further to evaluate their potential as biological control agents of kudzu bug. *Beauveria bassiana* is a common fungus, and different strains have been known to infect multiple arthropod species. It has occasionally been observed on kudzu bug in different areas of the southeastern United States since it was first documented in soybean fields in South Carolina in

2012 (Seiter et al., 2014).

Ecology/Biology of Kudzu Bug

The primary reproductive host plants of kudzu bugs are kudzu and soybean (Zhang et al., 2012). Overwintering adults begin laying eggs on kudzu vines in mid-April (Greenstone et al., 2014), although timing may vary depending upon latitude, temperature, or other factors. Little is known about the numbers of egg masses that can be laid by a female in a season. However, it has been reported that in a lifetime, one female kudzu bug can lay anywhere from 26 to 274 eggs (Eger et al., 2012). In eastern Tennessee, the average number of eggs laid per mass is 16.7 (personal research). The developmental period from egg to adult takes approximately six to eight weeks (Greenstone et al., 2014). Adults that develop on kudzu can later disperse into soybeans in mid-June to mid-July – these adults colonize young vegetative plants and will remain from fruiting until harvest (Greenstone et al., 2014). It has also been observed that first-generation *M. cribraria* nymphs can bypass kudzu altogether and complete development on early-planted soybeans (Del Pozo-Valdivia & Reisig, 2013). When females lay eggs on soybean, first-instar kudzu bugs typically eclose in 7 to 10 days and then complete development to adult in about six weeks (Ruberson et al., 2012; Zhang et al., 2012). After newly-hatched nymphs consume the contents of the endosymbiotic bacteria capsules laid with the eggs, they remain close to the egg mass for 1-2 days before scattering to feed on stems of the plant (Blount et al., 2016). In its invasive range of the United States, the kudzu bug appears to be bivoltine (Greenstone et al., 2014). In its native range, kudzu bug can have three generations per year by mid-October or even persist throughout the entire year if weather conditions are suitable (Eger et al., 2012). The first generation of kudzu bug is usually found only on kudzu in May and June while the second generation can be on kudzu and/or soybeans from July to August (Del Pozo-Valdivia & Reisig, 2013). In Tennessee, and potentially other locations, second-generation adult kudzu bugs can persist throughout September.

Soybean planting date has a significant effect on the number of egg masses laid throughout a season (Blount et al., 2016). In a study in Georgia, soybean planted in April had two generations of kudzu bug per season, those planted in May had a partial first and full second generation, and those planted in June and July had only a second generation (Blount et al., 2016). Even though early-planted beans have a longer period in the field and serve as host to more kudzu bugs than later-planted beans, soybean yield can be reduced as planting dates are delayed after May (Blount et al., 2016). Soybeans with a longer growing season (April and May planted beans) generally have higher yield potential than those planted later (June to July planting) (Blount et al., 2016). Although kudzu bug infestations in soybeans decrease in the later-planted fields, later planting does not guarantee a higher overall yield (Blount et al., 2016). Planting soybeans early in the season typically improves yield potential, but insecticides may have to be applied to control kudzu bugs (Blount et al., 2016). Later-planted soybeans will most likely avoid insecticidal treatments for kudzu bug, but yield potential will generally be less, and later-planted soybean are more prone to other insect pests, such as stink bug and soybean looper (utcrops.com). Maturity group and environmental conditions are also factors that must be considered. Farmers will have to consider all aspects when deciding when to plant beans.

Preliminary studies of the kudzu bug in Knox County in 2014 led to questions about other plant species that may serve as hosts. Most of the documented host plants of kudzu bug are members of the legume family (Fabaceae), but some are non-leguminous (Eger et al., 2012). Although kudzu bugs have been observed on many leguminous and non-leguminous species, few actually serve as reproductive hosts. In other words, the complete life cycle (egg to adult) can be completed only on a few plant species.

Kudzu and kudzu bug are both classic examples of invasive species. Both species are not native to the United States, have a tendency to spread widely and rapidly, and cause great harm to the environment and human economy. Kudzu is present in such large quantities today that it is impossible to eradicate, however, managing this plant is possible if proper measures are taken. Kudzu bug is a newer invader. If proper studies are designed and conducted to learn more about its general habits and life cycle, effective management strategies will be developed for it as well.

Research Objectives

Research on the basic life history characteristics of the kudzu bug in the southeastern United States and knowledge about this pest in Tennessee is extremely limited. Thus, a two-year research project was initiated in 2014 to provide additional information on the kudzu bug to enhance long-term management efforts. The specific research objectives of this project are to:

1) Assess the ecology of the kudzu bug in Tennessee, including, but not limited to, seasonality, phenology, and possible alternate host plant associations,
2) Determine behavioral responses of the kudzu bug to host substrates, including kudzu, soybeans, and possible alternate host plants (common ragweed, *Ambrosia artemisiifolia*

[Asterales: Asteraceae], and bush honeysuckle, *Lonicera maackii* [Dipsacales: Caprifoliaceae]), and

3) Examine the relationship between the kudzu bug and potential biological control agents, including the egg parasitoid, *P. saccharalis*, and the entomopathogenic fungus, *B. bassiana*.

CHAPTER II
SEASONALITY AND PHENOLOGY OF KUDZU BUG IN EASTERN TENNESSEE

Introduction

The kudzu bug, *Megacopta cribraria* (Fabricius), is an invasive insect that has been present in the United States since Fall 2009 after appearing in residential areas in several counties in northern Georgia (Gardner et al., 2013b). Researchers have hypothesized about the seasonality and population density fluctuations of kudzu bug, but no research has been conducted to accurately assess these fluctuations on a weekly basis. In addition, the kudzu bug has only been present in Tennessee since 2012. Even if data on seasonality and phenology were present for other states, it is still important to examine these same biological characteristics in Tennessee before proper solutions for pest management can be determined and implemented in the state. Climatic and topographic differences will also have an influence on kudzu bug phenology and seasonality.

Therefore, a study was designed to assess the seasonality and phenology of the kudzu bug in Tennessee. Questions to be answered in this study included: What is the phenology and seasonality of the kudzu bug in eastern Tennessee – when does it first emerge, when is each life stage found, what differences in population densities occur, and when do seasonal peaks in densities of each life stage occur?

Materials and Methods

To obtain data for this entire study, several stages (eggs, nymphal kudzu bugs, and adult kudzu bugs) of field-collected specimens were counted and examined closely. Identification and separation of nymphal stages (one through five) were determined from images found at www.kudzubug.org. Egg masses were collected from the field, and eggs per mass were counted in the laboratory. Weekly sampling in 2014 was conducted only in Knox County, Tennessee. In 2015, five additional counties were added to assess differences among latitudes.

Assessment of Kudzu Bugs (Immatures and Adults)

To determine seasonality and population density of kudzu bug, multiple sites were visited within Knox County, Tennessee in 2014 and 2015 (Table 1, Fig. 5). Three locations (north, south, and west regions of Knox County) were visited with two individual sites established at each location in 2014. The number of sites was expanded to three at each location in 2015 to provide a greater understanding of phenology (Table 2). In addition to these nine Knox County sites, five sites outside of Knox County (Blount, Monroe, McMinn, and Polk Counties, Tennessee and Murray County, Georgia [one site per county]) were sampled in 2015 (Table 2). These five sites were established to determine if differences in population levels occurred across small-scale latitudinal gradients. Altogether, 14 sites in six counties were sampled to assess seasonality and phenology of kudzu bug in 2015 (Fig. 5).

Weekly sampling began on 22 July 2014 and on 18 May 2015 to monitor population fluctuations (Table 2). In 2015, kudzu sites were visited in spring prior to the beginning of sampling to observe for presence of kudzu bugs. Once kudzu plants were fully green and thriving and kudzu bugs began to move back into kudzu, weekly sampling was initiated. Sampling continued in both years until kudzu bugs were no longer present in kudzu patches. Sampling was terminated on 7 November 2014 and 16 November 2015. Sampling during 2014 began once bugs were already present in kudzu patches. Overall seasonality of kudzu bug was

Table 1. Sites regularly visited to assess kudzu bug population density in kudzu in eastern Tennessee, 2014 and 2015.

Site	Latitude / Longitude	Years Sampled
KnoxSRT (South, River Towne Apartments)	N 35.942995 W 83.926313	2015
KnoxSFD (South, Fort Dickerson Park)	N 35.945703 W 83.916920	2015
KnoxSBB (South, Bayou Bay Restaurant)	N 35.96883 W 83.82430	2014 2015
KnoxWAU (West, Aubrey's Restaurant)	N 35.942584 W 84.095780	2014 2015
KnoxWBC (West, Ball Camp Baptist Church)	N 35.95657 W 84.12556	2014 2015
KnoxWCO (West, Country Oaks Apartments)	N 35.951081 W 83.991177	2015
KnoxNLA (North, Lambert Family Property)	N 36.00985 W 84.03224	2015
KnoxNUT (North, UT Medical Plaza Halls)	N 36.069874 W 83.926533	2014 2015
KnoxNHS (North, Halls Senior Center)	N 36.076025 W 83.918947	2014 2015
Blount County	N 35.74912 W 83.96170	2015
Monroe County	N 35.56232 W 84.29740	2015
McMinn County	N 35.28909 W 84.54333	2015
Polk County	N 35.16464 W 84.68258	2015
Murray County (GA)	N 34.95133 W 84.73602	2015

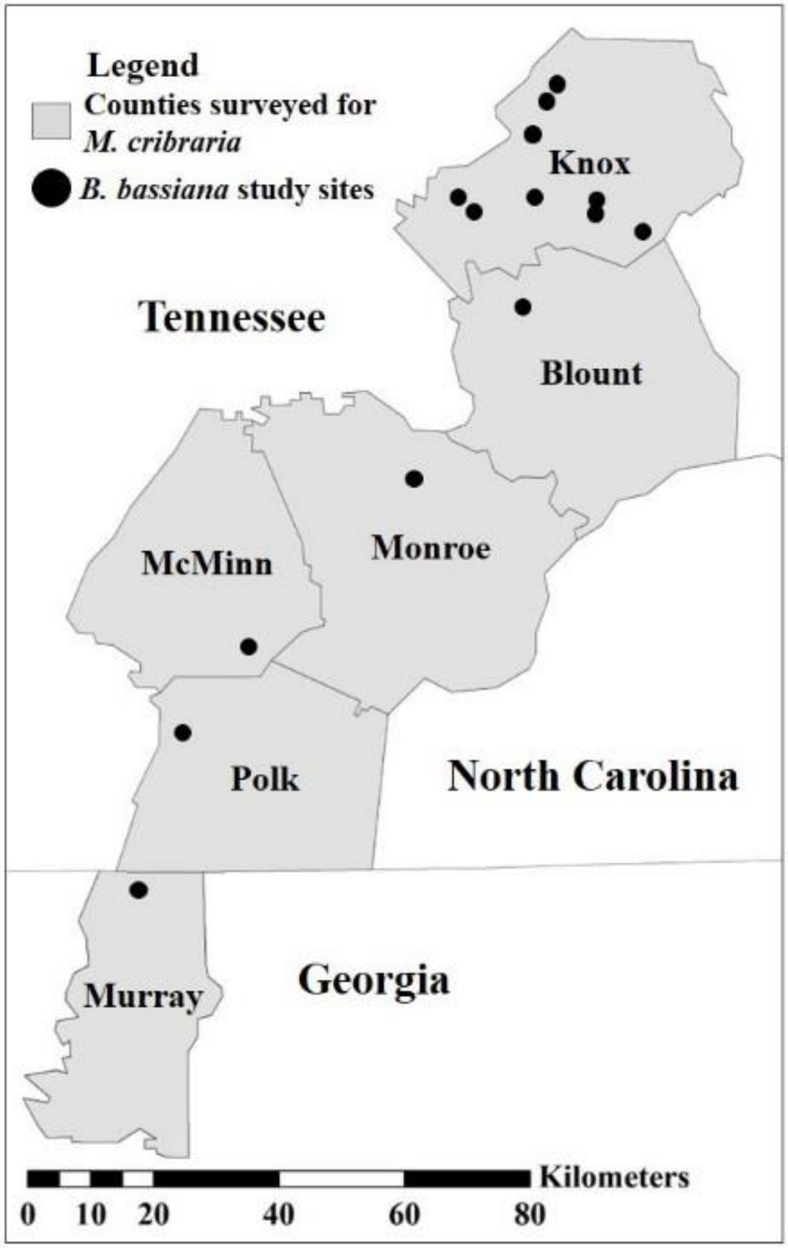

Figure 5. Map of counties visited to assess kudzu bug phenology and seasonality, 2015.

Table 2. Sampling dates to assess kudzu bug population density in kudzu in eastern Tennessee, 2015.

Week, 2014	**Sampling Date**	**Week, 2015**	**Sampling Date**
		1	18 May
		2	25 May
		3	4 June
		4	9 June
		5	15 June
		6	22 June
		7	29 June
		8	6 July
		9	13 July
1	22 July	10	20 July
2	29 July	11	27 July
3	5 August	12	6 August
4	12 August	13	12 August
5	19 August	14	18 August
6	26 August	15	24 August
7	2 September	16	31 August
8	9 September	17	7 September
9	16 September	18	14 September
10	23 September	19	21 September
11	30 September	20	28 September
12	7 October	21	5 October
13	14 October	22	12 October
14	21 October	23	19 October
		24	26 October
		25	2 November
		26	9 November
		27	16 November

determined from the onset of emergence to overwintering. Upon visiting a kudzu site, five sweep-net samples were made where each sample consisted of five sweeps of the net through kudzu foliage using a standard sweep net (38 cm in diameter). Therefore, 25 total sweeps were conducted in kudzu at each site. After each sweep-net sample was taken, contents of the net were placed into 3.8 liter resealable plastic bags and transported to the laboratory. This procedure ensured accurate counts of all life stages of kudzu bugs. In the laboratory, samples were poured onto a plastic white plate (approximately 30 cm diameter). Kudzu bugs were separated by life stage (nymphal stages one to five and adults). Kudzu bug adults were further separated into groups of either male or female. Once kudzu bugs were separated to their appropriate life stage and/or sex, they were counted and the numbers were recorded. Data recorded included the number of kudzu bugs of each life stage per sweep-net sample. Data were summarized as the average number of bugs (by county and across all sites) of each life stage per sweep-net sample per week.

Assessment of Kudzu Bugs (Eggs)

To gain a better understanding of the temporal incidence of oviposition by kudzu bugs in the field, as well as the number of egg masses and eggs laid per mass throughout the sampling season, eggs were collected as often as they were observed in the field. Assessment of eggs occurred only in 2015, when eggs were present in the field from 18 May to 17 September. The method of collecting eggs upon visiting a kudzu patch was to thoroughly examine kudzu foliage and collect the first 10 egg masses found. Eggs were typically laid on terminal ends of kudzu vines, so they were easily detached to bring into the laboratory. Other sites of oviposition included upper and lower surfaces of kudzu leaves. When eggs were collected, they were placed into a 100 mm petri dish with all eggs from the same site in the same dish. Petri dishes were labeled, closed, and sealed with parafilm. After transportation to the laboratory, petri dishes were stored in the laboratory at room temperature (approximately 22.2-23.3^{o} C) for an extended period of time to ensure that eggs had adequate time to hatch (if eggs were viable). Egg masses remained completely undisturbed during this time. No moist filter paper or moisture of any kind was added to petri dishes to prevent mold formation. After approximately three months, egg masses were examined. Data recorded included number of eggs per mass and percent of eggs hatched per mass. Data were summarized as the average number of eggs laid per mass per week (across all sites) and the average percent of eggs hatched per week.

Results and Discussion

Assessment of Kudzu Bugs (Immatures and Adults)

Kudzu Bugs in Knox County, 2014

Kudzu bug populations were sampled for 14 weeks in 2014 with general population fluctuations documented throughout the season. Kudzu bugs were found from 22 July to 21 October 2014 (Fig. 6). No pronounced population density trends were evident during the season in nymphal stages one through four. This lack of obvious trends could be attributed to

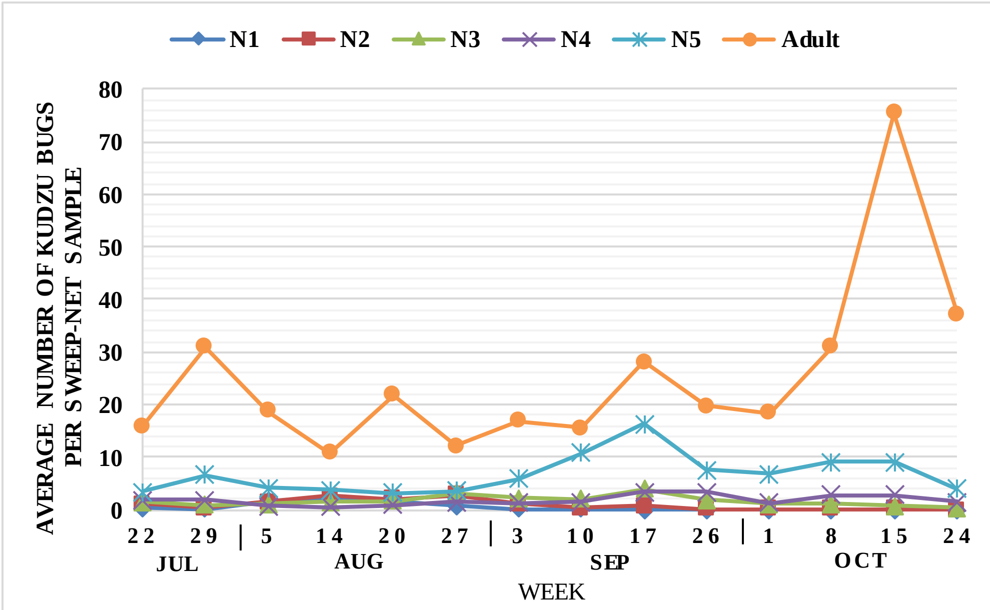

Figure 6. Average number of kudzu bugs per sweep-net sample per sampling week, Knox County, 2014.

several reasons. First, these four instars are small (2-4 mm) which may make it difficult for them to be captured in large numbers, or consistently, in a sweep net. Second, they may inhabit areas lower in the kudzu canopy that may not always be easily accessible with a sweep net. When in the field, it may seem somewhat misleading to collect smaller numbers of these lower instar nymphs since they should be abundant on foliage, especially when seeing large numbers of hatched/hatching egg masses are observed.

In contrast to nymphal stages one through four, last instar kudzu bugs exhibited pronounced trends in population densities throughout the season (Fig. 6). Population densities of stage five nymphs peaked on 17 September 2014 (week 9), which was approximately one month before the highest peak in population densities of adults on 14 October (week 13) (Fig. 6). After the peak in stage five nymphs, population densities declined for two weeks, leveled for two weeks, and declined at the end of the season.

Adult population densities in 2014 were much different than those of nymphs (Fig. 6). Throughout most of the sampling season, adult population densities were consistent (weeks 1-12; 22 July through 7 October), with a slight increase in numbers on 29 July (week 2) and 15 October 2014 (week 13). This population increase can most likely be explained by the maturation of the first and second generations of kudzu bugs. The second peak in population densities was much larger than the first. This difference in population density peaks can be explained due to two specific events. The first relates to soybean maturing and harvesting. In eastern Tennessee, soybean harvest time is typically from late September to late October. Soybeans are the most suitable alternate host for survival and development of kudzu bug. Typically, some adult kudzu bugs at the end of the first generation will move into soybean as soon as the plants become attractive; the exact reason for this movement is currently undetermined, however, it is believed to result from the plant's growth and maturation from vegetative stage to reproductive stage. Since soybeans typically mature before kudzu is killed by frost, a large number of kudzu bugs would be expected to return to kudzu with the maturation of soybean, as well as the maturation of kudzu bug in soybean fields. The presence of soybean fields near kudzu patches increases the volume of reproductive host plant material available to kudzu bugs, thereby contributing to larger population densities overall. The second possible reason for the movement of kudzu bugs back into kudzu is the occurrence of a freezing temperature event. The first freeze occurred on 4 October (between weeks 11 and 12) during the 2014 sampling season. For the two following sampling periods (7 and 14 October; weeks 12 and 13), population densities of kudzu bug in kudzu dramatically increased while those in soybean decreased. Although a large number of kudzu bugs were collected in kudzu on the last sampling date (21 October; week 14; the third collection after the freeze event) their densities had declined from the earlier peak that occurred (14 October; week 13) after the freeze event. The influx of kudzu bugs back into kudzu patches is most likely because kudzu provides a suitable environment that can also provide sufficient food before kudzu bugs begin overwintering.

Kudzu Bugs in Knox County, 2015

Sampling of kudzu bug populations began much earlier in 2015 than in 2014, which provided a season-long assessment (27 weeks) of kudzu bug population density fluctuations (Table 2, Fig. 7). Few kudzu bugs were found on kudzu during the first six weeks of sampling (18 May to 22 June) as they moved into kudzu from overwintering sites. Beginning with week six (22 June), nymphal kudzu bugs were collected, indicating that oviposition and egg hatch had begun. Starting at week seven (29 June), adult densities began to increase. Since eggs can be laid as soon as kudzu bug adults inhabit kudzu patches, the increase in adult population densities is consistent with the observation of Ruberson et al. (2012) that development from a hatched nymph to an adult requires four to six weeks. For the next four weeks (weeks 8-11; 6 July to 27 July), adult population densities consistently increased until reaching a peak, which is most likely a result of the development of the first generation of hatched insects. Once this peak in adults occurred, large numbers of adult kudzu bugs left kudzu patches and moved elsewhere for a portion of the season (most likely to soybeans, or potentially other suitable alternative host plants). The movement was probably influenced by high population densities in kudzu or the

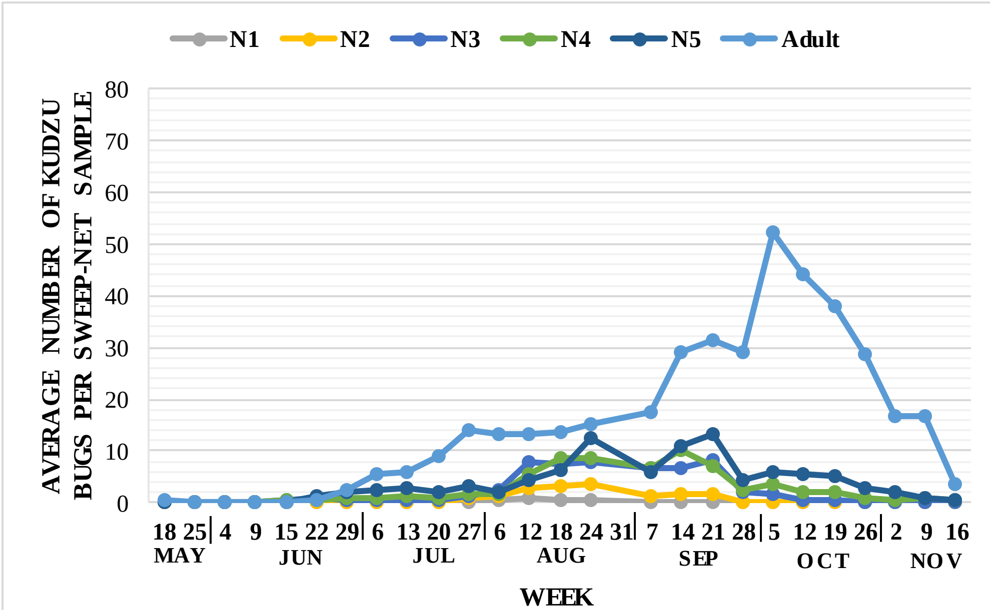

Figure 7. Average number of kudzu bugs per sweep-net sample per sampling week, Knox County, 2015.

availability of other host plants. Because there are not many soybean fields in Knox County, kudzu bugs may be moving onto other alternate host plants.

After the initial peak (week 11; 27 July), population densities plateaued for three weeks (weeks 12 through 14; 6 to 18 August) (Fig. 7). Four weeks later, populations peaked again (week 18; 14 September). These fluctuations of population densities are consistent with the expected development time from hatch to adult (Ruberson et al., 2012). This peak signifies the end of the second generation of adult kudzu bugs. Once these two peaks in adult densities occurred (weeks 11 and 18; 27 July and 14 September), high numbers of kudzu bugs were collected. Population densities were consistent for one week (week 19; 21 September) and then increased for two weeks (weeks 20 and 21; 28 September to 5 October). Three weeks after the second adult population peak, the largest peak in adults was recorded (week 21; 5 October). This third peak was most likely due to two factors. The first was that temperatures were consistently declining, possibly causing a late-season peak in adult densities, as kudzu bugs moved from other plants (probably soybean) to kudzu patches while plants were still viable enough to provide suitable amounts of food to obtain sufficient nutrition before overwintering. The first freezing temperature event occurred within two weeks of the highest population peak (between weeks 22 and 23; between 12 and 19 October). One week after the peak (week 22; 12 October), population density levels declined. Collections on 19 October (week 23) were somewhat different from the previous collections on 12 October (week 22) (approximately 25-30 bugs collected as compared to 35-40 the previous week and ~40 at peak). Freezing temperatures appeared to impact population densities. In addition, some kudzu bugs were probably also relocating to suitable overwintering locations. The second contributing factor to high population densities in kudzu bug late in the season is soybean maturation and harvest in eastern Tennessee. Soybean harvesting typically occurs from mid-September to late October (weeks 19 to 24 during the 2015 sampling period) (Fig. 7). Thus, the highest population peak in kudzu would be expected when populations increase on soybeans, and then return to kudzu because soybean is no longer a suitable host.

An additional interesting observation is that densities of stage five nymphs were similar to those of adults from 24 to 31 August (weeks 15 and 16). This similarity could be attributed in part to the movement of adults away from kudzu patches after the end of the first generation of kudzu bugs. Adults are more mobile than nymphal stages and are more likely to relocate to a more suitable habitat for growth and development. Because nymphs are not able to move easily from their habitat, their population densities could reach similar levels in kudzu patches than adult kudzu bugs.

Comparisons of Adults in Knox County 2014 and 2015

The same trend in adult densities during peak weeks is evident throughout both sampling seasons (27 July 2014 and 27 July 2015) in Knox County (Fig. 8). Initial peaks were found at week 11 (27 July) with slight fluctuations until a second, somewhat less pronounced, peak at 14 September 2014 and 21 September 2015. During both years, population densities decreased slightly before increasing to their highest peaks. In 2014, the highest peak was on 12 October and was about 50% larger than the peak in 2015 that occurred on 5 October (peaks of ~75 adult kudzu bugs per sweep-net sample in 2014 and at 55 kudzu bugs per sweep-net sample in 2015). The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Clavicipitaceae) (see Chapter IV) was prevalent during late season in 2015 and most likely a contributing factor to the late-season decrease in population densities in 2015.

Comparisons of Nymphs, Knox County, 2014 and 2015

In 2015, population density trends can be seen in certain nymphal stages (Fig. 7), unlike 2014, where nymphal kudzu bug numbers were never great enough to yield any visible trends in population densities (Fig. 6). Stage one nymphs were not collected in high enough densities in 2014 or 2015 to express seasonal trends. As stated earlier, low numbers of smaller stage nymphs (nymphal stages one through four) may be due to the fact that sometimes it is harder to collect insects of such a small size when collecting with a sweep net. In 2015, stage two nymphs had a slight increase in population density from 12 to 24 August (weeks 13, 14, and 15). After this time, densities slightly declined and stage two nymphs were no longer present after 28

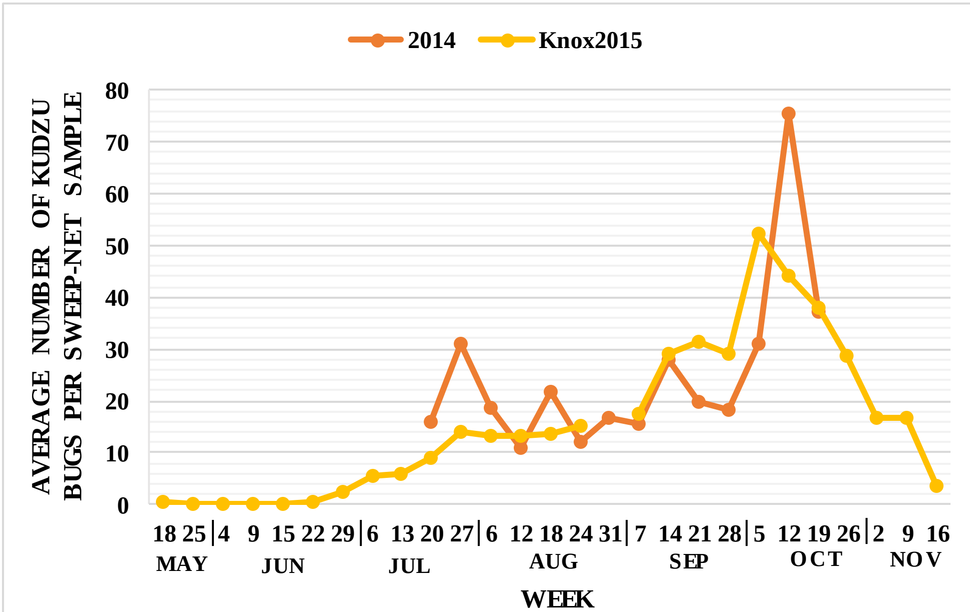

Figure 8. Average number of adult kudzu bugs per sweep-net sample per week in Knox County, 2014 and 2015.

September (week 20). Stage three nymphs had a seven-week plateau from 12 August to 21 September (weeks 13 to 19), after which they decreased consistently until sampling ended. Densities of stage four nymphs increased around 12 August (week 13) for two weeks (August 18 to 24), decreased for one week (7 September; week 17), increased again (14 September; week 18), and consistently declined for the remainder of the season. Stage five nymphs, which were the most prevalent nymphal stage collected, were first found on 22 June (week 6) and were consistently collected (around 3 kudzu bugs per sweep-net sample) until 12 August (week 13) when densities steadily increased for three weeks. Densities then declined for one week (18 August; week 14), increased for two (24 and 31 August; weeks 15 and 16), and declined consistently for the duration of the sampling season. Stage five nymphs are the most expected nymphal stage to show population trends since they are largest in size and easiest to collect in a sweep net.

Comparisons of Knox County 2014 and All Counties Overall 2015

The average number of adult kudzu bugs in sweep-net samples in Knox County (average of the six regularly sampled sites) in 2014 are compared with the overall averages of adult kudzu bugs per sweep-net sample across all regularly visited sites (14 sampled sites in six counties) in 2015 in Figure 9. The timing of population density peaks were similar both years, but the peak population density was higher in 2015.

The latter one-third of the 2015 sampling season yielded an unexpected result in kudzu patches. On 1 September 2015, the entomopathogenic fungus *B. bassiana* was found infecting and killing kudzu bugs at several of the sampling sites. As was previously discussed, soybean

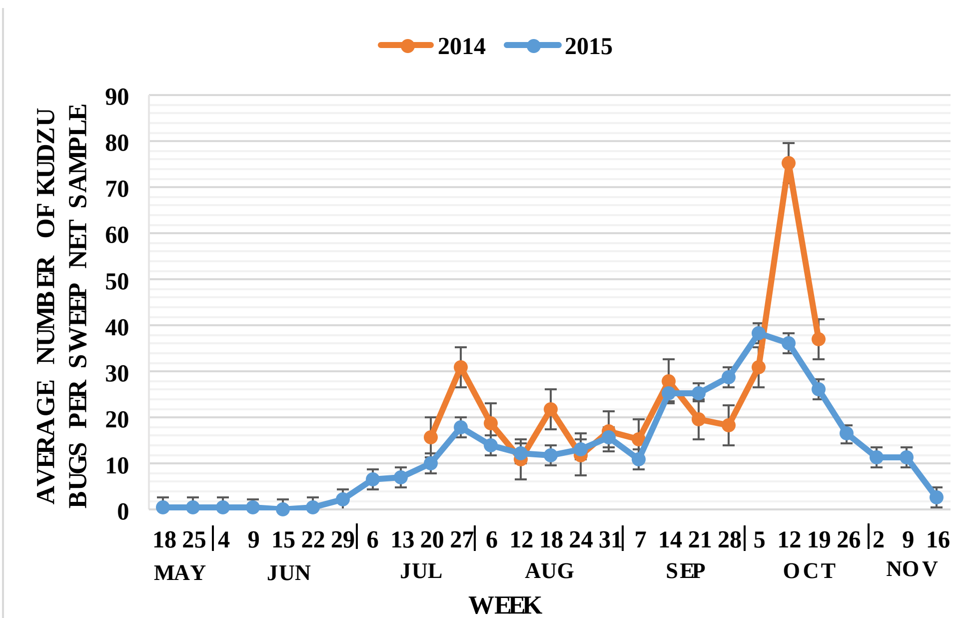

Figure 9. Average number of adult kudzu bug per sweep-net sample per week in Knox County 2014 and all 14 sites, 2015.

maturation and harvest in eastern Tennessee typically occurs from 21 September to 26 October (week 19 to 24). The highest peaks during both seasons were found during this time period. However, the presence of *B. bassiana* seems to be a reason for the lower population densities at the time of highest peak in 2015. Another distinct difference between the two sampling seasons was the final sampling date. The first freezing event of both seasons occurred around the same time period (between 12 and 19 October), but in 2014, bugs were not present in the field long after this event. In 2015, freezing temperatures occurred and then densities of kudzu bugs slowly declined over 4 weeks. Kudzu also remained viable for a longer period after the freezing temperature event in 2015 than in 2014.

Comparisons of Adult Populations by County, 2015

Adult population densities in Murray and Blount Counties were higher than in the other counties (Fig. 10). Densities peaked in Murray County on 27 July (week 11) and again on 7 September (week 17), but this latter peak was 50% lower than the earlier peak. Densities in Blount County follow the general trends of those discussed earlier for Knox County (Figs. 8 and 9), however, the highest peak is approximately 50% greater than the next highest peak, which was found in Murray County on 27 July (week 11). This large increase in kudzu bug adult density is greater than any other peak density in 2014 or 2015.

All counties had similar population densities until 6 July (week 8), at which time population levels increased. All counties followed a similar trend of low population densities at the beginning of the season, and a consistent decline in population densities after reaching peak densities. However, all counties have distinctly different dates of peak population densities. This difference could be due, in part, because these counties are along different latitudinal gradients.

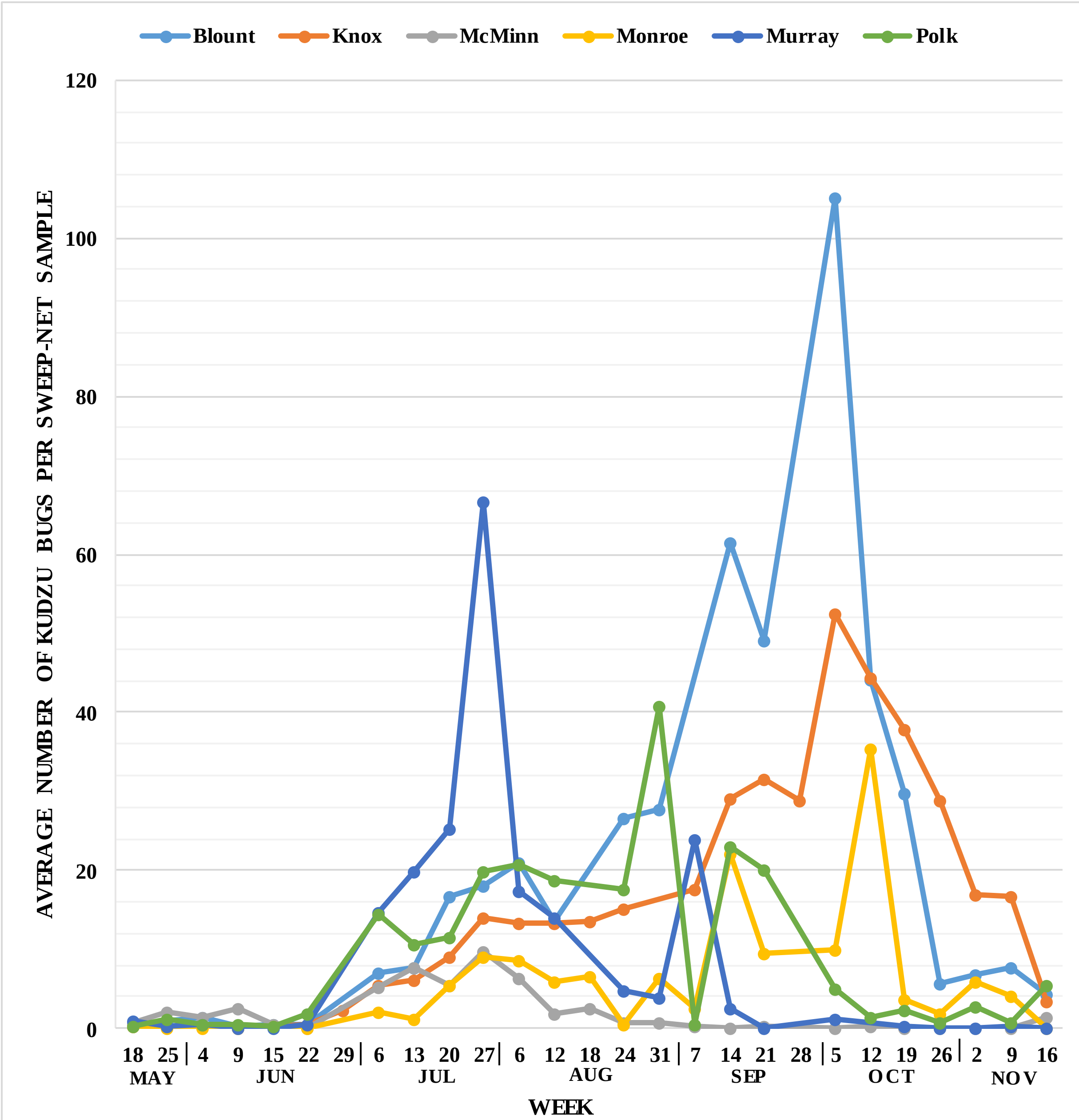

Figure 10. Average number of adult kudzu bugs per sweep-net sample per week per county, 2015.

Population densities could be influenced by areas surrounding the different kudzu patches. All sites in Knox and Blount Counties were in urban areas, and it would be unlikely for soybean to influence population densities in kudzu at these sites. Sites in Monroe, McMinn, Polk, and Murray Counties, however, were located in rural areas, where soybean had a higher likelihood to influence population density of kudzu bugs. In kudzu patches in Blount, Polk, and Murray Counties, the effects of *B. bassiana* (see Chapter IV) were much more noticeable than in the other three counties.

Comparisons of Population Densities across Counties Outside of Knox County, 2015

While kudzu bug population densities in Knox County in 2015 (Fig. 7) had trends similar to the overall average kudzu bug population densities in 2015 (Fig. 11), this trend was not seen at the other sampling sites in counties outside of Knox County. For example, the highest level of adult population densities (approximately 110 adults per sweep-net sample) was found in Blount County (Fig. 10), where the peak in adult densities almost doubled the next highest county peak (approximately 65 in Murray County) (Fig. 10). Interestingly, population densities peaked at different weeks in all counties throughout the sampling season, with the exception of Murray and McMinn Counties, which both peaked on 27 July (week 11). Population density peaks were less pronounced in Blount County throughout the sampling season. In Blount County, from August 24 to 31 (weeks 15 and 16), densities of stage five nymphs were higher than densities of adults from August 24 to 31 (Fig. 12). In addition, from 12 to 31 August (weeks 13 through 16), densities of stage three nymphs were higher than adult densities. From 12 to 24 August (weeks 13 through 15), densities of stage four nymphs were higher than adult densities. For a single sampling week (12 August; week 13), densities of stage two nymphs were higher than adults.

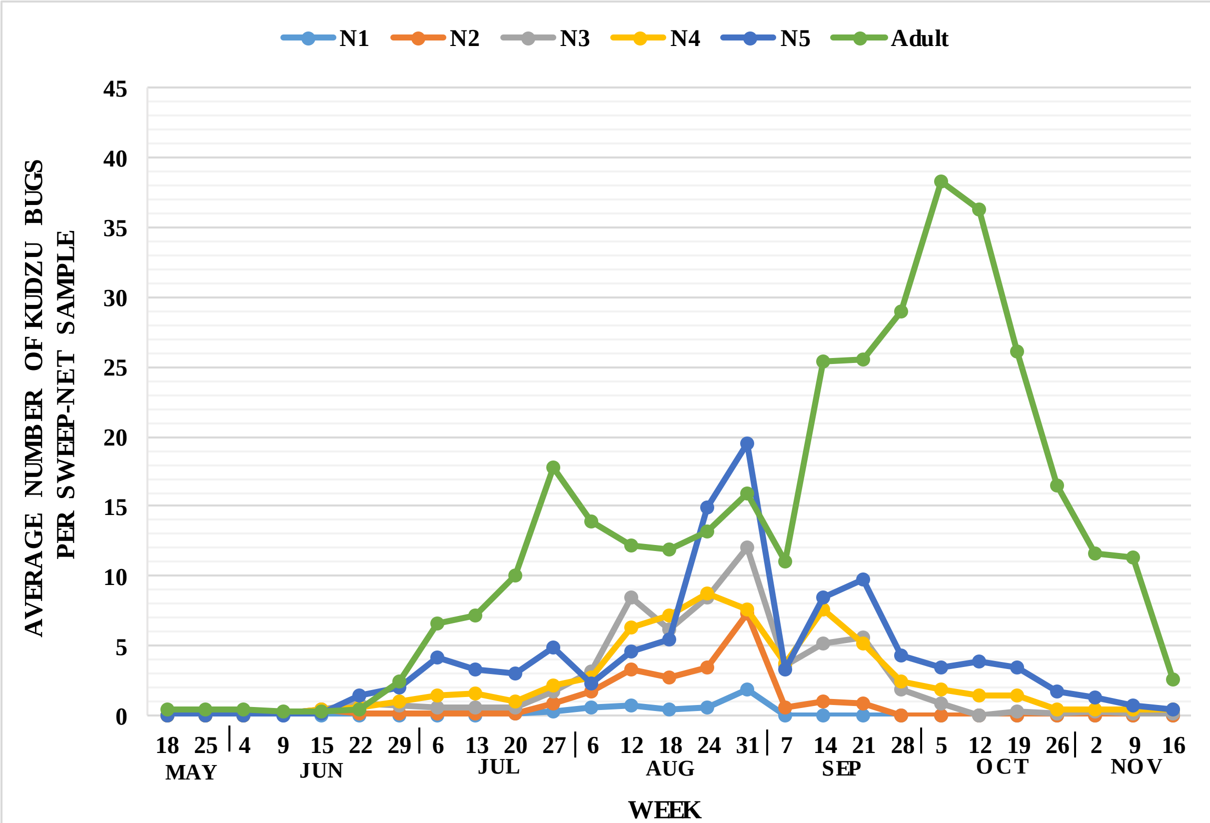

Figure 11. Average number of kudzu bugs per sweep-net sample across all 14 sites, 2015.

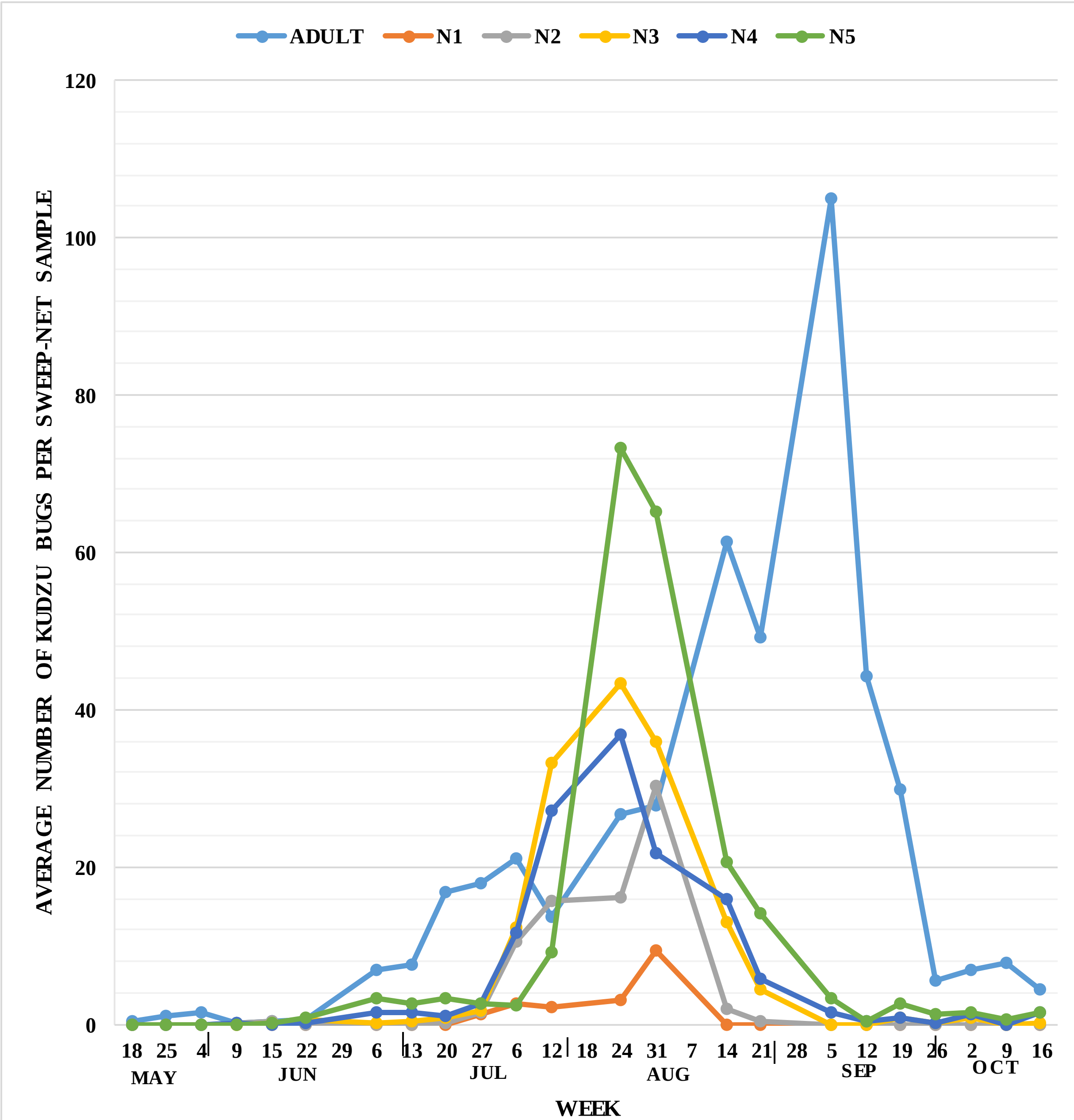

Figure 12. Average number of kudzu bugs per sweep-net sample, Blount County, 2015.

Adult densities are frequently (if not always) higher than any nymphal stage of kudzu bug. Perhaps these differences occurred during a transitional period where a large number of adult kudzu bugs were exiting kudzu patches to migrate to alternate host plants. It could have also resulted from overcrowding of adults and nymphs since this site had considerably higher densities across all life stages for the duration of the season.

Densities of adults in McMinn County exhibited trends that were different from other counties (Fig. 13). Adults initially appeared in higher densities in McMinn County than in other counties on 25 May and 9 June (weeks 2 and 4). However, population densities were noticeably lower at this site throughout the sampling season. Adult densities peaked at about 10 kudzu bugs per sweep-net sample on 27 July (week 11), which was the earliest adult peak. However, this peak density was low, especially compared to more than 100 adults per sweep-net sample in Blount County. Adult densities decreased for six weeks until remaining consistently low (0-2 adults per sweep-net sample) for the duration of the sampling season. On several occurrences (12 to 31 August; weeks 13-16), densities of nymphs were higher than those of adults.

Beauveria bassiana was present on kudzu bugs in McMinn County, but it was not as prevalent or as densely spread throughout this kudzu patch as in other counties. Therefore, *B. bassiana* is not believed to be a factor in explaining why population densities were consistently low in McMinn County.

Adult densities in Monroe County (Fig. 14) are different from overall population densities in all counties (Fig. 10). Three distinct population peaks (27 July, 14 September, and 12 October; weeks 11, 18, and 22) are evident throughout the sampling season. This trend is similar to the overall population density of adult kudzu bugs (Fig. 10). Nymphs collected from Monroe

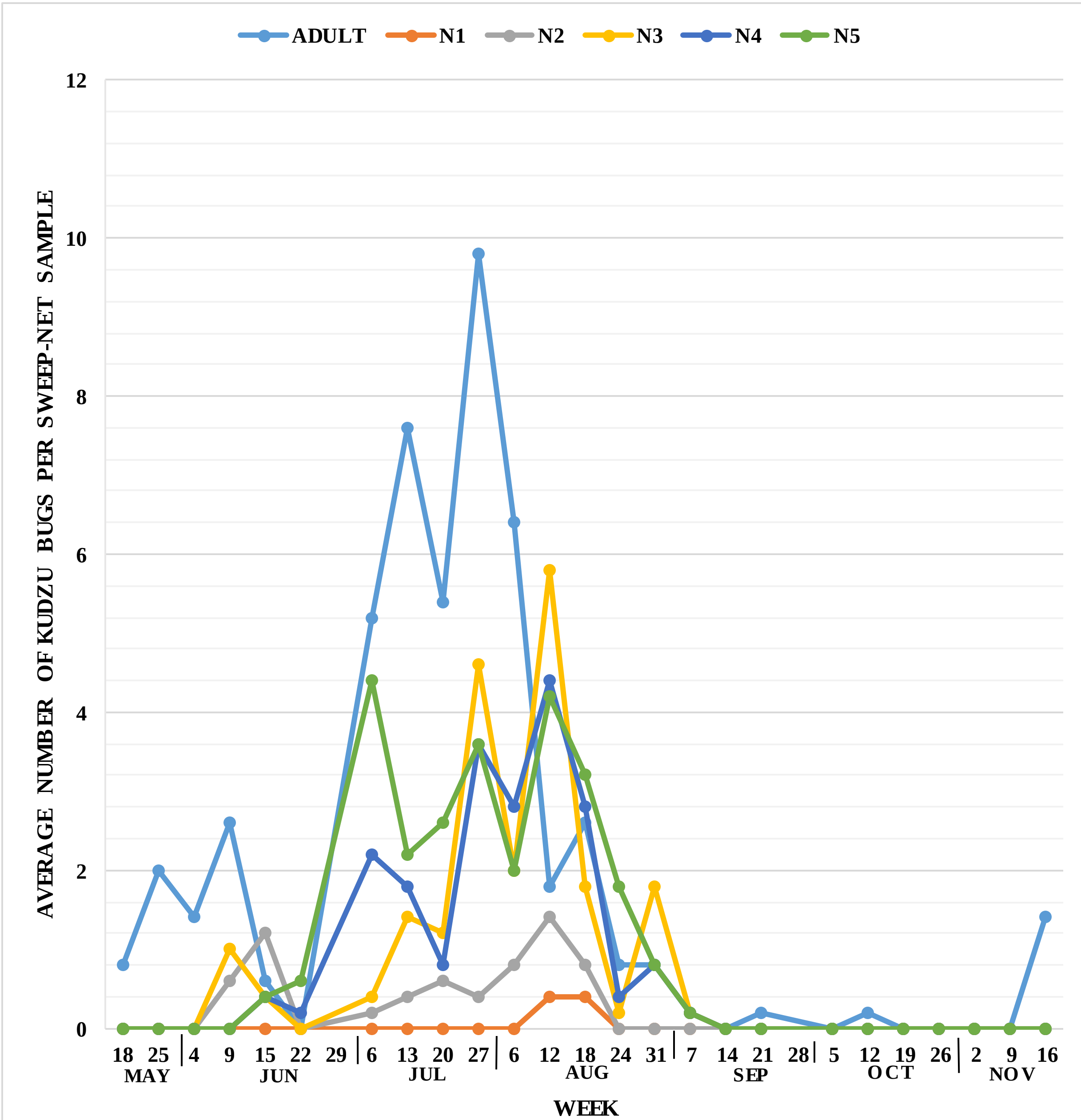

Figure 13. Average number of kudzu bugs per sweep-net sample, McMinn County, 2015.

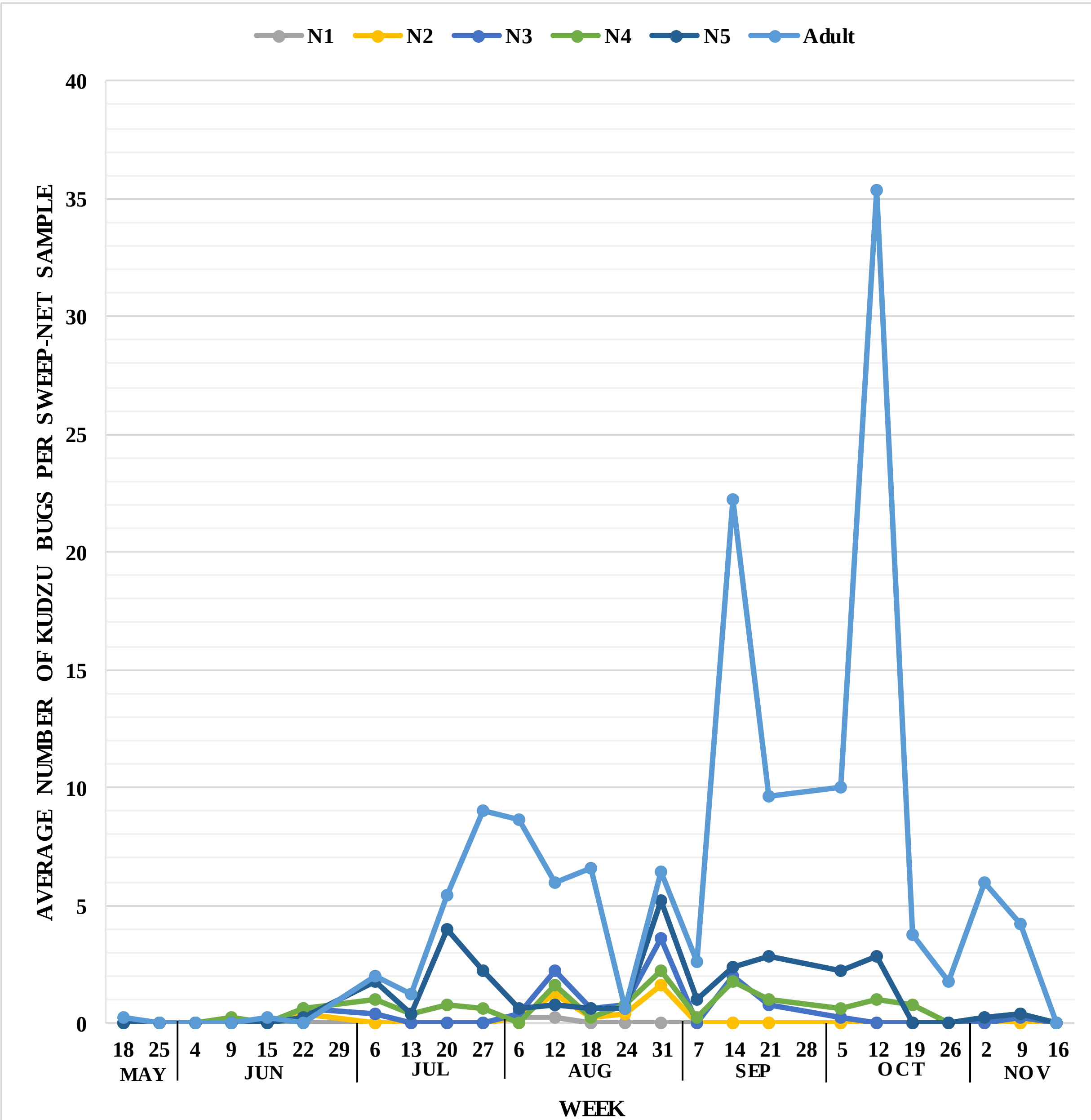

Figure 14. Average number of kudzu bugs per sweep-net sample, Monroe County, 2015.

County never reached sufficient densities to establish trends among different stages. Densities consistently remained at 5 individuals or less per sweep-net sample. An interesting occurrence is the difference in numbers of adults from 12 (36 per sweep-net sample) to 19 October (4 per sweep-net sample) (week 22 to 23). Between the two sampling dates, the first freeze event occurred in Knox County, so a similar freeze event probably occurred in the other counties. If a similar freeze event occurred in Monroe County, it would be one factor affecting the rapid decline in adult densities. Population density differences could also be influenced by agricultural practices or varieties of crops present in the vicinity of kudzu patches in different counties. *B. bassiana* was also present at this site, although it was somewhat later in the sampling season. Combined with the effects of freezing weather, this fungus could have played a large role in killing adult kudzu bugs at the end of the sampling season. In addition to adult densities dropping after week 22, nymphal kudzu bugs were low in population densities after this date. All nymphal stages decreased to near-zero after 19 October (week 23).

Kudzu bugs in Polk County (Fig. 15) exhibited a different trend in population densities than those in other counties, as well as overall averages for all counties (Fig. 11). First, kudzu bugs were almost non-existent in kudzu on 7 September (week 17), when only two nymphs and two adults were collected. Adult density peaked earlier on 31 August (week 16). Smaller peaks in adult population densities occurred prior to the highest peak, but all population densities corresponded with the Ruberson et al. (2012) observation that four to six weeks are required for nymphs to molt to adulthood. Peaks in Polk County are closer to four weeks versus six weeks or more. On 14 September (week 18), adult population densities averaged around 25 bugs per

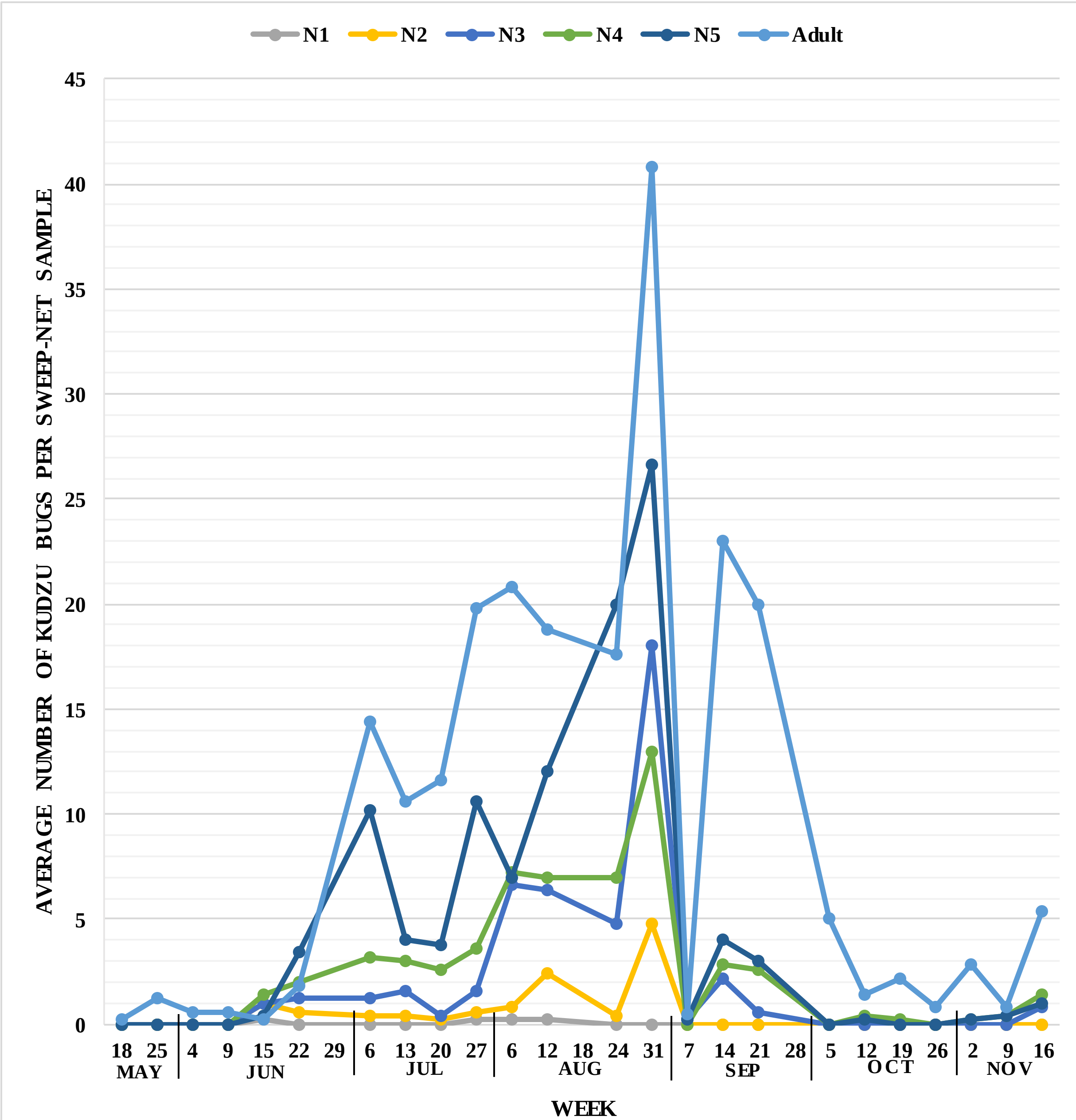

Figure 15. Average number of kudzu bugs per sweep-net sample, Polk County, 2015.

sweep-net sample. After this week, populations steadily declined until they were at or less than five for the duration of the sampling season.

From 14 September (week 18) onward, less than five individuals of each nymphal stage were found per sweep-net sample. Densities of nymphs were higher than adults on 15 and 22 June and 24 August (weeks 5, 6, and 15) but these differences were not large like at other sites. An important occurrence at Polk County, beginning on 31 August (week 16), was the presence of *B. bassiana*. As soon as the fungus was detected, adult and nymph populations declined. Even discounting the low densities on 7 September (week 17), populations still declined after the presence of the fungus was discovered.

In Murray County (Georgia), the fungus *B. bassiana* was found at high levels throughout the latter half of the sampling season (Fig. 16). All stages of kudzu bug were affected by *B. bassiana*, which was first detected on 1 September (week 16), but may have been present earlier. In this week, the fungus was detected in Polk and Murray Counties. The numbers of kudzu bugs collected per sweep-net sample in Murray County suggested that populations may have been affected prior to the initial detection of *B. bassiana*. Densities of kudzu bug in Murray County peaked at week 11, which was similar to McMinn County. Although populations peaked in these two counties about the same time, kudzu bugs in McMinn County were not as heavily infected with the fungus as those in Murray County.

Only in the beginning of the season and prior to any adult population density peaks did nymphal kudzu bugs ever appear higher than adults. These high nymphal densities are most likely due to adult kudzu bugs leaving kudzu to find alternate plants, possibly due to overcrowding in kudzu patches. This finding also supports the theory that *B. bassiana* had a

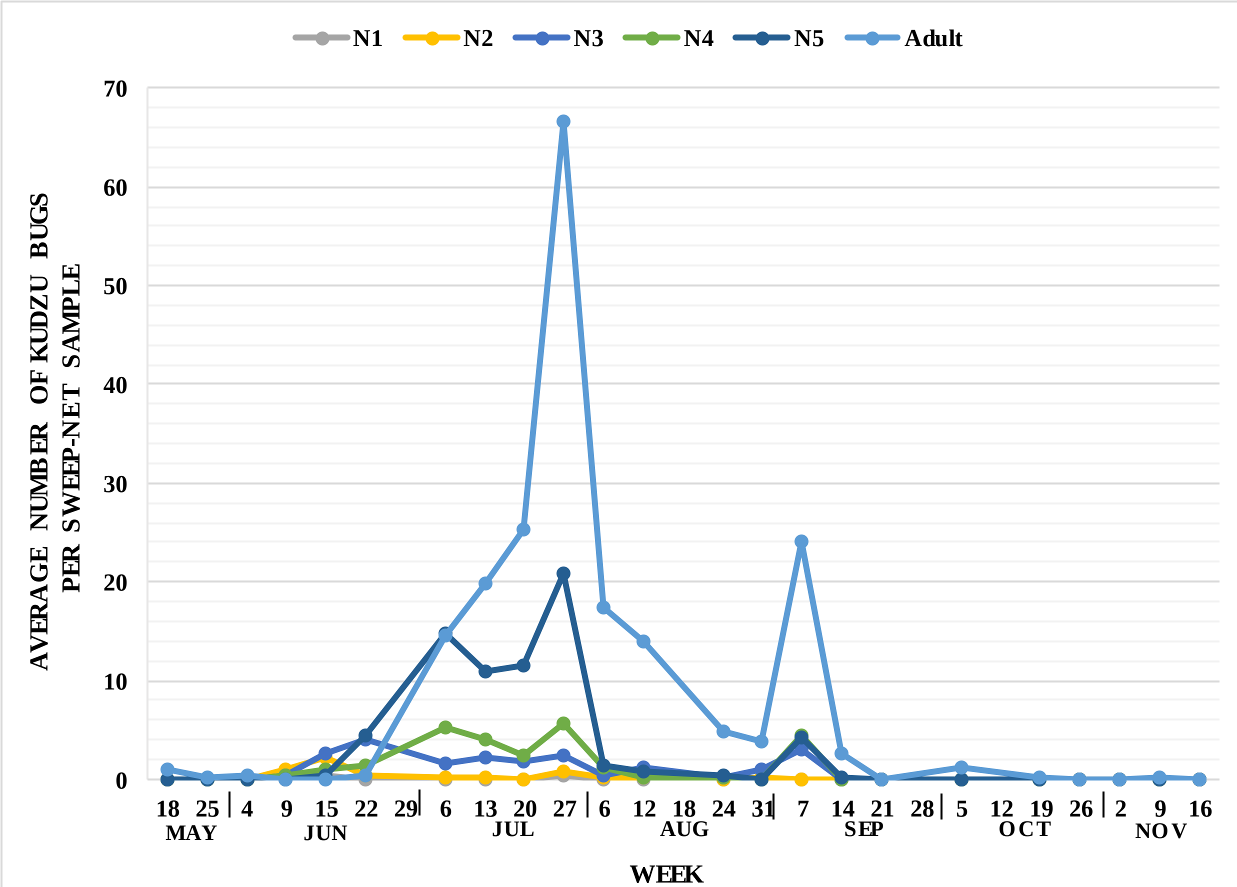

Figure 16. Average number of kudzu bugs per sweep-net sample, Murray County (GA), 2015.

great impact on kudzu bugs at this site. From a study that closely examined the impact of *B. bassiana* on densities of adult and nymph kudzu bugs, it was noted that nymphal kudzu bugs were more affected by the fungus than adults (see Chapter IV). Considering this finding, it is logical that once adult kudzu bug populations become established, their numbers would consistently surpass the population densities of any nymphal stage. It is still somewhat surprising that few kudzu bugs of any stage were collected after 14 September (week 18), even though sampling for insects continued for 27 weeks.

Stage five nymphs in Murray County had strong population densities through 6 August (week 12), which is approximately the same time that adult populations began to decline (Fig. 16). With few nymphal kudzu bugs collected, low numbers of adults would be expected. Infection of nymphs by *B. bassiana* would result in lower densities of adults in the latter portion of the season because fewer nymphs would be present to survive and molt to adulthood.

Comparisons of Population Densities of All Nymphal Stages by County, 2015

Different nymphal life stages of kudzu bug exhibited different trends in population density throughout the sampling season (Figs. 17-21). Large numbers of stage one nymphs were not expected because they are small and difficult to collect in a sweep net (Fig. 17). However, substantial numbers were collected at two sites – Monroe and Blount Counties. For stage one nymphs, trends in population densities were best delineated in Monroe County, where they did not exhibit peaks in population densities until the latter half of the season, specifically on 14 September and 12 October (weeks 18 and 22). This trend is consistent with the general trend of adult peak population densities, since adult densities were different from site to site. It is not understood why these nymphs peaked in late season instead of early season. It is understandable

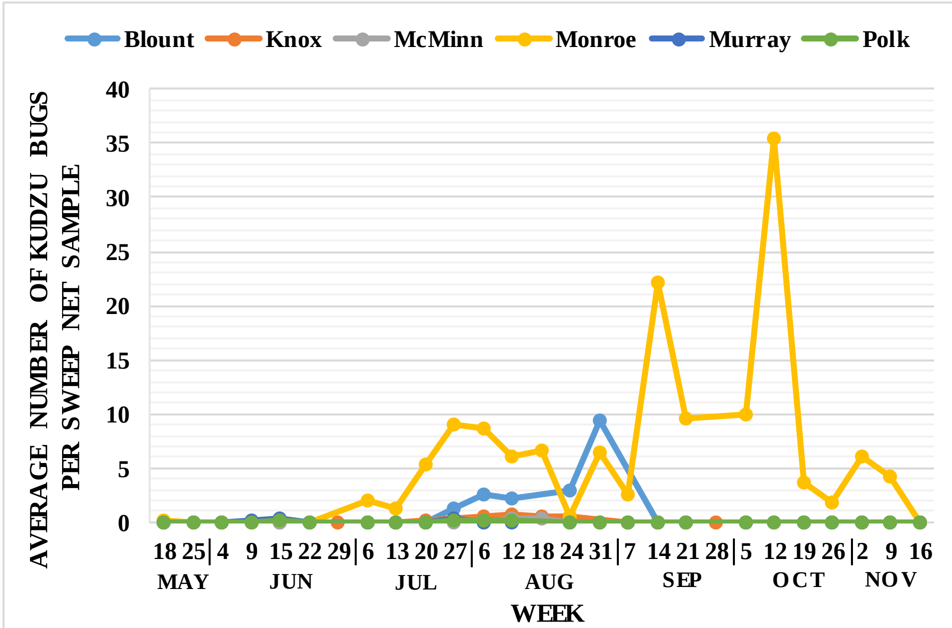

Figure 17. Average number of stage one nymphs per sweep-net sample, 2015.

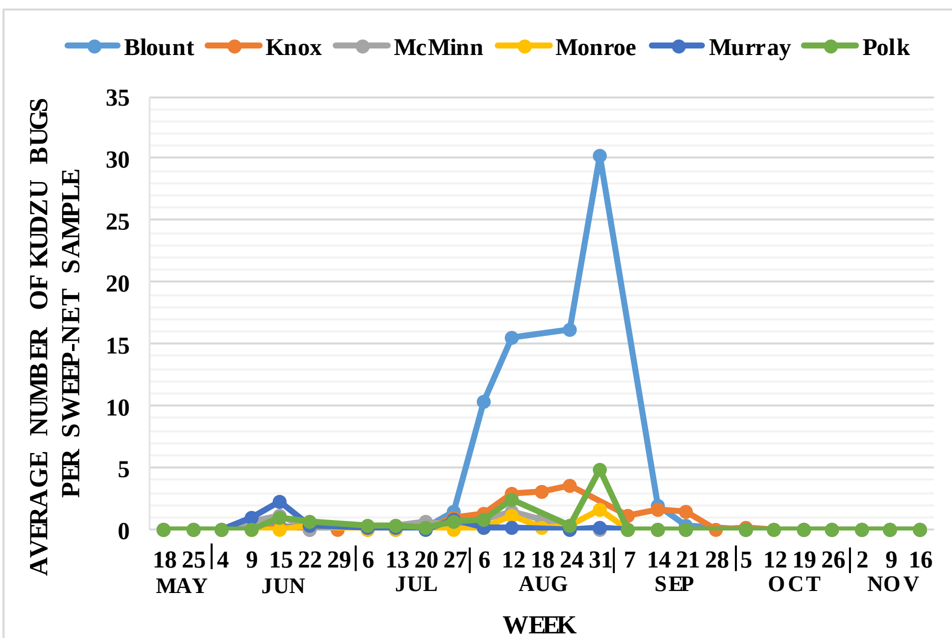

Figure 18. Average number of stage two nymphs per sweep-net sample, 2015.

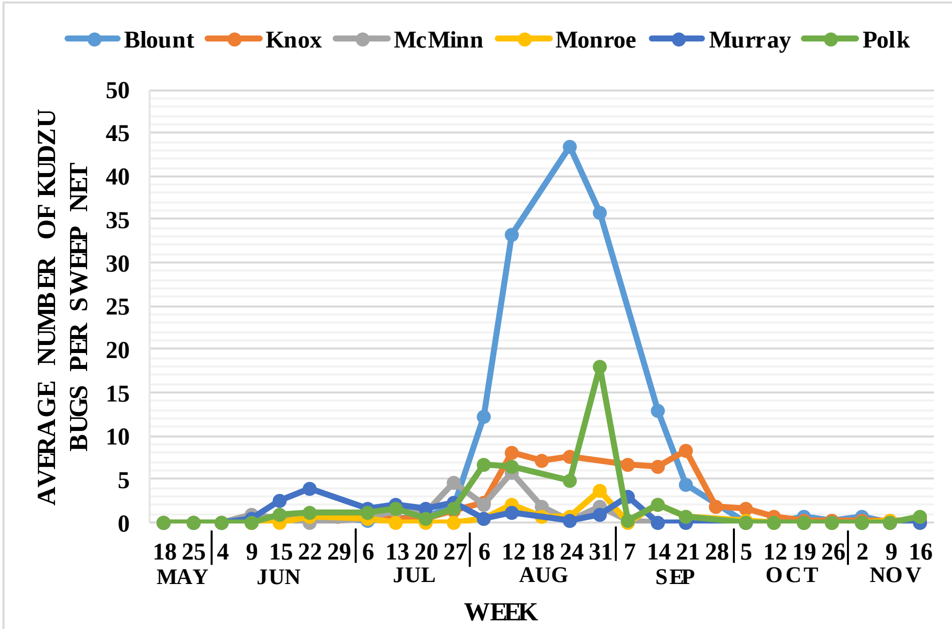

Figure 19. Average number of stage three nymphs per sweep-net sample, 2015.

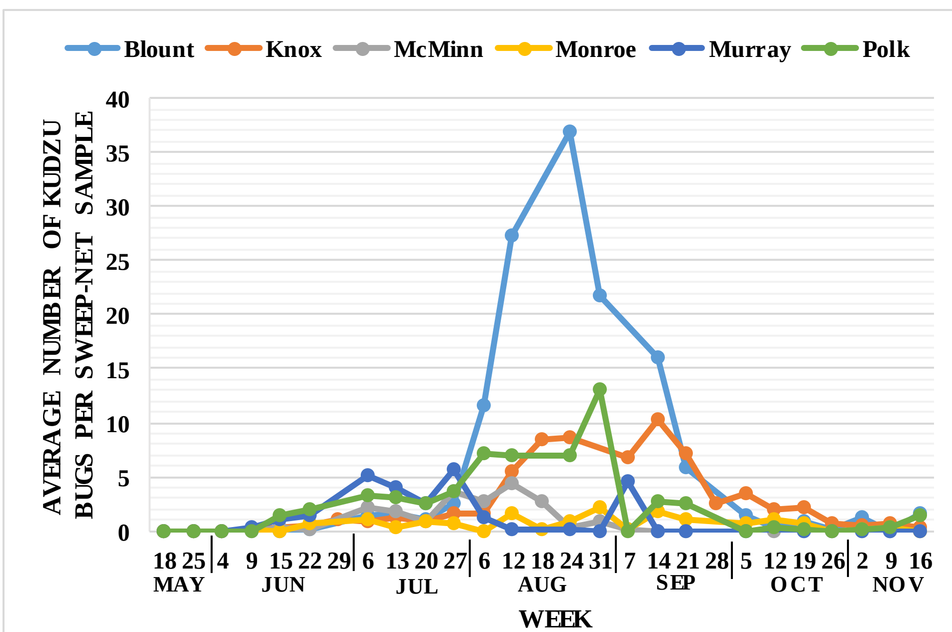

Figure 20. Average number of stage four nymphs per sweep-net sample, 2015.

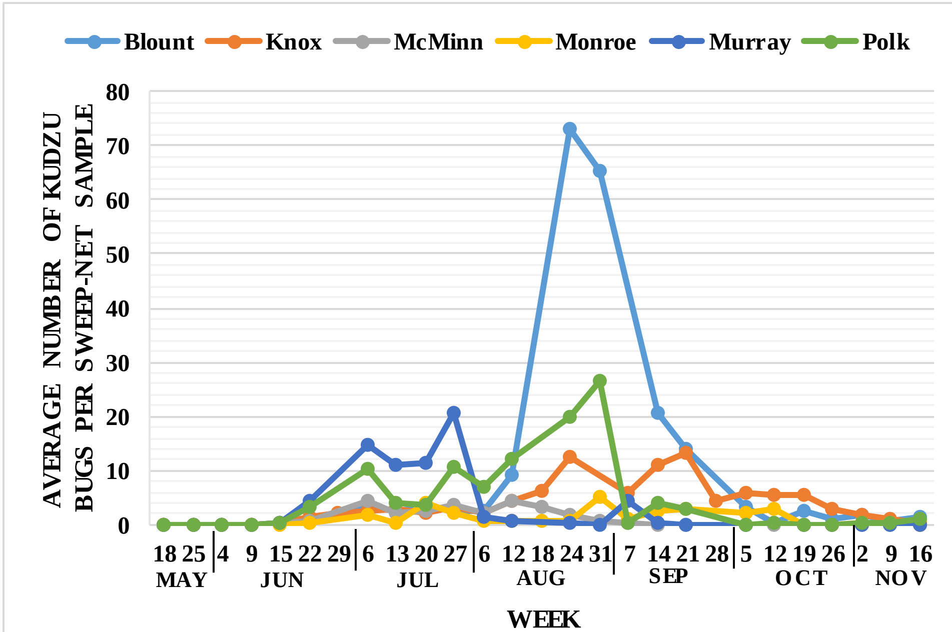

Figure 21. Average number of stage five nymphs per sweep net sample, 2015.

that adult densities build as nymphs molt to adulthood. Also, adults can fly, and they can leave patches and come back in late season to increase numbers. Nymphs of any stage do not have the ability to fly, so an increase in numbers of kudzu bugs at stage one would not be influenced by a mass influx of insects back into the kudzu patch. These numbers are strictly based on nymphs that have just hatched from eggs. For these reasons, it is interesting that they have a late-season peak in population densities (Fig. 17). Also, from 6 July (week 8) forward, stage one nymphs were present at least at one site every week. The same trend was not evident for other nymphal stages (Figs. 18-21). Although *B. bassiana* was eventually present at the Monroe County site, it was not as prevalent there as it was at other sites. Therefore, it would not have been expected to have as detrimental of an effect on nymphs at this site.

Stage two nymphs (Fig. 18) did not exhibit the same late-season peak in population densities as stage one nymphs. However, similar to stage one nymphs, stage two nymph populations only exhibited a trend at one collection site, Blount County, where they increased and peaked beginning on 27 July (week 11) and ending on 31 August (week 16). In contrast to stage one nymphs persisting throughout the duration of the sampling season in Monroe County, stage two nymphs disappeared after 21 September (week 19). The highest number of stage two nymphs were collected from Blount County on 31 August (week 16), and nymphal populations declined drastically after that time. Densities of kudzu bug in Blount County were heavily affected by *B. bassiana*; its greater impact on nymphs than on adults explain the rapid decline in nymphal densities.

Stage two nymphs appeared in smaller numbers but at the same time of the year in other counties (Fig. 18). While kudzu in Knox and Polk Counties had stage two nymphs present in numbers of 5 or less per sweep-net sample, these counties had the same population trends as Blount County. Polk County also had the highest number of stage two nymphs collected in kudzu on week 16. Similar to the adult population densities, stage two nymphs were no longer present in Polk County after week 16. Knox County had the highest number collected on week 15 and numbers subsequently declined.

An interesting observation can be drawn from stages three (Fig. 19), four (Fig. 20), and five (Fig. 21). While some stages were collected in larger numbers (i.e., stage five collected in higher numbers than other stages), all three of these nymphal stages exhibited the same general population trends at the same sampling sites throughout the sampling season. Densities of all three stages (three, four, and five), peaked on 24 August (week 15) in Blount County; similarly, all three stages peaked in Polk County on 31 August (week 16). For each of the three stages in Knox County, numbers increased beginning on 12 August (week 13) and remained relatively high through 21 September (week 19); after 21 September (week 19), numbers of all three life stages (three, four, and five) continuously declined through the end of the season.

With *B. bassiana* first appearing in the field on 1 September (week 16), it likely impacted densities of stages three, four, and five similar to the other nymphal stages. After densities of each nymphal stage peaked in different counties, densities continuously declined through the end of the sampling season. In contrast to stages one and two, life stages three, four, and five were found in higher numbers throughout the entire season.

Comparisons of Adult Males and Females Across all Counties, 2015

Overall, densities of adult female kudzu bugs (Fig. 22) were consistently lower throughout the sampling season than males (Fig. 23). While both sexes exhibited the same fluctuations in population density trends, number of bugs collected were markedly different. Two noticeable differences in densities between sexes occurred in Polk County. The first difference was on 6 July (week 8) when female population densities (n = 8.6) were approximately 50% greater than male densities (n = 5.8). The second difference was on 2 November (week 25), and although adult population densities were relatively low, females (n = 1.6) outnumbered males (n = 1.2) by approximately 30%. It is interesting to see the differences in densities and trends among counties, as no counties exhibited the same trends.

Interestingly, female population densities were higher at the beginning of the season but started to decrease around 6 July (week 8) when overall population density began to increase

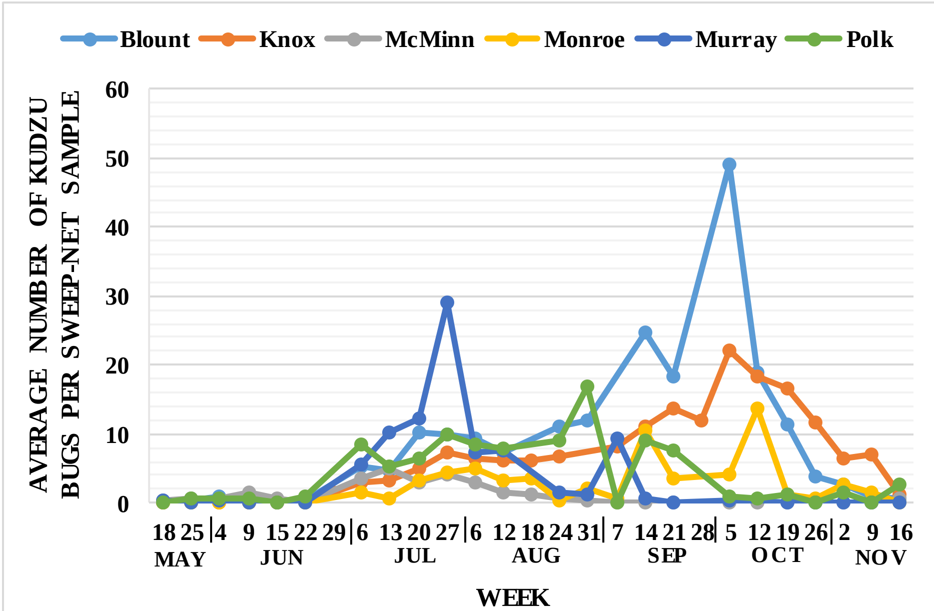

Figure 22. Average number of adult female kudzu bugs per sweep-net sample per sampling week, all counties, 2015.

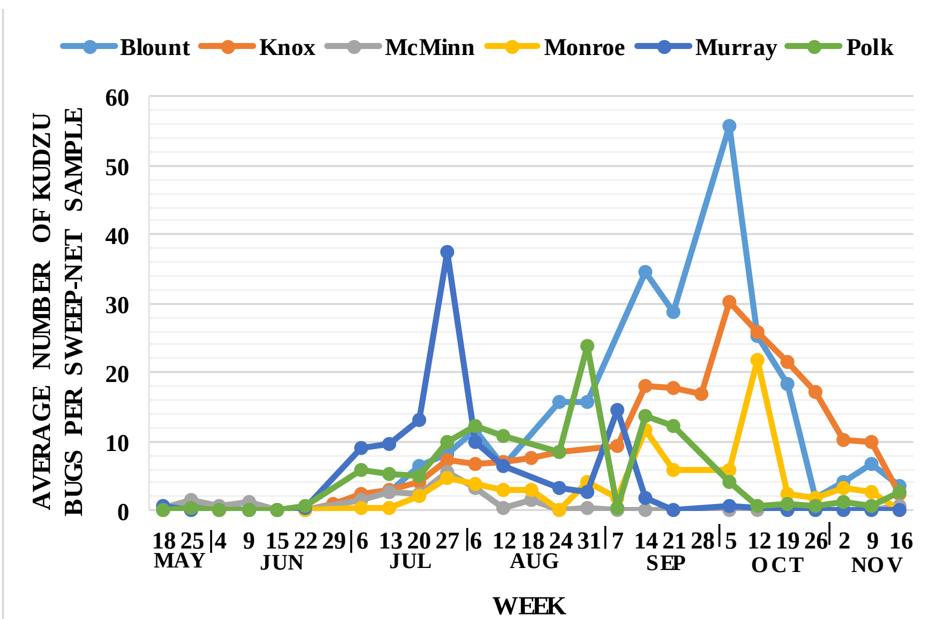

Figure 23. Average number of adult male kudzu bugs per sweep-net sample per sampling week, all counties, 2015.

throughout all of the sampling sites. The higher initial level of adult female densities indicates that female kudzu bug adults were more successful at overwintering than males.

In Knox County, population densities of adult female and male kudzu bugs compared across all counties were more or less consistent throughout the entire season (Figs. 22 and 23, respectively). Prior to the first population peak (27 July; week 11), female densities were slightly higher than males. At the time of the first population density peak, the numbers of males and females were basically the same, with male densities slightly greater than females. After this initial peak, male population densities remained higher than female densities for the duration of the sampling season. At the conclusion of sampling (16 November; week 27), population densities were similar, with densities of males slightly higher than those of females.

Morphologically speaking, female kudzu bugs are slightly larger in size than males. This size difference is important because it may cause difficulties in counting insects when sampling in the field. For example, when in the field, it is easier to see the larger females as human eyes are normally drawn to the larger of the two insects. While it seemed as though more female kudzu bugs were present in the field throughout the sampling period, the number of females and males were similar, with male population densities slightly higher for the majority of the sampling season (Fig. 24).

Assessment of Kudzu Bugs (Eggs)

In 2015, eggs were found from 18 May to 17 September 2015 (weeks 1 through 18). The average number of eggs per egg mass (16.70) was fairly consistent throughout the season. The

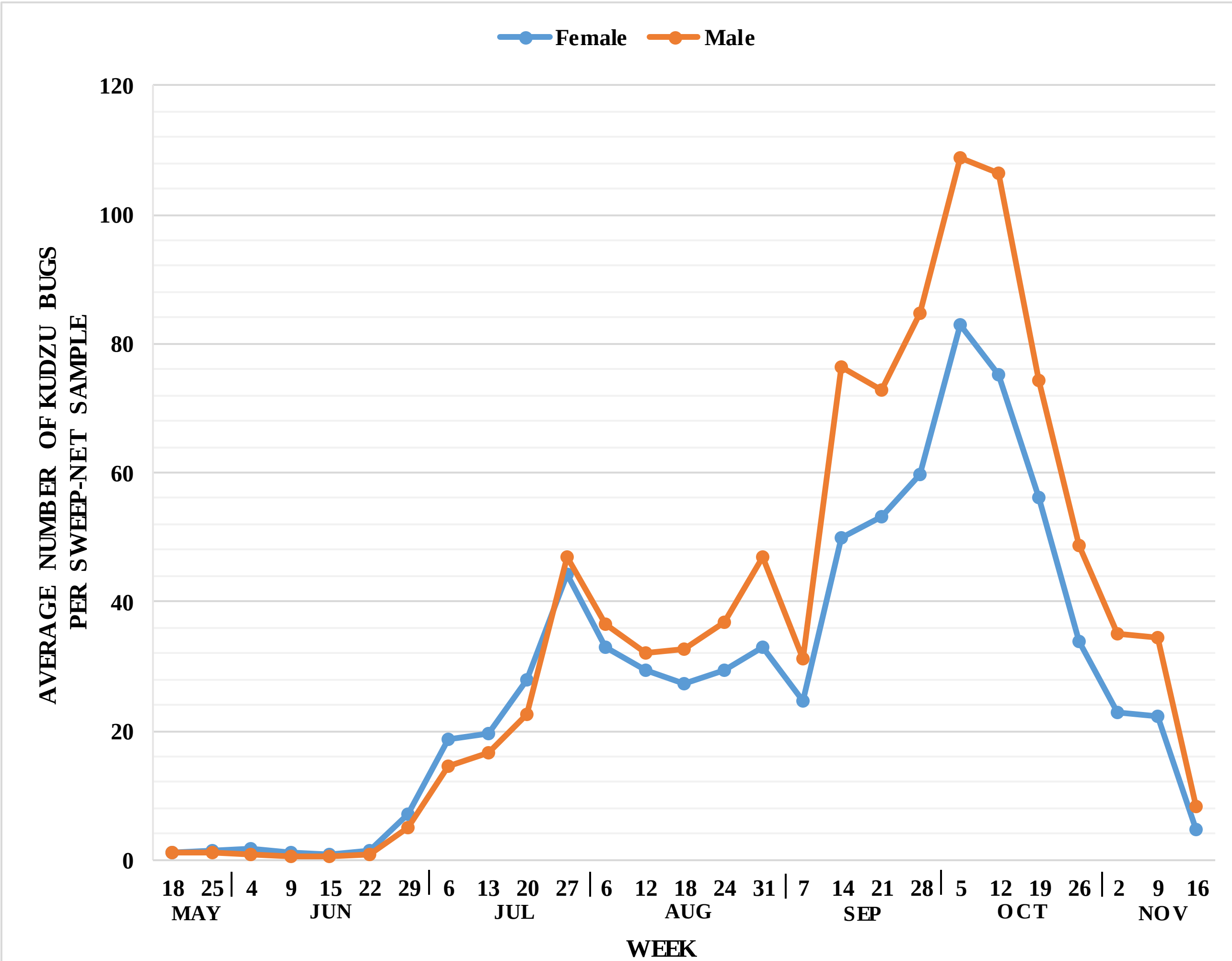

Figure 24. Average number of adult male and female kudzu bugs per sweep-net sample per sampling week, all counties, 2015.

average size of egg masses increased slightly overall for the first 13 weeks then fluctuated until the end of egg-laying on 14 September (week 18) (Fig. 25). The number of eggs per mass ranged from 13.55 on 9 June (week 4) to 20.25 on 14 September (week 18). The highest percentage of eggs hatched per mass (66.52%) occurred on 18 May (week 1) (Fig. 25), and the lowest percentage of eggs hatched per mass (20.81%) occurred on 6 July (week 8). This number was 50% less than the next lowest hatch percentage, which was 30.86% on 14 September (week 18). Although there was a general trend of an increasing size of egg masses as the season progresses, no distinct trend in the percentage of eggs hatched per mass was observed during the sampling season. Overall, 47.90% of 43,707 eggs in 2,619 field-collected egg masses hatched. This low percentage of eggs hatched may be attributed to potential pathogen infection (potentially *B. bassiana*; it is unknown how egg development is influenced by the fungus), fertilization with non-viable sperm, improper storage of eggs in the laboratory, predation of eggs by enemies in kudzu patches, or unsuitable environmental conditions in the field around the time period in which eggs were laid.

Summary

Many trends in seasonality and phenology of kudzu bugs (adults and immatures) were documented at the 14 sampling locations in six counties in eastern Tennessee. Adults moved from overwintering sites into kudzu in early May, where adult kudzu bugs were found until November. Populations of adult kudzu bug peaked at a substantially greater number in Knox County in 2014 compared to 2015 (and higher than all counties in 2015). The phenology of populations was apparent with later nymphal stages (stages three through five) but was not readily apparent with smaller nymphs, because it is more difficult to monitor younger stages.

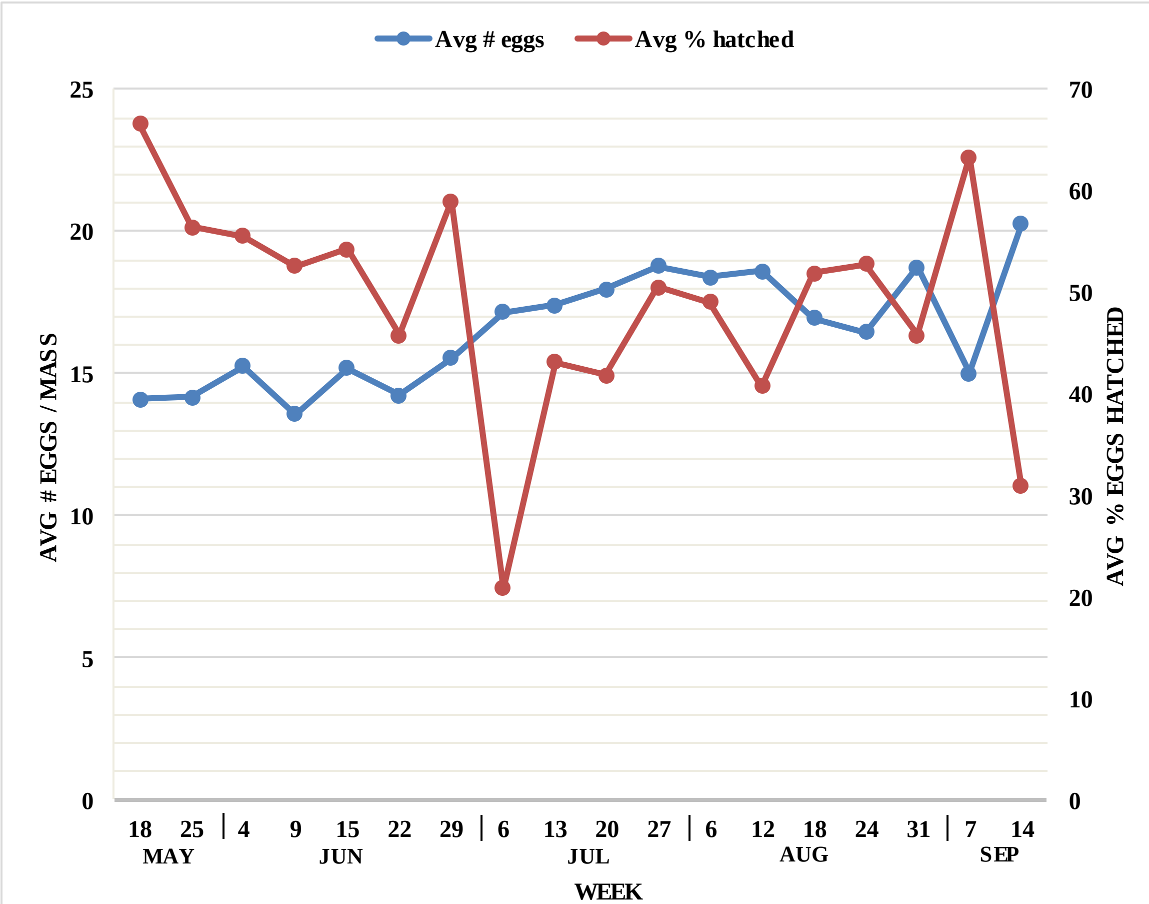

Figure 25. Average number of eggs and percent of eggs hatched per mass, 2015.

Development from nymph to adult occurred in four to six weeks, corresponding with the observation of Ruberson et al. (2012). Two generations of kudzu bug were obvious throughout the sampling period in both years at all locations. The first generation of kudzu bugs developed on kudzu while the second generation developed on kudzu and/or soybean. As temperatures declined in the fall, adult kudzu bugs moved back to kudzu to feed before seeking overwintering sites. Eggs were laid from mid-May to mid-September in kudzu patches. The average number of eggs laid per mass was 16.7.

The fungus *Beauveria bassiana* impacted late-season population densities of adult and nymph kudzu bugs throughout the different counties in 2015. Differences in landscape and types of agriculture present in the various counties likely affected kudzu bug population densities at sites visited outside of Knox County. This new knowledge on the life cycle of kudzu bug will enhance management programs directed at managing this invasive species, reducing its impacts on agriculture and humans.

CHAPTER III
BEHAVIORAL RESPONSES OF KUDZU BUG TO HOST PLANT SPECIES

Introduction

In Fall 2009, the kudzu bug, *Megacopta cribraria* (Fabricius) (Hemiptera: Heteroptera: Plataspidae) was first discovered as an invasive pest in the United States. It was detected due to its bothersome behavior to homeowners in several counties in northern Georgia, where it had become well established in large numbers in kudzu, *Pueraria montana* variety *lobata* (Willd.) Maesen and S. Almeida (Fabales: Fabaceae), that neighbored residential areas. Kudzu bugs were likely present in these kudzu patches throughout the summer of 2009, but they were not detected until they invaded homes to overwinter.

Kudzu is the main host plant species (i.e., sufficient for normal growth, development, and reproduction) for kudzu bug. Soybeans, *Glycine max* (L.) Merrill (Fabales: Fabaceae), are another suitable host plant for kudzu bugs. Thus, this invasive insect has the potential to spread to any area where kudzu and soybean are present. Because kudzu is present across most of the southeastern United States, the kudzu bug poses a tremendous threat to homeowners and soybean farmers throughout the region. Kudzu bug was detected in Tennessee in 2012 and is now known to be present in more than 60 counties.

In Summer 2014, kudzu bugs were observed in field sites in Knox County, TN on several plant species including bush honeysuckle (*Lonicera maackii* [Rupr.]; Family Caprifoliaceae) and common ragweed (*Ambrosia artemisiifolia* L.; Family Asteraceae) (personal observation). Eggs, nymphs, and adult kudzu bugs were present on these plants. The presence of kudzu bugs on these plants was frequent, occurring for several consecutive weeks, and did not seem to be coincidental events. If other commonly occurring plant species can suitably host kudzu bugs, these additional host plants could provide a greater pathway for spread of kudzu bug across the United States than originally estimated. Also, these plants are particularly interesting since they are non-leguminous. Kudzu bug has been known to have host plants that are not legumes (Eger et al., 2012), however, the extent of utilization of these non-leguminous hosts is unclear. Thus, it is important to learn about the activity and behavior of kudzu bugs on these alternate plants.

The specific objectives of this study were to determine behavioral responses of the kudzu bug to host substrates, including kudzu, soybeans, and the observed possible alternate host plants bush honeysuckle and common ragweed (hereafter referred to as ragweed). Questions to be answered in this objective include: What choice will the kudzu bug make when offered different plant species? Will a non-documented alternate host plant be more attractive than an existing host plant? When given only one plant species, will kudzu bugs find the plant attractive or try to avoid it?

Materials and Methods

The plant species assessed for this objective were kudzu, soybean, bush honeysuckle, and ragweed. Two different experiments were conducted: no-choice and choice tests.

No-choice Tests

Plant foliage of four test plant species – kudzu, soybean, bush honeysuckle, and ragweed (Fig. 26) – was field-collected, placed into water vases (3.8 liter volume) and taken to the laboratory. Pieces of foliage (approximately 8 cm in diameter) were clipped and placed into 100 mm petri dishes on top of moistened pieces of filter paper. Only adult kudzu bugs were used in

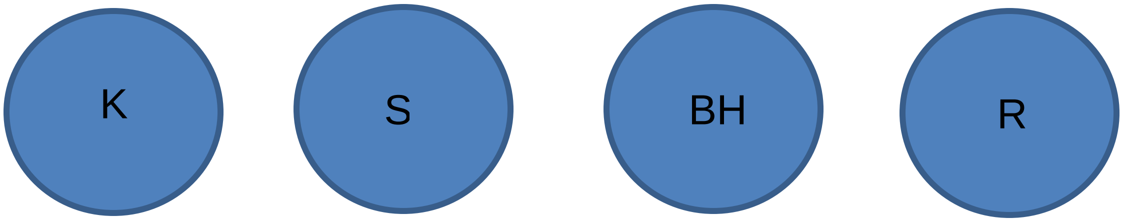

Figure 26. The no-choice test options included kudzu (K), soybean (S), bush honeysuckle (BH), and ragweed (R).

this study. Insects were collected and pooled from the nine regularly-visited collection sites in Knox County, TN (see Chapter II). Kudzu bugs were maintained in the laboratory on kudzu. Twenty-four hours prior to conducting the study, kudzu bugs were removed from host plant material, placed into 3.8 liter containers with moistened paper towels, and allowed to fast (i.e., no food was provided). After this time, three different treatments were applied to the test foliage in the petri dishes – one male, one female, and a paired male and female kudzu bug. Treatments were replicated ten times, with a total of 120 petri dishes per test period (3 treatments x 4 plant species x 10 replications = 120 petri dishes). Petri dishes were observed after specified time periods (1, 2, 4, 8, 12, 24, 48, 72, and 96 hours post setup) to record activity level and location of kudzu bugs in petri dishes. Recorded observations included kudzu bug activity level – bugs were classified as 'active' or 'inactive.' Active classifications consisted of many different types of activities: feeding on plant material, drinking from filter paper, walking on dish or on plant, and actively moving antennae. Inactive classifications were made when kudzu bugs were not moving at all in the petri dish and antennae were pointed down. Kudzu bug location on the plant material (i.e., on leaf, on stem, or off plant) was also recorded. The entire study was repeated on three separate occasions. These tests were conducted under room temperature and a 14:10 L:D photoperiod.

Choice Tests

Plant foliage of the four test plant species – kudzu, soybean, bush honeysuckle, and ragweed – was field collected, placed into water vases (3.8 liter volume) and taken to the laboratory. Foliage with a generous amount of stem (approximately 15 cm in length) was placed into a 12 dram plastic vial containing water. Parafilm was placed over the plastic vial prior to plant placement to seal the opening so that kudzu bugs would not move into water in the tubes. The vials were filled to two-thirds of their volume with water (approximately 8 drams) to sustain the plant material for the duration of the test.

To explore kudzu bug plant choice across the four plant species, plants were grouped into every possible combination, making 11 total choice combinations (Fig. 27). Three treatments were employed for this test – one male, one female, and a paired male and female kudzu bug. Treatments were replicated three times, with a total of 99 containers per test period (3 treatments x 11 plant combinations x 3 replications). Plant foliage secured in plastic vials (12 dram) were placed into 3.8 liter volume plastic containers with mesh holes (3 holes, each 7 cm in diameter) in the containers for ventilation. The appropriate treatment (male, female, or male and female) was placed on the foliage in each container. Containers were observed after specified time periods (1, 2, 4, 8, 24, 48, 72, and 96 hours post-setup), and the plant chosen (kudzu, soybean, bush honeysuckle, ragweed, or no plant), activity level (as previously described), and location of kudzu bugs in containers (i.e., on leaf, on stem, or off plant) were recorded. The tests were conducted under ambient laboratory temperatures and a photoperiod of 14:10 L:D.

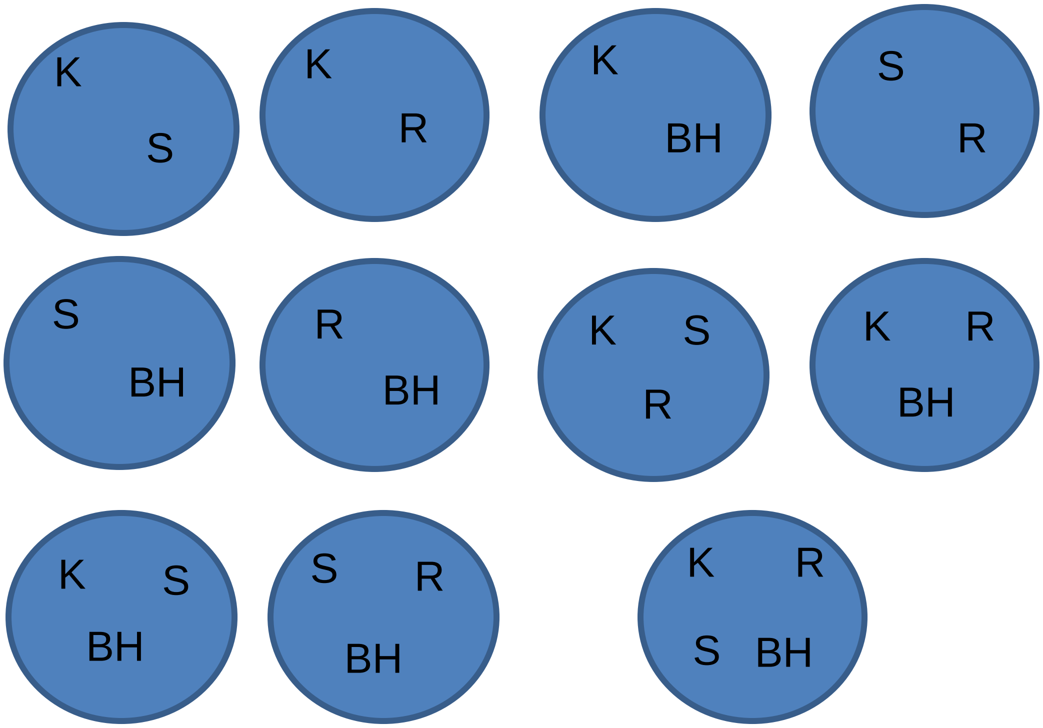

Figure 27. The choice test options included all 11 possible combinations of four host plant species (kudzu [K], soybean [S], bush honeysuckle [BH], and ragweed [R].

Data Analysis

Data for all tests were analyzed using SAS System GLIMMIX ANOVA. The estimation technique was a restricted maximum likelihood. Degrees of freedom method and fixed effects were both Kenward-Roger. A significance level of 0.05 was used for mean separation.

In the no-choice experiment, data included only insects that were classified as 'active' throughout the testing period. Analyses compared overall percent activity level of adults by hour, plant species, and sex; percent activity level for insects that chose 'stem' as their location by hour, plant species, and sex; percent activity level for insects that chose 'leaf' (upper or lower surface of foliage area) as their location by hour, plant species, and sex; and percent activity level of those adults that were active but not on any plant surface, denoted as 'off,' and analyzed by hour, plant species, and sex.

In the choice experiment, data included only insects that were classified as 'active' throughout the testing period. These results were separated into overall percent activity level of adults by hour, sex and location on plant; percent activity level for insects that chose bush honeysuckle by hour, sex, and location on plant; percent activity level for insects that chose kudzu by hour, sex, and location on plant; percent activity level for insects that chose ragweed by hour, sex, and location on plant; percent activity level for insects that chose soybean by hour, sex, and location on plant; and percent activity level of those adults that were active but not on any plant surface, denoted as 'no plant,' by hour, sex, and location on plant.

Results and Discussion

No-Choice Tests

Overall

Overall results showed a general trend in percent adult activity relative to the observation (hour) period. As the amount of exposure time increased, insects were less active in petri dishes (Table 3). For example, 79.6% of the kudzu bugs were classified as active after 1 hour, but only 41.0% were classified as active at the 96 hour observation period. This decrease in activity is expected as kudzu bugs become more adapted and acclimated to conditions within the petri dish over time. With decreasing levels of activity over the study period, kudzu bugs may have found the foliage suitable and rested on it instead of actively moving around the petri dish to locate a more suitable substrate. As stated earlier, bugs were starved for 24 hours prior to initiation of the study. Thus, they may have fed on plant material initially and were less active after they fed.

Table 3. Overall comparisons of locations of 'active' adult kudzu bugs in no-choice tests by exposure time.

	Overall[1]	Location of Active Adult		
		Stem	Leaf	Off
Hour[2]	$F_{(8, 3663)} = 11.52$, **P < 0.0001**	$F_{(8, 3622)} = 1.12$, **P = 0.3454**	$F_{(8, 3622)} = 48.12$, **P < 0.0001**	$F_{(8, 3622)} = 51.48$, **P < 0.0001**
1	79.61 ± 8.69 **AB**[3]	0.83 ± 0.73 **B**	26.04 ± 2.99 **E**	73.12 ± 3.15 **A**
2	80.19 ± 6.56 **A**	1.87 ± 0.73 **AB**	29.79 ± 2.99 **DE**	68.33 ± 3.15 **B**
4	69.02 ± 6.33 **ABC**	2.08 ± 0.73 **AB**	33.33 ± 2.98 **D**	64.58 ± 3.15 **B**
8	63.51 ± 6.16 **BC**	2.29 ± 0.74 **AB**	46.54 ± 2.99 **C**	51.16 ± 3.15 **C**
12	62.07 ± 6.18 **BC**	2.29 ± 0.74 **AB**	55.55 ± 2.99 **B**	42.15 ± 3.15 **D**
24	65.79 ± 5.69 **BC**	3.15 ± 0.74 **A**	57.97 ± 2.99 **B**	38.88 ± 3.16 **D**
48	57.34 ± 6.55 **C**	1.90 ± 0.74 **AB**	67.47 ± 3.00 **A**	30.64 ± 3.16 **E**
72	24.46 ± 6.21 **E**	2.42 ± 0.75 **AB**	69.97 ± 3.02 **A**	27.59 ± 3.18 **E**
96	40.96 ± 5.97 **D**	2.90 ± 0.76 **A**	69.51 ± 3.04 **A**	27.62 ± 3.20 **E**

[1]Column headings designate all active insects (overall), insects active on stems of foliage (stem), insects active on leaf foliage (leaf), and insects active off of plants (off).

[2]Hour observations are time (in hours) since kudzu bugs were placed into petri dishes.

[3]Mean percent active ± standard error; means followed by the same letter within columns are not significantly different (P > 0.05).

Overall, insects were significantly less active on ragweed when compared to the other plant species, since only 54.4% of kudzu bugs were classified as active on ragweed throughout the testing period (Table 4). Activity of kudzu bugs was similar on kudzu (64.6%), soybean (62.2%), and bush honeysuckle (60.1%) throughout the testing period. As for sex, percent activity levels of paired males and females in petri dishes were not significantly different (Table 5). However, males paired with a female were significantly more active in petri dishes than lone males or lone females. This activity may be partially attributed to having male and female adult kudzu bugs together in a petri dish, where males may be more likely to search for solitary space rather than shared space or may be more active to gain the attention of the female for mating.

Stem

For insects that were active on stems of plants, percent activity level did not change much over the course of the study, ranging from less than 1.0% at hour 1 to 2.9% at the end of the study (Table 3). Percent activity of adults was significantly different at three observation periods; percent activity at hours 24 and 96 was significantly greater than at hour 1 (Table 3).

When examining the activity of kudzu bugs on stems of different plant species, significantly fewer adults were active on ragweed than on kudzu or bush honeysuckle (Table 4). Percentage of active kudzu bugs on stems of ragweed was lower than, but not significantly different from, those on stems of soybean plants. It is interesting to note that the lowest activity levels of kudzu bugs on stems and overall (across all plant locations) were found on ragweed. When petri dishes containing ragweed were opened in the laboratory, a strong odor was detected, unlike when examining dishes of the other plant species. This odor could be a reason for low activity levels with ragweed overall in the laboratory.

Table 4. Comparisons of adult kudzu bugs that were classified as 'active' in no-choice tests by plant species.

	Overall	Location of Active Adult Stem	Leaf	Off
Plant[1]	$F_{(3, 473)} = 5.52$, **$P < 0.0010$**	$F_{(3, 464)} = 2.45$, **$P = 0.0633$**	$F_{(3, 464)} = 5.98$, **$P = 0.0005$**	$F_{(3, 464)} = 6.98$, **$P < 0.0001$**
BH	60.07 ± 4.55 **A**[2]	2.88 ± 0.74 **A**	47.40 ± 3.37 **B**	49.72 ± 3.54 **A**
K	64.60 ± 4.56 **A**	2.90 ± 0.74 **A**	60.31 ± 3.37 **A**	36.77 ± 3.55 **B**
R	54.40 ± 4.60 **B**	0.65 ± 0.75 **B**	46.81 ± 3.38 **B**	52.53 ± 3.55 **A**
S	62.24 ± 4.56 **A**	2.33 ± 0.74 **AB**	48.22 ± 3.37 **B**	49.45 ± 3.55 **A**

[1]Plant = bush honeysuckle (BH), kudzu (K), ragweed (R), and soybean (S).

[2]Mean percent activity ± SE; means followed by the same letter within columns are not significantly different (P > 0.05).

Table 5. Comparisons of locations of adult kudzu bugs that were classified as 'active' by sex in no-choice tests.

	Overall	Location of Active Adult Stem	Leaf	Off
Sex[1]	$F_{(3, 473)} = 3.68$, **$P = 0.0122$**	$F_{(3, 464)} = 1.30$, **$P = 0.2730$**	$F_{(3, 464)} = 0.24$, **$P = 0.8679$**	$F_{(3,464)} = 0.13$, **$P = 0.9427$**
PF	61.53 ± 4.56 **AB**[2]	2.14 ± 0.74 **AB**	52.10 ± 3.37 **A**	45.76 ± 3.54 **A**
PM	64.68 ± 4.57 **A**	2.29 ± 0.74 **AB**	49.69 ± 3.37 **A**	48.00 ± 3.55 **A**
F	56.64 ± 4.55 **B**	3.11 ± 0.75 **A**	49.49 ± 3.37 **A**	47.40 ± 3.55 **A**
M	58.46 ± 4.58 **B**	1.22 ± 0.75 **B**	51.46 ± 3.38 **A**	47.32 ± 3.55 **A**

[1]Sex corresponds with the gender of insects used. PF (paired female) and PM (paired male) are insects that were together in a petri dish. F (female) and M (male) are kudzu bugs that were lone insects in petri dishes.

[2]Mean percent activity ± SE; means followed by the same letter within columns are not significantly different (P > 0.05).

When comparing activity between the sexes of kudzu bugs on stems of plant species (Table 5), activity of lone males was significantly less than activity of lone females. No other statistical differences were detected among sexes.

Leaf

Kudzu bugs were more active on leaf foliage (includes both upper and lower leaf surface) than on the stem (Table 3). This finding is expected since leaf foliage provides the largest portion of plant material for kudzu bug to use as a resting substrate. Interestingly, percent activity on leaf foliage increased throughout the study, ranging from 26.0% at hour 1 to 69.5% at hour 96.

Kudzu bug was most active on leaf foliage of kudzu (Table 4). Percent activity of kudzu bugs was significantly greater on leaf foliage of kudzu than on leaf foliage of bush honeysuckle, ragweed, or soybean. This finding is expected since kudzu is the most common and most preferred host plant of kudzu bugs.

Among the sexes, no significant differences in activity of kudzu bug on leaf foliage were documented (Table 5). Activity of females may be expected to be higher than males, but only if they were gravid and seeking oviposition sites. It was not determined if gravid female kudzu bugs were used in this study.

Off

During this study, kudzu bugs classified as 'off of plant' were significantly less active as time increased, ranging from 73.1% at hour 1 to 27.6% at hour 96 (Table 3). This reduced activity is expected because as time passes, one would expect adult kudzu bugs to be located on plants instead of roaming around petri dishes. Presence on plant material suggests that kudzu bugs acclimated to the plant material within the petri dish.

Kudzu bugs classified as 'off the plant' were significantly more active in petri dishes that contained bush honeysuckle, ragweed, and soybean than on kudzu (Table 4). Kudzu is the most preferred host plant, so kudzu bugs exposed to kudzu plant material are expected to be on the plant and not classified as off the plant.

No significant differences in activity of kudzu bugs off of the plant species were observed among the sex classifications (Table 5). Percentages of kudzu bugs classified as 'off the plant' ranged from 45.8% for paired females to 48.0% for paired males.

Choice Tests

Overall

When all plant species were compared overall, the activity level of kudzu bugs declined consistently for the first 8 hours of the study, increased slightly at 24 hours, and declined for the duration of the study (Table 6). This activity pattern suggests changes within plants over time could likely influence kudzu bug behavior. The activity of kudzu bugs ranged from 91.0% at 1 hour to only 47.2% at the conclusion of the study. The lowest activity (40.21%) occurred 8 hours after initial exposure.

When examining location choices among all plant species, the percent of active kudzu bugs on leaf foliage was significantly greater than on the stem (50.6% and 44.8%, respectively) (Table 6). Although 79.5% of the kudzu bugs were classified as 'off the plant,' they were not significantly different from those on the stem or on the leaf.

Females that were paired with a male were significantly more active than lone females (Table 6). No other differences in activity were detected among the sexes. It is probable that

Table 6. Overall comparisons of adult kudzu bugs that were classified as 'active' by time of exposure, location on plant, and sex in choice tests.

	Overall
Hour[1]	$F_{(7, 2374)} = 64.10$, **P < 0.0001**
1	90.99 ± 6.51 **A**[2]
2	81.32 ± 6.51 **B**
4	41.07 ± 6.49 **FG**
8	40.21 ± 6.52 **G**
24	55.86 ± 6.47 **CD**
48	51.80 ± 6.49 **DE**
72	58.02 ± 6.50 **C**
96	47.20 ± 6.49 **EF**
Location[3]	$F_{(2, 2765)} = 3.24$, P = 0.0392
Leaf	50.64 ± 8.59 **A**
Off	79.52 ± 17.27 **AB**
Stem	44.76 ± 8.82 **B**
Sex[4]	$F_{(3, 604.2)} = 3.00$, P = 0.0303
PF	62.58 ± 6.38 **A**
PM	57.88 ± 6.35 **AB**
F	54.45 ± 6.39 **B**
M	58.31 ± 6.37 **AB**

[1]Hour observations are time (in hours) since kudzu bugs were introduced into petri dishes.
[2]Mean percent activity ± SE; means followed by the same letter within columns are not significantly different (P > 0.05).
[3]Location corresponds with where active insects were on plants during times of observation.
[4]Sex corresponds with the gender of insects used. PF (paired female) and PM (paired male) are insects that were together in a petri dish. F (female) and M (male) are kudzu bugs that were lone insects in petri dishes.

females paired with males were more active than females alone, as they may have been engaging in courtship activities with a male present.

Bush Honeysuckle

The percent of kudzu bugs on bush honeysuckle ranged from 28.0% at 1 hour to 33.2% at 2 hours, with only 32.5% of adults active at the end of the study (Table 7). No obvious trends in activity of kudzu bugs over time were found on bush honeysuckle. When considering different parts of bush honeysuckle, activity levels on leaf and stem portions of the plant were statistically similar (Table 8). However, activity of kudzu bugs classified as off the plant were significantly lower than those on leaf foliage or on stems. Activity of the sexes on bush honeysuckle was statistically similar (Table 9). Percent of active kudzu bugs ranged from 29.5 to 33.0% for females paired with males and for females alone, respectively.

Kudzu

No general trend in adult activity was observed in relation to time (Table 7). Activity was significantly lower at 48 hours (30.0%) than at 72 hours (34.3%) and 96 hours (35.2%). Activity at all other time periods were statistically similar. Activity level fluctuated between 32.1% at hour 1 to 35.2% at the end of the study. Kudzu is a documented host plant of kudzu bug, so one would expect similar activity over time.

Activity levels of kudzu bugs on the three locations within the petri dish were all significantly different (Table 8). Activity of kudzu bugs was significantly greater on stems (56.3%) than on leaf foliage (40.8%). The lowest activity (0.3%) of kudzu bugs were those classified as off of the kudzu plant. The stem is the vascular system of the plant and is where

Table 7. Comparisons of adult kudzu bugs that were classified as 'active' by time of exposure on four plant species in choice tests.

	Bush Honeysuckle[1]	**Kudzu**	**Ragweed**	**Soybean**
Hour[2]	$F_{(7, 1250)} = 2.45$, P < 0.0001	$F_{(7, 1199)} = 1.42$, P = 0.1939	$F_{(7, 1298)} = 1.84$, P = 0.0767	$F_{(7, 1260)} = 1.24$, P = 0.2766
1	27.98 ± 3.58 **BC**[3]	32.11 ± 2.16 **AB**	21.35 ± 2.01 **A**	21.29 ± 2.55 **A**
2	33.20 ± 3.56 **A**	30.60 ± 2.16 **AB**	18.82 ± 2.00 **ABC**	19.72 ± 2.53 **A**
4	31.18 ± 3.56 **ABC**	31.79 ± 2.14 **AB**	17.97 ± 1.99 **ABC**	21.54 ± 2.52 **A**
8	31.67 ± 3.56 **ABC**	33.69 ± 2.14 **AB**	16.98 ± 1.99 **BC**	19.61 ± 2.52 **A**
24	32.42 ± 3.55 **ABC**	32.22 ± 2.14 **AB**	16.72 ± 1.98 **C**	21.03 ± 2.51 **A**
48	32.78 ± 3.54 **A**	29.95 ± 2.12 **B**	20.83 ± 1.97 **AB**	18.55 ± 2.51 **A**
72	28.32 ± 3.55 **C**	34.33 ± 2.14 **A**	18.55 ± 1.99 **ABC**	21.07 ± 2.54 **A**
96	32.50 ± 3.55 **AB**	35.19 ± 2.13 **A**	16.53 ± 1.99 **C**	17.96 ± 2.52 **A**

[1]Columns designate percent of the insects active on bush honeysuckle, kudzu, ragweed, and soybean after the specified hours of exposure.

[2]Hour observations are time (in hours) since kudzu bugs were introduced into petri dishes.

[3]Mean percent activity ± SE; followed by the same letter within columns are not significantly different (P > 0.05).

Table 8. Comparisons of adult kudzu bugs that were classified as 'active' by location on plant material of four plant species in choice tests.

	Bush Honeysuckle[1]	**Kudzu**	**Ragweed**	**Soybean**
Location[2]	$F_{(2, 419.9)} = 112.50$, P < 0.0001	$F_{(2, 412.9)} = 128.26$, P < 0.0001	$F_{(2, 415.4)} = 84.95$, P < 0.0001	$F_{(2, 321.7)} = 50.05$, P < 0.0001
Leaf	45.89 ± 3.76 **A**[3]	40.83 ± 2.40 **B**	36.89 ± 2.15 **A**	29.02 ± 2.81 **A**
Off	0.29 ± 3.66 **B**	0.32 ± 2.38 **C**	-0.78 ± 2.08 **C**	0.18 ± 2.66 **B**
Stem	47.59 ± 4.23 **A**	56.32 ± 3.04 **A**	19.31 ± 2.90 **B**	31.09 ± 3.23 **A**

[1]Columns designate percent of the insects active on the leaf (foliage) or stem of bush honeysuckle, kudzu, ragweed, and soybean, as well as those active off the plant.

[2]Location corresponds with where active insects were on plants during times of observation.

[3]Mean percent activity ± SE; means followed by the same letter within columns are not significantly different (P > 0.05).

Table 9. Comparisons of adult kudzu bugs that were classified as 'active' by sex on four plant species in choice tests.

	Bush Honeysuckle[1]	**Kudzu**	**Ragweed**	**Soybean**
Sex[2]	$F_{(3, 524.4)} = 0.23$, $P = 0.87$	$F_{(3, 509.4)} = 0.97$, $P = 0.40$	$F_{(3, 491.4)} = 1.67$, $P = 0.17$	$F_{(3, 488.4)} = 0.35$, $P = 0.78$
PF	29.47 ± 4.23 **A**[3]	33.00 ± 3.13 **A**	17.24 ± 2.91 **AB**	19.90 ± 3.27 **A**
PM	30.81 ± 4.10 **A**	28.63 ± 2.94 **A**	21.71 ± 2.72 **A**	21.15 ± 3.17 **A**
F	32.98 ± 4.21 **A**	35.86 ± 3.17 **A**	13.86 ± 2.90 **B**	21.53 ± 3.30 **A**
M	31.77 ± 4.19 **A**	32.46 ± 3.12 **A**	21.06 ± 2.87 **AB**	17.81 ± 3.22 **A**

[1]Columns designate percent of the insects active on each plant species, as well as those active off the plant.

[2]Sex corresponds with the gender of insects used. PF (paired female) and PM (paired male) are insects that were together in a petri dish. F (female) and M (male) are kudzu bugs that were lone insects in petri dishes.

[3]Mean percent activity ± SE; means followed by the same letter within columns are not significantly different ($P > 0.05$).

kudzu bugs feed primarily. Thus, one would expect a larger percent of insects to be active on this portion of their preferred host plant.

When examining sexes of kudzu bugs, activity of all sexes was significantly similar throughout the duration of the choice test (Table 9). Percent activity of kudzu bugs ranged from 28.6% for paired males to 35.9% for lone females. Because kudzu is the known preferred host plant of kudzu bug, all sexes are expected to find the plant suitable.

Ragweed

No general trends in activity level of kudzu bugs on ragweed were observed in relation to the time period in which observations were made (Table 7). The percent of active kudzu bugs ranged from a high of 21.4% at hour 1 to 16.5% at the end of the study (hour 96). The percent of active kudzu bugs on ragweed at hour 1 was significantly greater than at 8 (16.9%), 24 (16.7%), and 96 hours (16.5%).

Activity of kudzu bugs was significantly different across locations in the petri dish (Table 8). Kudzu bugs were significantly more active on foliage (36.9%) than on the stem (19.3%). Kudzu bug activity off the plant was significantly lower than on either location on the plant.

Significant differences in kudzu bug activity were observed among sexes (Table 9). Paired male kudzu bugs, which were the most active (21.7%), were significantly more active than lone females (13.9%).

Soybean

Activity of kudzu bugs on soybean foliage was not significantly different across time periods. Over the duration of this choice test, activity of kudzu bugs on soybean gradually declined from 21.3% to 18.0%, at 1 hour and at 96 hours, respectively (Table 7). This similarity could be expected as kudzu bugs acclimated to soybean, which is considered a suitable host plant for kudzu bug.

Activity of kudzu bugs on leaf foliage (29.0%) and stems (31.1%) of soybean plants were not significantly different (Table 8). Activity of kudzu bugs classified as off of soybean plants was significantly different from those on leaves and stems.

Similarly, when compared among sexes, activity of kudzu bugs on soybean foliage did not differ (Table 9). Activity ranged from 17.8% for lone males to 21.5% for lone females. This activity is similar to the activity levels across sexes with kudzu, another common host plant, and is consistent with the knowledge that these two plant species are known, suitable host plants for kudzu bug. Kudzu and soybean plants have similar statistical responses of active kudzu bugs during the testing period. However, when examining the actual percentage of adults active on each plant throughout the duration of the testing period, activity on kudzu was 30 to 60% higher on kudzu than on soybean.

No Plant Choice

As this experiment progressed over time, kudzu bugs became significantly less active when classified as off of plants (Table 10). Percent activity ranged from 61.2% at the beginning to 43.5% at the end of the experiment. This finding is somewhat counterintuitive because kudzu bugs off of plants would be expected to remain active in efforts to locate a suitable host plant.

When compared among sexes, differences in activity of kudzu bugs when not on any plant were observed (Table 10). Paired females were significantly more active off of plants than lone females. However, activity of paired females was not significantly different than activity of paired males or lone males.

Table 10. Comparisons of adult kudzu bugs that were classified as 'active' but were not located on plant material by time of exposure and sex in choice tests.

	Off of Plant
Hour[1]	$F_{(7, 2158)} = 11.62, P < 0.0001$
1	61.20 ± 18.44 **A**[2]
2	51.41 ± 18.44 **B**
4	45.32 ± 18.44 **C**
8	45.00 ± 18.44 **C**
24	41.28 ± 18.44 **C**
48	43.55 ± 18.44 **C**
72	43.79 ± 18.44 **C**
96	43.46 ± 18.44 **C**
Sex[3]	$F_{(3, 390.1)} = 2.30, P = 0.0767$
PF	51.92 ± 18.54 **A**
PM	48.18 ± 18.54 **AB**
F	41.56 ± 18.54 **B**
M	45.84 ± 18.54 **AB**

[1]Hour observations are time (in hours) since kudzu bugs were introduced into petri dishes.
[2]Mean percent activity ± SE; means followed by the same letter within columns are not significantly different (P > 0.05).
[3]Sex corresponds with the gender of insects used. PF (paired female) and PM (paired male) are insects that were together in a petri dish. F (female) and M (male) are kudzu bugs that were lone insects in petri dishes.

Choice Test Evaluated by Plant Species

Comparisons of all 11 choice combinations yielded interesting and inconsistent results (Table 11). Although the activity of kudzu bugs was similar at each interval throughout the choice test experiment (data not shown), activity within different plant combinations illustrated interesting differences between kudzu and soybean (Fig. 28). Percent adult activity tended to be lowest on ragweed.

For the BR combination (bush honeysuckle and ragweed), ragweed had the highest percentage of active kudzu bugs; however, they were not significantly different from each other or from those off the plant. In the KB combination (kudzu and bush honeysuckle), the highest percentage of kudzu bugs were significantly more active on kudzu than bush honeysuckle. With the KR combination (kudzu and ragweed), kudzu had the highest percent of active kudzu bugs, but activity of kudzu bugs on the two plant species was not statistically different. When kudzu and soybean were paired (KS combination), a higher percentage of kudzu bugs was active on soybean, but this difference was not significant. In the SB combination (soybean and bush honeysuckle), bush honeysuckle had the highest percent of active insects, but values were not significantly different. When soybean and ragweed were paired together (SR), soybean had the highest percentage of active insects, but plant species were not significantly different.

Table 11. Insects that were classified as 'active' during choice tests. Each combination of plant species is separated to show activity level on each individual plant species (kudzu [K], soybean [S], bush honeysuckle [BH], ragweed [R], and off the plant [X]).

Plant Combination	Plant	Mean Percent Activity ± Standard Error[1]
BR	B	57.96 ± 11.25 **DE**
BR	R	67.61 ± 11.37 **BCDE**
BR	X	36.28 ± 16.58 **BCDE**
KB	B	59.09 ± 11.36 **CDE**
KB	K	85.01 ± 11.20 **A**
KB	X	48.19 ± 16.62 **ABCDE**
KR	K	67.10 ± 11.04 **BCDE**
KR	R	61.30 ± 11.92 **BCDE**
KR	X	36.79 ± 16.62 **BCDE**
KS	K	70.98 ± 10.81 **ABCD**
KS	S	72.32 ± 11.78 **ABCDE**
KS	X	42.56 ± 16.65 **ABCDE**
SB	B	71.70 ± 11.08 **ABCD**
SB	S	65.68 ± 11.66 **BCDE**
SB	X	37.05 ± 16.61 **BCDE**
SR	R	57.98 ± 11.12 **DE**
SR	S	74.75 ± 11.62 **ABCD**
SR	X	39.04 ± 16.39 **BCDE**

[1]Means followed by the same letter within columns are not significantly different ($P > 0.05$).

Table 11 (continued). Insects that were classified as 'active' during choice tests. Each combination of plant species is separated to show activity level on each individual plant species (kudzu [K], soybean [S], bush honeysuckle [BH], ragweed [R], and off the plant [X]).

Plant Combination	**Plant**	**Mean Percent Activity ± Standard Error[1]**
KBR	B	61.26 ± 11.18 **CDE**
KBR	K	71.04 ± 11.91 **ABCDE**
KBR	R	62.64 ± 12.13 **BCDE**
KBR	X	39.15 ± 16.73 **ABCDE**
KBS	B	62.08 ± 11.22 **CDE**
KBS	K	64.62 ± 12.24 **BCDE**
KBS	S	76.39 ± 12.50 **ABCD**
KBS	X	41.14 ± 16.34 **ABCDE**
KSR	K	75.17 ± 10.84 **ABC**
KSR	R	58.76 ± 12.67 **CDE**
KSR	S	75.64 ± 12.43 **ABCD**
KSR	X	43.31 ± 16.61 **ABCDE**
SBR	B	60.86 ± 11.13 **CDE**
SBR	R	69.83 ± 16.39 **ABCDE**
SBR	S	81.18 ± 11.99 **AB**
SBR	X	39.51 ± 16.63 **ABCDE**
KSRB	B	58.15 ± 11.75 **CDE**
KSRB	K	66.62 ± 11.65 **BCDE**
KSRB	R	51.46 ± 12.58 **E**
KSRB	S	62.96 ± 15.08 **ABCDE**
KSRB	X	35.42 ± 16.78 **BCDE**

[1]Means followed by the same letter within columns are not significantly different ($P > 0.05$).

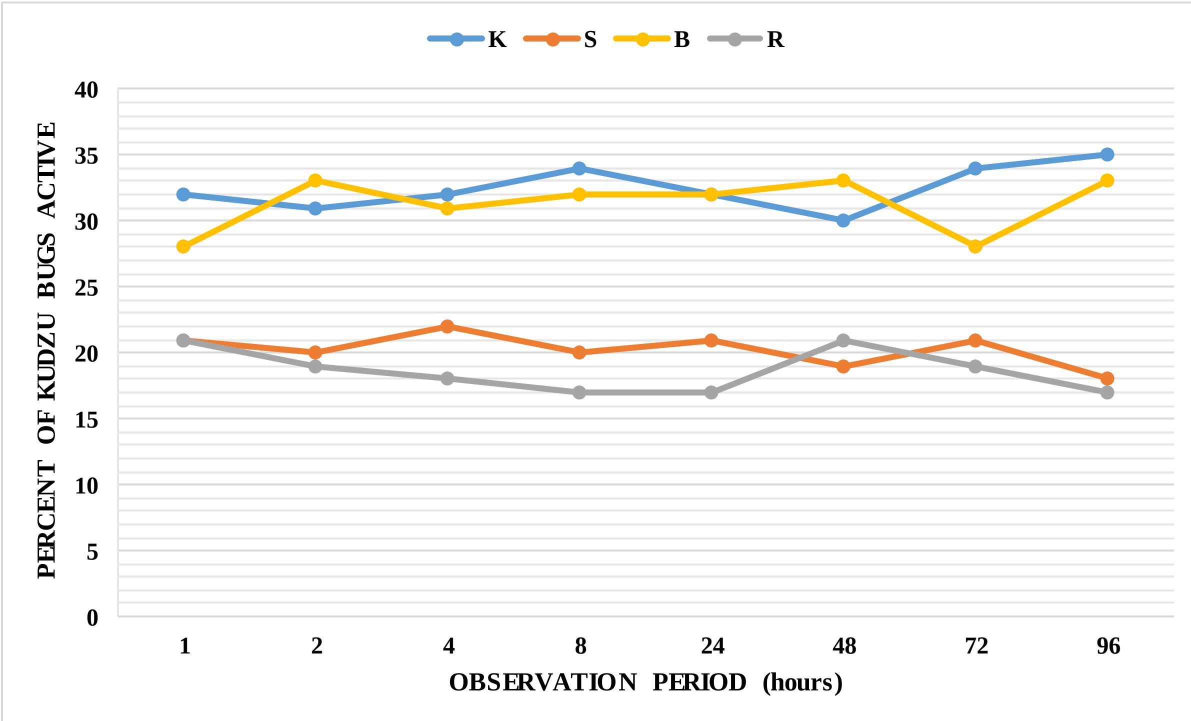

Figure 28. Percent of kudzu bugs active on four plant species throughout the choice test.

In the KBR (kudzu, bush honeysuckle, and ragweed) combination, kudzu had the highest percentage of active insects but was not significantly different from any other plant options (bush honeysuckle, ragweed, or off the plant). When kudzu, bush honeysuckle, and soybean were combined (KBS), soybean had the highest percentage of active insects, but again, no significant differences were documented. In the KSR combination (kudzu, soybean, ragweed), activity on kudzu and soybean was similar but was not significantly different from the other options. With the SBR (soybean, bush honeysuckle, and ragweed) plant combination, soybean had the highest percentage of active insects, and activity on soybean was significantly greater than activity on bush honeysuckle. However, percentages of kudzu bugs active on ragweed and active off the plant are both statistically similar to the percentage of kudzu bugs active on bush honeysuckle and soybean.

When all plants were combined (KSRB; kudzu, soybean, ragweed, and bush honeysuckle), kudzu bug activity was highest on kudzu. However, this activity was not significantly different from insects active on the other three plant species or off of the plant altogether. This expresses that kudzu plants are still the most suitable host plants to kudzu bugs.

Summary

Adult kudzu bugs were active on all four plant species in both experiments. In the no-choice experiment, kudzu bugs were significantly less active on ragweed than on kudzu, soybean, or bush honeysuckle. On all plant species, kudzu bug was 15 to 50 times more active on leaves than on stems. Adults were most active within the first 24 hours. Paired male and female kudzu bugs were more active overall, possibly suggesting movement for mate location. In the choice experiment, activity of insects throughout the duration of the experiment was consistently higher on kudzu and bush honeysuckle than on ragweed or soybean. The similarities of activity of kudzu bug on kudzu and bush honeysuckle, as well as the similarities of activity on soybean and ragweed, were not expected. Kudzu was the most preferred host plant while ragweed was the least preferred. Future studies will need to be conducted to further evaluate the suitability of these alternate host plants for reproduction and survival of kudzu bug to understand their influences on this invasive pest species.

CHAPTER IV
ASSESSMENT OF BIOLOGICAL CONTROL OF KUDZU BUG

Introduction

Kudzu bug, *Megacopta cribraria* (F.) (Hemiptera: Plataspidae), is native to Asia and was discovered in nine northeastern counties in Georgia (United States) in mid-October 2009 (Jenkins et al., 2010). It is the only representative of the family Plataspidae in the continental United States and has been documented in 13 states and the District of Columbia (kudzubug.org). Kudzu bugs are attracted to legumes, especially kudzu, *Pueraria montana* var. *lobata* (Willd), and soybean, *Glycine max* (L.) Merrill, where they feed by sucking sap from stems, petioles, and leaves (Halbert & Eger, 2010) (Zhang et al., 2012).

Since the kudzu bug is an invasive species in its new expanded range, no host-specific natural enemies are present to reduce population levels. Several biological control organisms exist as possible tools to reduce populations of kudzu bug. One is a parasitic wasp, *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygastridae), which is of the same native origin as kudzu bug. *P. saccharalis* is an egg parasitoid. Other options include the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, and two native dipteran species, *Strongygaster triangulifera* (Loew) and *Phasia robertsonii* (Townsend) (Diptera: Tachinidae).

The fungus infects immatures and adult kudzu bugs, while *S. triangulifera* and *P. robertsonii* have only been found to parasitize adult kudzu bugs. The two dipterans are generalist parasitoids and commonly parasitize other species of insects. *P. saccharalis* is the only biological control agent that is currently specific to kudzu bug in its invasive range. This small-sized wasp (~1 mm in size) parasitizes members of the family Plataspidae, and currently, kudzu bug is the only representative of this family in the continental United States. *P. saccharalis* is actually known to parasitize only four species (Gardner et al., 2013a). This host-specific characteristic makes it a prime candidate for biological control of kudzu bug. Naturalized populations of *P. saccharalis* have been found in several areas of the southeastern United States – including Alabama, Georgia, Florida, and Mississippi – which further shows that it is a suitable candidate for biological control of kudzu bug in this new, expanded range (Gardner et al., 2013b).

Beauveria bassiana is a well-documented, generalist entomopathogenic fungus that is known to infect many different arthropod species. It has been observed on kudzu bug in different areas of the southeastern United States since it was first documented in soybean fields in South Carolina in 2012 (Seiter et al., 2014). Interestingly, the fungus has been found to infect only kudzu bugs in the areas where it has been documented in kudzu patches in Tennessee.

In a 2013 study in Auburn, AL, *S. triangulifera* parasitized approximately 5% of collected kudzu bugs (Golec et al., 2013). A single occurrence of parasitism of kudzu bug by *P. robertsonii* was observed in 2012 in Tifton, GA (Ruberson et al., 2012). While both incidences of parasitism by these native species are encouraging, they are both generalists and more research is needed to evaluate their status as natural enemies of kudzu bug. Of these four species of natural enemies, the parasitic wasp, *P. saccharalis*, and the entomopathogenic fungus, *B. bassiana*, are the best candidates for biological control of kudzu bug thus far.

While completing another study (see Chapter II), a fungal pathogen infecting immature and adult kudzu bugs in kudzu was first observed in Polk County, TN on 1

September 2015. The fungal pathogen was visually identified as *B. bassiana* by Mary Dee of the Department of Entomology and Plant Pathology at the University of Tennessee, Knoxville and later confirmed by Dr. Maribel Portilla of the USDA-ARS in Stoneville, MS. *B. bassiana* has been known to infect kudzu bugs in its natural and invasive range (Ruberson et al., 2012; Seiter et al., 2014). Despite recorded infections of kudzu bugs by *B. bassiana* in South Carolina in 2012 and Georgia in 2013, little is known about its impact on population densities. In addition, little information on the natural enemies of kudzu bug in Tennessee is available. This study was designed to catalogue and observe natural enemies of *M. cribraria* in Tennessee. If natural enemies were present, their seasonalities and phenologies were assessed, as well as their relationship with and impact on kudzu bug populations. Information collected on natural enemies will assist in the future assessment, development, improvement, and implementation of kudzu bug management programs, including biological control.

Materials and Methods

During population monitoring of kudzu bug on kudzu in 2015 (refer to Chapter II), several studies were developed and initiated to survey and assess natural enemies of kudzu bug in Tennessee. The primary focus was to monitor for presence of the parasitic wasp, *P. saccharalis*, since it was reported in the southeastern United States but not documented in Tennessee. Egg masses of kudzu bug were collected from 18 May until 17 September 2015, when eggs were no longer present in kudzu patches. Upon visiting each of the 14 regularly sampled sites (see Chapter II), kudzu bug egg masses were collected and taken to the laboratory for observation. Ten egg masses were collected from each site per visit when possible. Because eggs were laid on plant material, small portions of kudzu were collected so that egg masses would stay intact. On each sampling date, all egg masses from each site were placed into petri dishes (100 x 15 mm) that were sealed with parafilm, taken to the laboratory, and left alone until most were hatched (three months or more so that all viable eggs were allowed to hatch). No moisture was added to petri dishes to prevent mold development.

After allowing sufficient time for hatching, egg masses and overall contents of petri dishes were examined under a microscope to observe for presence of kudzu bug immatures, as well as *P. saccharalis* or other egg parasitoids. Unhatched egg masses were examined closely for overall color – if color was suspicious (eggs grey in coloration), a fine insect pin (Bioquip insect pins, size 0) was used to rupture the egg to examine the contents inside. When kudzu bug eggs are parasitized by *P. saccharalis*, they appear dark grey to black. It is believed that eggs infected by *B. bassiana* can cause developing kudzu bugs to die in the egg, therefore also causing a darkened egg coloration (personal communication, Ian Knight, University of Georgia).

Yellow pan traps were used to monitor for parasitoids at eight of the 14 sampled kudzu sites (Bauer et. al., 2011). In Knox County, traps were placed at three sites (one site each in north, south, and west Knox County [see Chapter II for further description]). Pan traps were also placed at each of the sites outside of Knox County (five total, one each in Blount, Monroe, McMinn, Polk [Tennessee], and Murray County [Georgia]) (Fig. 5). Traps were placed into kudzu patches on the first sampling date, 18 May 2015, and left for the duration of the season. At each site selected for parasitoid monitoring, three pan traps were placed in different locations throughout the kudzu patch. Traps consisted of a yellow bowl (355 mL volume, 18 cm diameter) mounted to a wooden stake (1 m off ground surface). A 50:50 solution of propylene glycol and water (approximately 236 mL per pan trap) was placed in each pan trap. Traps were monitored, contents collected, and the propylene glycol and water solution was replaced on each weekly sampling date. The contents of each trap were poured into a paint strainer to capture all collected specimens and taken to the laboratory for sorting and processing. Upon sorting, all suspect wasp specimens were placed in vials (2 dram / 7.4 ml) containing a 95% EtOH solution. These were identified using an existing taxonomic key (Eger et al., 2012) as well as sent to Richard Evans (USDA-ARS, Stoneville, MS) for confirmation.

The confirmation of *B. bassiana* on kudzu bug on 1 September 2015 in Polk County, TN led to the development of a six-week study to monitor the prevalence of this pathogen and the associated mortality on kudzu. This study was initiated on 16 September 2015 and concluded on 19 October 2015.

Infection of kudzu bug by *B. bassiana* was monitored weekly at two locations in eastern Tennessee – one site each in Polk (N35.16464 W 084.68258) and Blount (N 35.74912 W 083.96170) Counties (Fig. 29). At each location, 10 collection sites were designated. Two 40 m transects, separated by at least 5 m, were measured. In each transect, one collection site was established every 10 m. Each transect had a total of five collection sites. At each collection site each week, approximately 1.91 m of kudzu stems and foliage containing infected and non-infected kudzu bugs were clipped and placed into a 38-cm diameter net, secured in 26.8 x 27.3 cm plastic bags, and taken to the laboratory to ensure accurate counts of insects. In the laboratory, all immature and adult kudzu bugs were examined and counted; the percent of kudzu bugs infected by *B. bassiana* was calculated (Table 12). Bugs were classified as ‘infected’ when they had fungal mycelia emerging from the body.

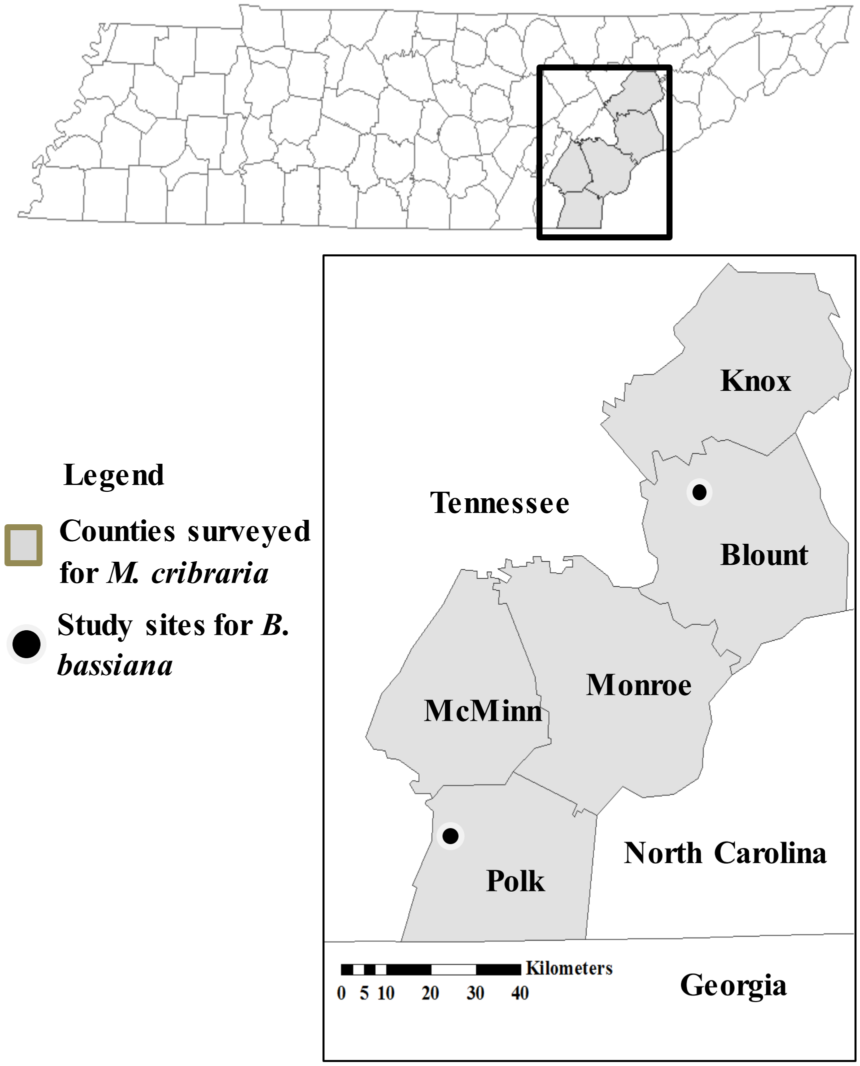

Figure 29. Locations of *Beauveria bassiana* study sites and counties monitored for kudzu bug phenology.

Table 12. Number (and percent infected) of kudzu bugs (immatures, adult, and total) at sampling locations in Blount and Polk Counties, TN, 16 September to 19 October 2015.

	Number Collected			% Infected		
County	**Immature**	**Adult**	**Total**	**Immature**	**Adult**	**Average**
Blount	645	1,016	1,661	71.30	25.20	43.11
Polk	176	532	708	90.91	49.06	59.46
Total	821	1,548	2,369	75.50	33.40	47.99

Results and Discussion

Pan Traps

No kudzu bug egg parasitoids, including *P. saccharalis*, were found in the pan trap surveys. From 18 May to late September, approximately 200 pan trap collections were processed to check for presence of parasitoids. While at least 15 other species of parasitic wasps were recovered from pan traps, none of those species were believed to have emerged from kudzu bug eggs.

Assessment of Egg Parasitoids

No egg parasitoids were reared from any of the field-collected kudzu bug egg masses. Approximately 2,600 egg masses were collected in kudzu patches from 18 May to 17 September 2015. Each egg mass contained an average of 16.7 eggs; thus, 43,703 eggs were collected and evaluated. A dark grey to black coloration was seen in the eggs several times, but rupturing the eggs only found dead unhatched kudzu bugs.

Assessment of Beauveria bassiana

Overall mortality levels of both adult and immature kudzu bugs infected by *B. bassiana* increased throughout this study (Fig. 30). Mortality of immature kudzu bugs ultimately reached 100% by the end of sampling in Polk County on 19 October 2015. Adult infection levels at both sites started near zero and increased over the season. Unfortunately, the percent of infected adults did not increase as much throughout the season as the percent of infected immatures. Throughout the duration of the season, the average mortality level of adults was only 33.4% while the total mortality level of immatures was 75.5% (Table 12). Adult mortality level was only 25.2% in Blount County yet was almost double this number, 49.1%, in Polk County. Average immature mortality was 71.30% in Blount County yet reached 90.9% in Polk County. No distinct difference in the percentages of adult male and adult female mortality levels was observed.

As a result of the high mortality of immature kudzu bugs during 2015, population densities of emergent adult kudzu bugs in 2016 may be lower than those previously recorded. Furthermore, although the impact of *B. bassiana* on kudzu bugs was closely monitored at only two sites, the pathogen was eventually documented at 12 additional sites in three other counties (Knox, McMinn, and Monroe) that also were sampled regularly in 2015 (Fig. 30). It is important to note that these regularly visited sites were sampled on 15 dates in 2015 before *B. bassiana* was discovered. Sites visited over a 14-week period in 2014 never revealed signs that the entomopathogen was present, even though sampling occurred at the same time that the pathogen was discovered in 2015. In addition, many different insect species were present in these same kudzu patches but they were unaffected by *B. bassiana*. In fact, many insect

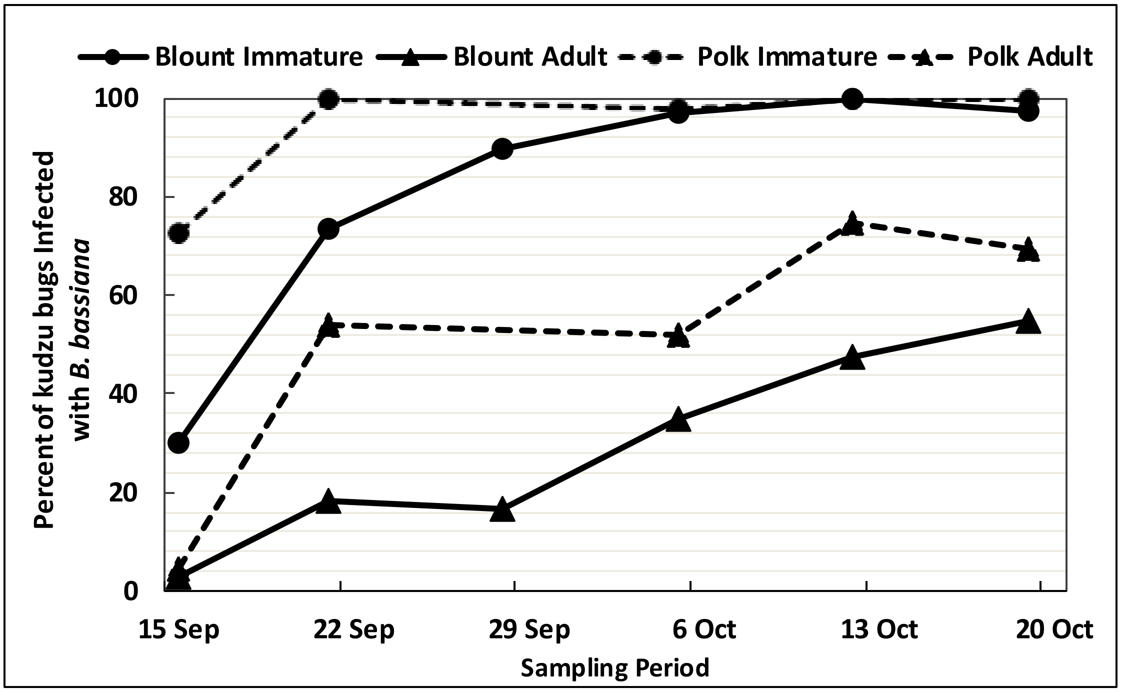

Figure 30. Percent of kudzu bugs infected with *Beauveria bassiana* in Blount and Polk Counties in eastern Tennessee, 2015.

species of the order Hemiptera (true bugs, piercing-sucking mouthparts) were occupants of these patches, yet were not killed or infected by the fungus.

Summary

No kudzu bug egg parasitoids were recovered from the pan trap surveys or egg collections. While other species of parasitoids were recovered from pan traps, only kudzu bugs were recovered from the eggs. This research will be repeated in future years to continue the monitoring efforts for kudzu bug egg parasitoids. Hopefully *P. saccharalis* will continue to be found in other areas of the southeastern United States. If this is the case, it could continue to spread and eventually become established throughout the new range of kudzu bug, including more northern areas in the United States.

Although the phenomenon of the epizootic entomopathogen *B. bassiana* on kudzu bug has been observed in other areas of the southeastern United States and infects kudzu bug in Asia, prevalence or percent mortality of kudzu bug has not been closely examined (Seiter et al., 2014). This study illustrates the impact of *B. bassiana* on immature and adult kudzu bugs. The presence of *B. bassiana* will contribute to greater overall management against kudzu bug. Future efforts will consist of monitoring for *B. bassiana* on a wider scale throughout Tennessee, including the examination of infection levels in several additional counties, as well as continuing to conduct sampling to assess the impact on emergent population levels in 2016. The information gained from this study will help to better address the potential for biological control of kudzu bug.

CHAPTER V
CONCLUSIONS

The kudzu bug, *Megacopta cribraria* Fabricius (Hemiptera: Heteroptera: Plataspidae), is an invasive species from Asia that first appeared in the United States in fall of 2009. Since then, it has spread rapidly to 13 states and the District of Columbia. Kudzu bug was first found in Tennessee in 2012 and has since spread rapidly across the state. Kudzu bugs are nuisance pests of homeowners and agricultural pests of soybean (*Glycine max* (L.) Merrill; Fabales: Fabaceae). These insects can cause great yield losses in soybean plots if plants are left untreated. Kudzu bugs undergo two generations per year in their expanded range; the first generation develops on kudzu (*Pueraria montana* variety *lobata*, [Willd.] Maesen and Almeida; Fabales: Fabaceae) and the second generation can develop on kudzu and/or soybean. Several methods of control exist to manage populations of kudzu bugs, including several different types of insecticides. More information was needed to assess seasonality and phenology of kudzu bug in Tennessee, thus, a 2-year research study was initiated in 2014.

Population density studies from 2014 and 2015 revealed distinct trends throughout the sampling season. Both years, bugs were present in kudzu patches from early May to mid-November. Development of kudzu bug from egg hatch to adult eclosion takes four to six weeks, and this period was consistent with population density fluctuations in eastern Tennessee. Population densities peaked three times throughout the season, the first two peaks represented the end of the first and second generations of kudzu bug development. After the conclusion of the first generation, kudzu bugs moved to locations with other suitable host plants (most likely soybeans). The third population peak occurred near the end of the season and most likely resulted from the movement of kudzu bugs back into kudzu patches before beginning to overwinter. Eggs, which averaged 16.7 laid per mass, were present on kudzu from mid-May to mid-September in 2015.

Behavioral responses of kudzu bugs were observed on four plant species to evaluate influence of these plants on adult kudzu bugs. Kudzu and soybean were evaluated because they are known host plants of kudzu bug. Bush honeysuckle (*Lonicera maackii* [Rupr.]; Family Caprifoliaceae) and common ragweed (*Ambrosia artemisiifolia* L.; Family Asteraceae) were evaluated because immature and adult kudzu bugs were observed inhabiting these species, as well as ovipositing on foliage of both plants. Based on insect behavior, kudzu was the most preferred plant species and common ragweed was the least preferred. Over the duration of the study, activity levels were similar on kudzu and bush honeysuckle; these activity levels were higher than those on soybean and ragweed.

Paratelenomus saccharalis (Dodd) (Hymenoptera: Platygastridae), an egg parasitoid of insects in the family Platygastridae, have been found in Georgia, Alabama, Mississippi, and Florida. Efforts (via pan trap and egg collections) to locate them in Tennessee proved unsuccessful. However, another biological control agent of kudzu bug was found at several locations in Tennessee in 2015.

The fungus *Beauveria bassiana* (Balsamo) Vuillemin was found in kudzu patches infecting and killing adult and nymph kudzu bugs. By the end of the sampling season, mortality of kudzu bug nymphs reached 100%, while adult mortality was only 69%. Of all adult and immature kudzu bugs collected, 33% and 75%, respectively, died due to fungal infection. This high mortality level greatly reduced the number of overwintering adults, and as a result, may impact emerging population densities of adult kudzu bugs in 2016. These lower numbers of overwintering kudzu bugs will mitigate impacts of this invasive species as a pest of soybeans and a nuisance to homeowners.

This study provided valuable new information about the kudzu bug in Tennessee. The information gained will be used in the future to better manage kudzu bug population levels and mitigate its impact as an agricultural pest and as a nuisance.

REFERENCES

Bauer LS, Hansen J & Gould J (2011) Yellow pan traps: A simple method for trapping larval parasitoids released for biological control of the emerald ash borer. http://nrs.fs.fed.us/ disturbance/invasive_species/eab/local-resources/downloads/YPT_Method.pdf.

Beardsley J, John W & Fluker S (1967) *Coptosoma xanthogramma* (White), (Hemiptera: Plataspidae) a new pest of legumes in Hawaii. Proceedings, Hawaiian Entomol. Soc. 19: 367-372.

Blount JL, Buntin GD & Roberts PM (2016) Effect of planting date and maturity group on soybean yield response to injury by *Megacopta cribraria* (Hemiptera: Plataspidae). J. Econ. Entomol. 109: 207-212. doi:10.1093/jee/tov314.

Britt K, Standish C, Grant J & Vail K (2016) Invasive pest and fall home invader: Kudzu bug. University of Tennessee Extension: 1-4 pp. https://extension.tennessee.edu/ publications/Documents/W358.pdf

Brown AM, Huynh LY, Bolender CM, Nelson KG & McCutcheon JP (2014) Population genomics of a symbiont in the early stages of a pest invasion. Mol. Ecol. 23: 1516-1530. doi:10.1111/mec.12366.

(Committee) Definitions Subcommittee of the Invasive Species Management Committee (2006) Invasive species definition clarification and guidance white paper. National Invasive Species Council: 1-11 pp.

Del Pozo-Valdivia AI & Reisig DD (2013) First-generation *Megacopta cribraria* (Hemiptera: Plataspidae) can develop on soybeans. J. Econ. Entomol. 106: 533-535. doi:10.1603/ec12425.

Eger JE, Ames LM, Suiter DR, Jenkins TM, Rider DA & Halbert S (2012) Occurrence of the Old World bug *Megacopta cribraria* (Fabricius) (Heteroptera: Plataspidae) in Georgia: A serious home invader and potential legume pest. Insecta Mundi 121: 1-11.

Eviner VT, Garbach K, Baty JH & Hoskinson SA (2012) Measuring the effects of invasive plants on ecosystem services: Challenges and prospects. Invasive Plant Science and Management 5: 125-136. doi:10.1614/ipsm-d-11-00095.1.

Frye MJ, Hough-Goldstein J & Sun J-H (2007) Biology and preliminary host range assessment of two potential kudzu biological control agents. Environ. Entomol. 36: 1430-1440. doi:10.1093/ee/36.6.1430.

Fulton H (2010) The regulatory corner: Invasive against invasive - an exotic bug that attacks an invasive weed. Midsouth Entomol. 3: 65-66.

Gardner WA, Blount JL, Golec JR, Jones WA, Hu XP, Talamas EJ, Evans RM, Dong X, Ray C, Buntin GD, Gerardo NM & Couret J (2013a) Discovery of *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygastridae), an egg parasitoid of *Megacopta cribraria* F. (Hemiptera: Plataspidae) in its expanded North American range. J. Entomol. Sci. 48: 355-359.

Gardner WA, Peeler HB, LaForest J, Roberts PM, Sparks AN, Greene JK, Reisig DD, Suiter DR, Bacheler JS, Kidd KA, Ray C, Hu XP, Kemerait RC, Scocco EA, Eger JE, Ruberson JR, Sikora EJ, Herbert DA, Campana C, Halbert S, Stewart SD, Buntin GD, Toews MD & Bargeron CT (2013b) Confirmed distribution and occurrence of *Megacopta cribraria* (F.) (Hemiptera: Heteroptera: Plataspidae) in the southeastern United States. J. Entomol. Sci. 48: 118-127.

Golec JR & Hu XP (2015) Preoverwintering copulation and female ratio bias: Life history characteristics contributing to the invasiveness and rapid spread of *Megacopta cribraria* (Heteroptera: Plataspidae). Environ. Entomol. 44: 411-417. doi:10.1093/ee/nvv014.

Golec JR, Hu XP, Ray C & Woodley NE (2013) *Strongygaster triangulifera* (Diptera: Tachinidae) as a parasitoid of adults of the invasive *Megacopta cribraria* (Heteroptera: Plataspidae) in Alabama. J. Entomol. Sci. 48: 1-3.

Greenstone MH, Tillman PG & Hu JS (2014) Predation of the newly invasive pest *Megacopta cribraria* (Hemiptera: Plataspidae) in soybean habitats adjacent to cotton by a complex of predators. J. Econ. Entomol. 107: 947-954. doi:10.1603/ec13356.

Gurevitch J & Padilla DK (2004) Are invasive species a major cause of extinctions? TRENDS in Ecology and Evolution 19: 470-474. doi:10.1016/j.tree.2004.07.005.

Halbert S & Eger JE (2010) Pest alert - Bean plataspid, *Megacopta cribraria* (Fabricius) (Hemiptera: Plataspidae) an exotic legume pest established in Georgia. Florida Department of Agriculture and Consumer Services. DACS-P-01725.

Horn S & Hanula JL (2011) Influence of trap color on collection of the recently-introduced bean plataspid, *Megacopta cribraria* (Hemiptera: Plataspidae). J. Entomol. Sci. 46: 85-87. doi:10.18474/0749-8004-46.1.85.

Hosakawa T, Nikoh N & Fukatsu T (2014) Fine-scale geographical origin of an insect pest invading North America. PLoS ONE 9. doi:10.1371/10.1371/journal.pone.0089107.g001.

Hosokawa T, Kikuchi Y, Shimada M & Fukatsu T (2007) Obligate symbiont involved in pest status of host insect. Proc Biol Sci 274: 1979-1984. doi:10.1098/rspb.2007.0620.

Hulme PE (2009) Trade, transport and trouble: Managing invasive species pathways in an era of globalization. J. Applied Ecol. 46: 10-18. doi:10.1111/j.1365-2664.2008.01600.x.

Imai K, Miura K, Iida H, Reardon R & Fujisaki K (2010) Herbivorous insect fauna of kudzu, *Pueraria montana* (Leguminosae), in Japan. Florida Entomol. 93: 454-456. doi:10.1653/024.093.0323.

Jenkins TM & Eaton TD (2011) Population genetic baseline of the first plataspid stink bug symbiosis (Hemiptera: Heteroptera: Plataspidae) reported in North America. Insects 2: 264-272. doi:10.3390/insects2030264.

Jenkins TM, Eaton TD, Suiter DR, Eger JE, Ames LM & Buntin GD (2010) Preliminary genetic analysis of a recently-discovered invasive true bug (Hemiptera: Heteroptera: Plataspidae) and its bacterial endosymbiont in Georgia, USA. J. Entomol. Sci. 45: 1-2.

Mitich LW (2000) Kudzu [*Pueraria lobata* (Willd.) Ohwi]1. Weed Technol. 14: 231-235. doi:10.1614/0890-037x(2000)014[0231:kplwo]2.0.co;2.

Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T & Tsomondo T (2000) Economic and environmental threats of alien plant, animal, and microbe invasions. Agriculture, Ecosystems and Environment 84: 1-20.

Pimentel D, Zuniga R & Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Econ. 52: 273-288. doi:10.1016/j.ecolecon.2004.10.002.

Ruberson JR, Takasu K, David Buntin G, Eger JE, Gardner WA, Greene JK, Jenkins TM, Jones WA, Olson DM, Roberts PM, Suiter DR & Toews MD (2012) From Asian curiosity to eruptive American pest: *Megacopta cribraria* (Hemiptera: Plataspidae) and prospects for its biological control. Applied Entomol. and Zool. 48: 3-13. doi:10.1007/s13355-012-0146-2.

Seiter NJ, Grabke A, Greene JK, Kerrigan JL & Reay-Jones FPF (2014) *Beauveria bassiana* is a pathogen of *Megacopta cribraria* (Hemiptera: Plataspidae) in South Carolina. J. Entomol. Sci. 49: 326-330. doi:10.18474/0749-8004-49.3.326.

Seiter NJ, Greene JK & Reay-Jones FPF (2013) Reduction of soybean yield components by *Megacopta cribraria* (Hemiptera: Plataspidae). J. Econ. Entomol. 106: 1676-1683. doi:10.1603/ec13121.

Sharkey TD & Loreto F (1993) Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. Oecologia 95: 328-333.

Simberloff D (2008) We can eliminate invasions or live with them. Successful management projects. Biological Invasions 11: 149-157. doi:10.1007/s10530-008-9317-z.

Suiter DR, Eger JE, Gardner WA, Kemerait RC, All JN, Roberts PM, Greene JK, Ames LM, Buntin GD, Jenkins TM & Douce GK (2010) Discovery and distribution of *Megacopta cribraria* (Hemiptera: Heteroptera: Plataspidae) in northeast Georgia. J. Int. Pest Management 1: 1-4. doi:10.1603/ipm10009.

Zhang Y, Hanula JL & Horn S (2012) The biology and preliminary host range of *Megacopta cribraria* (Heteroptera: Plataspidae) and its impact on kudzu growth. Environ. Entomol. 41: 40-50. doi:10.1603/EN11231.

VITA

Kadie Elizabeth Britt was born on February 25, 1991 at Union Regional Medical Center in Monroe, North Carolina to her parents Tim and Tammy Britt. She grew up in the town of Marshville, North Carolina where she attended Marshville Elementary School, East Union Middle School, and Forest Hills High School. From August 2009 to May 2013, she attended Emory & Henry College in Emory, Virginia where she obtained her Bachelor of Science degree in Environmental Studies with a minor in Biology.

In the summer of 2012, Kadie took her first entomology course and fell in love with the subject. It is at that point that she decided to pursue an upper level degree in entomology and devote her life to science and research because she realized that science is what makes her happy. Kadie began a Master of Science degree in the Department of Entomology and Plant Pathology at the University of Tennessee in May of 2014 and concluded her program in August 2016.

Kadie loves anything science-related. She loves spending time with her boyfriend, Ked Byrd, as well as visiting with her parents, friends, and two cats, Lizzie and Stella.