

**Influence of Abiotic and Biotic Factors on Kudzu Bug Populations in Kudzu
in Eastern Tennessee**

**A Thesis Presented for the
Master of Science
Degree
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**Amy Marie Michael
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DEDICATION

To my parents – Thank you for loving me and supporting me through everything. Thank you for teaching me how to be grateful, humble, and kind.

To David – Thank you for being a constant source of inspiration and motivation. Thank you for teaching me how to be brave and find my own potential.

To my enormous family – Thank you for keeping me grounded. Thank you for teaching us all how to rely upon each other.

To my friends – Thank you for being the village that helped me rise. Thank you for teaching me how to laugh, how to cry, and how to live.

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ABSTRACT

The kudzu bug, *Megacopta cribraria* (F.) (Hemiptera: Plataspidae), was first discovered in the U.S. in 2009. Kudzu bug feeds primarily on invasive kudzu vine (*Pueraria montana* Lour. (Merr.) var. *lobata* (Willd.) [Fabales: Fabaceae]) but has become a pest of soybeans (*Glycine max* (L.) Merr. [Fabales: Fabaceae]) and homes throughout the South. Previous research has shown that established populations are bivoltine, and that the second generation of kudzu bugs are problematic for soybean growers in Tennessee. Most research to date has focused on management of this pest in soybeans. Large-scale ecological factors affecting kudzu bug populations have yet to be examined in depth within kudzu. This research aims to elucidate factors that impact kudzu bug populations. Research objectives are to: 1) investigate kudzu bug populations in eastern Tennessee, 2) elucidate abiotic factors that may impact kudzu populations, and 3) assess the impact of natural enemies on kudzu bug populations. Samples were gathered in kudzu patches in six eastern Tennessee counties to assess kudzu bug populations. Factors including climactic data were analyzed for population-level impacts. Natural enemies that were investigated included the entomopathogenic fungus, *Beauveria bassiana* (Bals.- Criv.) Vuill (Ascomycota: Hypocreales: Cordycipitaceae), and a previously undetected egg parasitoid of kudzu bugs (Hymenoptera: Encyrtidae). Percent infection by *B. bassiana* was estimated in each county via sampling along within-patch transects. Kudzu clippings were also screened for endophytic *B. bassiana*. Egg masses were collected from six counties and parasitoids allowed to emerge. Kudzu bug population densities have diminished substantially in the last four years in eastern Tennessee. Drought, temperature, and recent precipitation were all related to kudzu bug population densities. Egg parasitoids were recovered from kudzu bug eggs from early June – late

September 2017, and *B. bassiana* was recovered from late August – late October in both 2016 and 2017. Endophytic *B. bassiana* was observed in kudzu collected from all sites. The combination of these natural enemies may offer season-long biological control of *M. cribraria* and maintain kudzu bug populations below economically damaging levels for soybeans. This research will enhance understanding of factors driving kudzu bug population dynamics and aid in the refinement of management strategies.

TABLE OF CONTENTS

CHAPTER I Introduction and Literature Review	1
CHAPTER II Seasonality and Phenology of Kudzu Bug.....	12
Introduction.....	12
Materials and Methods.....	12
Results and Discussion	14
CHAPTER III Climatic Influences on Kudzu Bug Populations.....	20
Introduction.....	20
Materials and Methods.....	21
Results and Discussion	23
CHAPTER IV Three Years Later: <i>Beauveria bassiana</i> is a Persistent Entomopathogen of Kudzu Bug in Tennessee	27
Introduction.....	27
Materials and Methods.....	29
Results and Discussion	30
CHAPTER V First Record of a Parasitoid (Hymenoptera: Encyrtidae) on Eggs of <i>Megacopta cribraria</i> (Hemiptera: Plataspidae) in Tennessee and Initial Ecological Insights	35
Introduction.....	35
Materials and Methods.....	37
Results and Discussion	38
CHAPTER VI Conclusions	42
REFERENCES	47

APPENDIX.....	58
VITA	85

LIST OF TABLES

Table 1. Localities sampled for kudzu bug, Tennessee and Georgia, 2016 and 2017.....	60
Table 2. Numbers of kudzu bugs per life stage per year across 14 sites, 2016 and 2017.....	67
Table 3. Relative differences in kudzu bug densities between counties.....	68
Table 4. Week-wise differences in fifth-instar nymph densities, collected from kudzu in 2016 and 2017.....	70
Table 5. Week-wise differences in adult densities, collected from kudzu in 2016 and 2017.....	72
Table 6. Impact of factors on total kudzu bug numbers, weeks 1-19.	75
Table 7. Impact of factors on total kudzu bug numbers, weeks 20-31.	75
Table 8. Sites sampled for <i>Beauveria bassiana</i> in eastern Tennessee and Northern Georgia, 2016 and 2017.....	77
Table 9. Analysis of variance results for influence of site and week on percent infection with <i>B.</i> <i>bassiana</i> of kudzu bug by life stage.....	77
Table 10. Results of screening for endophytic <i>Beauveria bassiana</i> in kudzu, 21 Dec 2017.....	80

LIST OF FIGURES

Figure 1. Distribution of Kudzu in the United States (EDDMapS 2018).	59
Figure 2. Distribution of Kudzu Bug in the United States (Megacopta Working Group 2017)... 59	
Figure 3. Map of localities visited to assess kudzu bug populations, 2016 and 2017.	61
Figure 4. Adult kudzu bug population densities on kudzu in Knox County, TN over 4 years.	
*Data from 2014 and 2015 provided by Kadie Britt (2016).....	62
Figure 5. Estimated densities of kudzu bugs on kudzu by life stage in Knox County, TN in 2016	
(A) and 2017 (B).	62
Figure 6. Estimated densities of kudzu bugs on kudzu by life stage in Blount County, TN in 2016	
(A) and 2017 (B).	63
Figure 7. Estimated densities of kudzu bugs on kudzu by life stage in Monroe County, TN in	
2016 (A) and 2017 (B).	64
Figure 8. Estimated densities of kudzu bugs on kudzu by life stage in McMinn County, TN in	
2016 (A) and 2017 (B).	65
Figure 9. Estimated densities of kudzu bugs by life stage in Polk County, TN in 2016 (A) and	
2017 (B).	66
Figure 10. Estimated densities of kudzu bugs on kudzu by life stage in Murray County, GA in	
2016 (A) and 2017 (B).	67
Figure 11. Drought conditions in Tennessee throughout 2016 (left) and 2017 (right) (Artusa et al.	
2016). (A) Beginning of kudzu bug sampling [early May] (B) First generation of kudzu bug	
reaches adulthood [late Jun] (C) Week 20 [early Sep] (D) During the typical pre-	

overwintering peak [early Oct] (E) Kudzu bugs largely overwintering; end of sampling [mid-Nov 2016, late Oct 2017].	74
Figure 12. Sites surveyed for <i>Beauveria bassiana</i> , 2016 and 2017.....	76
Figure 13. Percent infection of adult kudzu bugs with <i>B. bassiana</i> by site and week, 2016 and 2017. Bars represent average percent infection $\pm$ SE at each collection location and horizontal brackets represent significant differences between means (Tukey's HSD, α = 0.05).	78
Figure 14. Percent infection of kudzu bug nymphs with <i>B. bassiana</i> by site and week, 2016 and 2017. Bars represent average percent infection $\pm$ SE at each collection location and horizontal brackets represent significant differences between means (Tukey's HSD, α = 0.05).	79
Figure 15. Number of kudzu bug eggs and egg masses collected per week across all localities in eastern Tennessee and northern Georgia, 2017.	81
Figure 16. Images of parasitoid recovered from eggs of <i>M. cribraria</i> in Tennessee and Georgia, 2017. (A) Female habitus. (B) Male habitus. Note the dimorphic antennal morphology. (C, D) SEM image detailing the female dorsal (C) and lateral (D) sclerites. (E) Female forewing. (F) Female dorsal mesosoma and detail of ultrastructure.....	82
Figure 17. Average parasitism rates in all counties, 2017. Shaded portion of graphs indicate opportunistic collections due to lower <i>M. cribraria</i> oviposition rates and are presented for conceptual purposes only.	83
Figure 18. Seasonality of parasitoid and <i>M. cribraria</i> emergence in Knox County, TN, 2017. ..	84

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Human existence has been intertwined with international trade for millennia, and we have come to rely upon introduced species in order to feed ourselves (Pimentel et al. 2005). The explosion of intercontinental trade that began with the Columbian Exchange and continues to this day has led to unprecedented introduction rates of non-native species (Hulme 2009, Mann 2011). When species are taken out of their native range, other species that have evolved to attack these species are often left behind. In the absence of natural enemies, these introduced species can thrive. Many of these introductions are intentional and are so successful that these species become embedded in the local economy and culture. Examples include introductions of tomatoes to Italy, potatoes to Ireland, and oranges to Florida (Mann 2011). However, many species also hitchhike on cargo, baggage, or even the transport vessel itself, and are accidentally introduced into new habitats (Hulme 2009). While the vast majority of these introductions cause no noticeable harm, some become serious invasive pests that dramatically alter the natural and human environments that they invade. According to the Invasive Species Advisory Committee Definitions Subcommittee: “Invasive species are those that are not native to the ecosystem under consideration and that cause or are likely to cause economic or environmental harm or harm to human, animal, or plant health” (ISAC 2006). Invasive species can cause massive ecological disruption by outcompeting or displacing native species, and are the chief cause of approximately 42% of Threatened or Endangered species classifications (Pimentel et al. 2005).

While imported goods can have an initial reprieve from natural enemies following their introduction, the arrival of their pests decades or centuries after their preferred hosts have

become established can have devastating impacts on the economy and human suffering (e.g., late blight of potato in Ireland and citrus greening disease in the U.S.) as those pests enjoy a similar newcomer's competitive advantage (Mann 2011, Simberloff et al. 2013). Pimentel et al. (2005) estimated the economic impact associated with managing invasive species at \$120 billion annually. The establishment of two such invasive species have resulted in serious economic and cultural consequences to the southeastern U.S.: Kudzu (*Pueraria montana* Lour. (Merr.) var. *lobata* (Willd.) (Fabales: Fabaceae)) and the kudzu bug (*Megacopta cribraria* (F.) (Hemiptera: Plataspidae)).

Kudzu

Kudzu is native to many parts of eastern Asia, and made its U.S. debut at the 1876 Centennial Exposition in Philadelphia (Mitich 2000). As popularity of the vine grew, the U.S. Department of Agriculture Soil Conservation Service promoted its planting as an erosion control and forage crop in southeastern states (Bailey 1939, Mitich 2000). However, the resilient and fast-growing vine eventually escaped cultivation and spread throughout the South, eventually earning the classification of federal noxious weed in 1999 (Mitich 2000). Kudzu and soybean (*Glycine max* (L.) Merr.) are related at the taxonomic level as members of the subtribe Glycininae (Frye et al. 2007). Like soybeans, kudzu produces trifoliate leaves and produces multiple seeds per pod. Unlike soybeans, kudzu has taproots up to 2-m long, and reproduces primarily by spreading adventitious shoots from tuberous roots. The semi-woody, climbing vines can grow as much as 10 cm a day, and have caused major problems in forest ecosystems and for man-made infrastructure, such as powerlines. While the above ground portions of the plant are

sensitive to frost and die back to the ground each winter, the roots and tubers remain viable underground, which allows the plant to regrow perennially (Mitich 2000).

Since being brought to the U.S., kudzu has spread to 31 states and the District of Columbia (EDDMapS 2018) (Figure 1). Like many invasive species, kudzu has been able to thrive in the relative absence of natural enemies. Arthropod diversity surveys have shown a greater diversity of herbivores and a greater number of feeding guilds on kudzu in China compared to the U.S. (Thornton 2004, Sun et al. 2006). A study conducted by Yang et al. (2014) showed that kudzu collected from an established stand in North Carolina and grown alongside native populations in China had lower resistance to feeding by herbivorous insects. However, plants from the invasive range compensated with a much higher growth rate than native kudzu and ultimately had more mass by the end of the experiment (Yang et al. 2014).

Rapid, climbing growth is the primary way that kudzu competes for canopy dominance, and this rapid growth has led to problems in many arenas. Kudzu will grow up and over trees, displacing native populations and causing millions of dollars in estimated losses to the timber industry each year (Mitich 2000, Weaver et al. 2015). This climb-and-cover strategy often leads kudzu to grow onto power lines and roads, presenting an unavoidable cost to infrastructure in southern states (Mitich 2000). Controlling foliage can offer short term relief from kudzu growth, but the large taproots can regenerate growth year after year. All of these issues combine to make kudzu management a costly endeavor that requires years of persistent, aggressive treatment (Miller 1996).

While some advances have been made recently in chemical control of kudzu, eradication often cannot be achieved with herbicides alone (Weaver et al. 2015). Difficulties in managing kudzu with mechanical or chemical means has led to exploration of potential biological control

by pathogens, insects, and grazing or burrowing mammals (Sun et al. 2006, Frye et al. 2007, Frye et al. 2012). However, many organisms that could be used as a biological control agent for kudzu also find the closely-related soybean to be a suitable alternate host and thus pose too great an economic risk to be released for biological control (Frye et al. 2007). These risks have been realized with several accidental introductions, such as soybean rust and kudzu bug. The fungal pathogen that causes soybean rust can survive in kudzu as an alternate host and often overwinters in kudzu in Gulf states (Allen et al. 2014), and the kudzu bug has an appetite for soybean, which has caused serious economic damage for southern soybean growers (Seiter et al. 2013b, Musser et al. 2017).

Kudzu Bug

The kudzu bug, also known as bean plataspid or lablab bug, is native to many countries in Asia, including China and Japan, where it feeds on kudzu and is an occasional pest of soybeans (Suiter et al. 2010). *Megacopta cribraria* is currently the only representative of the family Plataspidae present in the continental U.S. Like all true bugs, it possesses piercing-sucking mouthparts, which it inserts into stems, petioles, and undersides of leaves to feed on phloem (Zhang et al. 2012). Kudzu bug has been observed on many leguminous species in its native range (Eger Jr. et al. 2010). U.S. populations most likely came from the Kyushu region of Japan (Hosokawa et al. 2014).

Like native shield bugs (Hemiptera: Scutelleridae), kudzu bugs have large scutella that completely cover their wings, but are strongly convex dorsally with a flattened posterior, lending a distinct swollen square-like appearance (Eger Jr. et al. 2010). Adult kudzu bugs range from 3.5 to 6.0 mm in width, are olive green to light brown and are covered in dark brown punctation

(Eger Jr. et al. 2010). Adults are also sexually dimorphic: females are larger and have white markings on all sternites, while males are smaller and have white markings on the second and third sternites only (Eger Jr. et al. 2010). One female can lay anywhere from 49 to 160 individual eggs during her lifetime in lab conditions (Shi et al. 2014). Female kudzu bugs lay individual eggs adjacent to each other forming a chevron pattern, along with brown capsules containing the bacterial endosymbiont *Candidatus Ishikawaella capsulata* (Hosokawa et al. 2006). *M. cribraria* goes through five nymphal stages that are round and densely hairy in appearance and range from pale yellow-brown to green in color (Thippeswamy and Rajagopal 2005).

In its invasive range, *M. cribraria* populations have been shown to emerge from overwintering in April before producing two generations (Britt 2016). In Tennessee, the first generation develops almost exclusively on kudzu, while the second can develop on both kudzu and soybeans (Britt 2016). Second-generation adults begin to seek overwintering sites as temperatures drop in late September, often aggregating under tree bark or on white and tan structures (Suiter et al. 2010, Britt 2016). Adult females can mate before overwintering and maintain active sperm in their spermatheca throughout dormancy, and they are able to lay fertilized eggs with no loss in fecundity almost immediately after their emergence in spring (Golec and Hu 2015).

Impact of Kudzu Bug in the U.S.

While *M. cribraria* feeding can reduce the biomass of kudzu by about 32% in field cage studies (Zhang et al. 2012), its impact in the U.S. has been largely negative. In late 2009, this insect was first reported as a nuisance pest invading homes from adjacent kudzu patches in northeastern Georgia (Suiter et al. 2010, Gardner et al. 2013b). Adult kudzu bugs are particularly

attracted by white and tan structures as they seek overwintering refuges in the fall, which can lead to large aggregations on human residences (Suiter et al. 2010). Disturbed kudzu bugs also excrete a yellow-orange liquid, which has a strong odor and can cause skin irritation and rarely, allergic reactions (Ruberson et al. 2013, Seiter et al. 2013c). By the June following their initial observation, kudzu bugs had spread into dozens of additional counties, and soybean growers in Georgia observed kudzu bugs feeding on their plants (Suiter et al. 2010). The pest's identity was confirmed both morphologically by Eger et al. (2010) and genetically by Jenkins et al. (2010) within months of its initial discovery in Georgia. Since its initial discovery in the U.S., the kudzu bug has spread to 14 states and Washington, D.C., including Tennessee in 2012 (Figure 2) (Megacopta Working Group 2017).

Kudzu bugs were first known to reduce soybean yields in Tennessee in 2012, the first year they were detected in the state (Musser et al. 2013). Kudzu bugs can cause yield losses as high as 59% in field cage studies in the southern U.S., although this represents an extreme example (Seiter et al. 2013b). All yield losses caused by *M. cribraria* are from indirect damage by feeding on the phloem, which reduces the proliferation of nutrients throughout the plant (Seiter et al. 2013b). The combined cost of targeted treatment and loss of product to kudzu bug across seven Southern states was estimated at more than \$4 million in 2016, including over \$1.5 million in Tennessee (Musser et al. 2017). Current guidelines in Tennessee, like many adjacent states, recommend treating soybeans when an average of one nymph per sweep is present (Stewart and McClure 2016).

First-generation kudzu bugs can complete their life cycle on soybeans just as well as on kudzu and present a greater threat to early-planted soybeans in states farther south than Tennessee (Del Pozo-Valdivia and Reisig 2013, Blount et al. 2016). Due to later average

planting dates in Tennessee, the first generation of kudzu bugs typically develops on kudzu, and adults of this generation disperse to new sources of soybean and kudzu to feed and lay eggs (Zhang et al. 2012). Thus, in most years, it is the second generation of kudzu bugs that impacts soybean production in Tennessee.

Several insecticides already used to control stink bugs in soybeans also show efficacy against kudzu bugs, with carbamate and several pyrethroids causing the greatest mortality (Seiter et al. 2015a). Many insecticides including pyrethroids can be used as knockdown and short-term residual sprays for home infestations (Seiter et al. 2013c). However, chemical treatments in both situations are reactionary and remedial, mostly affecting second-generation kudzu bugs each season and do not target kudzu bugs in their source kudzu patches. These chemical strategies are therefore unlikely to make a lasting impact on the densities of overwintering adults and on resulting populations of kudzu bug in the following spring. Long-term, full-season reduction of *M. cribraria* requires the use of supplemental techniques as a part of an integrated pest management program. Ongoing attempts to manage kudzu now have the added benefit of reducing habitat availability for *M. cribraria*. Complete eradication of kudzu is unlikely to be adopted as a broad-scale control measure given the feasibility issues outlined above. Biological control may be more pragmatic and cost-effective in this case.

Biological Control of Kudzu Bug

The kudzu bug has several natural enemies throughout Asia, including at least seven species of parasitic Hymenoptera and one entomopathogenic fungus (Eger Jr. et al. 2010). Of these, the egg parasitoid *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygasteridae) was selected as the best candidate for classical biological control in the U.S. (Ruberson et al. 2013).

P. saccharalis is known to parasitize only four species of plataspids and demonstrates up to 80% parasitism of kudzu bug eggs in some situations (Ruberson et al. 2013). This parasitoid was under investigation for potential release by the USDA-ARS when specimens from a separate and unknown origin were found in 2013 in Georgia and Alabama (Gardner et al. 2013a).

Since its initial detection in the U.S., *P. saccharalis* has been explored as a control tactic in organic soybean production in Georgia (Tillman et al. 2016). However, the sustainability of *P. saccharalis* as a biological control agent is dubious given low, intermittent detection of this parasitoid in kudzu bug eggs in kudzu in the years following initial detection (Gardner and Olson 2016). While reduced kudzu bug population densities and oviposition play a role in detection of this parasitoid (Knight et al. 2017), this parasitoid was recovered from kudzu bug eggs in northern Georgia less frequently than from eggs in southern Georgia that were present in statistically similar densities over the course of a three-year study (Gardner and Olson 2016). After intensive collection and rearing of eggs from kudzu patches in Tennessee, it was determined that populations of *P. saccharalis* are not established in detectable numbers in Tennessee (Britt 2016). Temperature is likely a limiting factor for this wasp and could explain the distribution within and between the states where *P. saccharalis* has been detected.

The entomopathogenic fungus, *Beauveria bassiana* (Bals.- Criv.) Vuill (Ascomycota: Hypocreales: Cordecypitaceae), has also been providing biological control of kudzu bug in the U.S. (Seiter et al. 2014, Britt et al. 2016). This fungus was first observed to infect adult and nymph kudzu bugs in a kudzu patch in Georgia in 2010 (Ruberson et al. 2013). *B. bassiana* was first observed in kudzu patches in eastern Tennessee in Sep 2015 (Britt et al. 2016). A 6-week study was initiated to examine mortality caused by *B. bassiana* per life stage in both Blount and Polk Counties, using the same methods described in Ch. IV. In both counties, the level of

infection of kudzu bug by *B. bassiana* rose from the initiation of the study on 15 Sep 2015 to its termination on 19 Oct 2015, and that immature kudzu bugs were infected at higher rates than adults (Britt et al. 2016).

Beauveria bassiana has been shown to infect kudzu bugs on a density-dependent basis in field cage studies (Seiter et al. 2014), although the particular source of inoculation in the field is not known. This fungus has previously been shown to have systemic entomopathogenic activity when functioning as an endophyte by from treatments in tomatoes (Powell et al. 2009), and Russo et al. (2015) found that colonization is possible in soybean seedlings via sprays and seed treatments. *B. bassiana* may have been introduced as an endophyte to wild populations of kudzu. If this is the case, this scenario could potentially serve as a way to inoculate and protect commercial soybean production against kudzu bug.

Climate and the Kudzu Bug

Abiotic factors such as temperature are often the driving force influencing both the biology and behavior of insects. Temperature is often the primary determinant of development time, fecundity, and survival of poikilothermic organisms (Ratte 1984), and seasonal temperature regimes are one of the clearest factors in determining suitable geographical range for insects (Bale et al. 2002). Additional factors such as precipitation, drought, and host plant availability and quality, can also play a role (Bale et al. 2002, Huberty and Denno 2004). Observing population-level responses to these abiotic factors can be informative for predicting the potential establishment range and when pest population explosions are expected to occur.

The primary host plant for *M. cribraria*, kudzu, is most widely available in the southeastern U.S., but has been reported in the Pacific Northwest and in the eastern U.S. as far

north as New England (Figure 1). However, kudzu bugs can complete development on soybeans with no apparent fitness costs regardless of time of year (Del Pozo-Valdivia and Reisig 2013, Medal et al. 2016), and can cause economic damage to soybeans (Seiter et al. 2013c). This observation led to concern that kudzu bug could expand beyond the range of kudzu into major soybean commodity areas in the midwestern U.S. (Del Pozo-Valdivia and Reisig 2013). Liang et al. (2018) recently predicted that invasive populations of kudzu bug are capable of becoming established in the upper Midwestern U.S., as well as in Central and South America.

In contrast to these predictions, rates of new county reports of *M. cribraria* have decreased drastically since 2014 and it remains within a range smaller than that of kudzu itself (Figure 2). This slowed expansion suggests that kudzu bugs may have a more restricted possible invasive range compared to the kudzu vine, which may be due to abiotic factors described above (Liang et al. 2018). Previous research has been conducted in laboratory settings to determine how temperature and humidity impact kudzu bug biology (Shi et al. 2014, Golec and Hu 2015), but little has been done thus far to determine the impacts of abiotic factors on invasive populations of *M. cribraria* in the field.

Research Objectives

Much is still unknown about the ecology of *M. cribraria* populations in Tennessee. Findings from previous investigations (Britt 2016) have led to more questions, prompting the inception of a new two-year research project. Ultimate goals for this research are to develop treatment recommendations for growers that are based on sound, accurate ecological data and contribute to effective long-term control of this pest. Objectives of this research were to:

1. Monitor kudzu bug populations and phenology throughout eastern Tennessee, and compare observations to previous years,
2. Investigate population-level responses to climatic influences, such as severe drought,
3. Examine both the short-term and long-term impacts of the entomopathogenic fungus, *Beauveria bassiana*, on kudzu bug populations, and
4. Identify and monitor populations of kudzu bug egg parasitoids.

CHAPTER II SEASONALITY AND PHENOLOGY OF KUDZU BUG

Introduction

Kudzu bug (*Megacopta cribraria* (F.)) was first detected in the U.S. in 2009. This soybean pest dispersed quickly throughout the southeastern U.S., and was found in Tennessee in 2012 (Megacopta Working Group 2017). In its native range, the kudzu bug can have up to three generations per year (Wu et al. 2006). A previous two-year study conducted by Britt (2016) has shown that kudzu bugs are typically active from mid-May to mid-November and have two generations per year in Tennessee. Two additional years of observations serve not only to validate previously drawn conclusions, but also to assess changes that populations of *M. cribraria* may undergo in response to climactic factors such as extreme drought (see Ch. III) and recently arrived natural enemies (see Ch. IV and V).

Questions to be answered by this study include: How do kudzu bug population densities vary between years? Do kudzu bug populations exhibit peaks, and do these occur at consistent times of the year? Can any long-term trends be observed in kudzu bug populations? The ultimate goal of this study is to develop clear recommendations for soybean growers, as well as the general public, about which time of year to expect large numbers of kudzu bugs.

Materials and Methods

Population Density and Phenology Estimates

Roadside kudzu patches of 500 m² or greater were selected for this study. Sampling was conducted at three sites within three localities (West, North, and South) in Knox County, TN

(Table 1, Figure 3). Additional sampling was conducted in five counties to study broader geographical trends (Table 1), for a total of 14 localities in six counties (Knox, Blount, Monroe, McMinn, and Polk counties in Tennessee, Murray County in northern Georgia) (Table 1, Figure 3). Knox County sites were sampled weekly whenever possible in both 2016 (10 May – 18 Nov) and 2017 (28 Apr – 31 Oct). Additional counties were sampled every other week in 2016 (beginning 17 Aug due to workforce constraints, ending 16 Nov) and weekly in 2017 (8 May – 2 Nov).

Five samples were taken at each locality using a 38-cm diameter sweep net. Each sample consisted of five sweeps each, for a total of 25 sweeps per locality per sampling event. Plant and insect material from each sample were placed into sealable plastic bags with a 0.95 L capacity. Bags were then taken to the laboratory and stored in a walk-in refrigerator at 2°C until processing. Individual samples were emptied onto 32 cm x 23 cm rectangular white dishes to provide visual contrast for sorting. Nymphs were sorted by instar (I-V) and adults were sorted by sex. Number of kudzu bugs per life stage and sex of adults were recorded. Data are presented as average number per life stage per week.

Data Analysis

To assess population levels and variation over time, kudzu bug densities were analyzed using a mixed model zero-inflated Poisson regression with site as random effect using PROC GENMOD (SAS Institute Inc. 2008). Data were fitted to a zero-inflated Poisson distribution to fit the assumptions of the model. Wald's χ^2 test was used to make pair-wise comparisons of densities per life stage observed each week during 2016 and 2017. Pair-wise comparisons were also made among counties over both years. Wald's χ^2 test was again used to determine if

significant difference in population densities existed between counties. For this analysis, one subset of data must be used as a reference (expected value) that observations may be compared to in order to detect significant differences between additional subsets of data (observed values). For differences between counties, Polk County was selected as the reference because it borders Georgia and thus was likely the first county within our sampling range where kudzu bug was introduced. Week 31 was selected to measure differences between weeks because it exhibited the lowest numbers of kudzu bugs observed throughout the season.

Results and Discussion

Knox County

Within our most robust data set (Knox County), adult kudzu bug population densities have shown an overall decline since population monitoring commenced in 2014 (Figure 4). Phenological differences have also been observed in 2016 and 2017 compared to observations made in previous years (Britt 2016). Adults were observed at higher densities than nymphs throughout the season during both 2014 and 2015 in Knox County (Britt 2016), but adult and nymph densities tracked closer to each other in both 2016 and 2017 (Figure 5). In 2016, only one noticeable population peak was observed during week 19, as opposed to the three peaks observed by Britt (2016) (Figure 4). The peak thought to coincide with the end of the completion of the first generation (Britt 2016) was likely missed due to intermittent sampling. The observed peak likely coincides with the completion of the second generation. During this timeframe, densities of fourth and fifth instar nymphs were higher than adult densities for the first time since sampling began in Knox County. Furthermore, the third “pre-overwintering” peak of adults was not observed in 2016 (Figure 5A). The combination of these observations could indicate that

fewer kudzu bugs completed development in 2016, or that adults emigrated from kudzu in large numbers and did not aggregate within kudzu prior to overwintering. Either of these outcomes could be driven by abiotic stressors or the presence of natural enemies. These factors are explored in detail in the remaining chapters.

Population densities observed in 2017 more closely tracked those observed in 2016 than in previous years (Britt 2016), in particular with lower densities of adults (Figure 5B). Three population peaks were observed, and these fell within the expected timeframes for the completion of the first and second generations and the pre-overwintering aggregation. However, densities of fifth-instar nymphs exceeded adult densities again during weeks 19-21, approximately during the time that the second generation is expected to complete development (Figure 5B).

Blount County

Kudzu bug population densities appeared to be greater on average in 2017 compared with 2016 (Figure 6). A pre-overwintering population peak was not observed in 2016 (Figure 6A). First-instar nymph densities exceeded adult densities following the estimated development end point of the first generation. These likely marked the beginning of the development of the second generation. Kudzu bugs in Blount County exhibited the highest observed population densities of any site in 2017 (Figures 5 – 10), which may be due to higher oviposition or lower egg mortality rates in early July. Fifth-instar nymph densities also peaked around the end of the second generation and exceeded adult densities in late August. Adult densities were higher than nymphal densities for the remainder of the season, although a single consolidated pre-overwintering peak

was not observed (Figure 6B). This unevenness in the data may be an artifact of single-site sampling within the county.

Monroe County

Kudzu bug population densities in Monroe County were extremely low in 2016, never averaging more than one insect per life stage per sweep (Figure 7A). During 2017, adult densities were relatively high from early July – mid-August, at which point densities declined until mid-September, with a final peak in early October (Figure 7B). This peak is a bit offset from the increasing adult densities observed in Knox and Blount Counties during August and September. The second generation could have either reached development a few weeks ahead, in early August, or had reduced or delayed development causing them to reach adulthood later in September.

Many factors could be responsible for the decrease of kudzu bugs during this time. This site was almost always sampled in mid-day and is in full sun for most of the day, unlike Blount County, so it is possible that adults may have had reduced activity during sampling. Several significant rain events (remnants of storm systems from hurricanes Harvey and Irma) also could have suppressed kudzu bug populations in late August and early September. However, the entomopathogenic fungus *Beauveria bassiana* also becomes virulent to kudzu bugs during this time (see Ch. IV), and its incidence could be another driver for this shift.

McMinn County

Population densities of kudzu bug in McMinn County more closely matched observations from Monroe County than Knox or Blount Counties in 2017. Adult kudzu bug densities were highest throughout July, reaching 11.4 kudzu bugs/sweep on average on 6 Jul 2017 (Figure 8B).

Densities declined from late July until mid-September. Adult densities rose again on average until mid-October, but never reached more than 4 adult kudzu bugs/sweep. Observed densities during this time were similar to levels that were observed in 2016 (Figure 8A) and did not indicate a large pre-overwintering aggregation when compared to population peaks earlier in the season. Like the Monroe County site, this location also receives prolonged sun exposure. It is also worth noting that several grassy weeds were growing aggressively throughout the kudzu patch for the latter half of the season (personal observation, 19 Sep 2017). This incursion of non-native host plants could have caused a behavioral response in mated females and pre-overwintering adults to seek a uniform patch that could be more conducive to kudzu bug feeding and survival.

Polk County

Population densities of kudzu bugs in Polk County were low in 2016 (Figure 9A). In 2017, the phenological pattern of kudzu bug densities more closely resembled McMinn County than other previously discussed counties. Adult densities were highest from early July to mid-August and were not detected at more than 4 adults/sweep following this time. The Polk County site is more similar to the Blount County site in that it remains shaded for much of the day, so it is unlikely that sunlight or temperature avoidance caused lower kudzu bug densities in Polk County during this time. However, a noticeable amount of kudzu was physically removed along the edge of the patch later in the season (personal observation, 19 Sep 2017). Kudzu bugs typically aggregate near the edges of soybean fields (Seiter et al. 2013a), and it is likely that this same behavior occurs in kudzu. It is possible that vegetation removal could have also removed a substantial number of second-generation nymphs, resulting in a reduced second generation.

Murray County (GA)

Adult kudzu bug densities in Murray County in 2016 were relatively high throughout September and October (Figure 10A). Fifth instars also noticeably exceeded adult densities in late August and early September, similar to observations in Knox and Blount Counties in 2016. Above-ground kudzu growth was destroyed by a third party following 24 Oct 2016, and further sampling was not conducted that year. However, vegetative kudzu growth occurred at this site the following year and it was sampled for the duration of the season. Younger kudzu bug nymphs are often difficult to detect via sweep-net, but the removal of old-growth refugia and abundance of new growth seems to have compensated for this usual impediment to detection. More distinct peaks of younger instars (I-III) were observed at this site compared to others (Figure 10B). Adult densities here peaked in early July, mid-September, and late October. Fifth instars reached the highest density of any life stage in early September, in the week immediately prior to a peak observed in adults. While the later season peaks were not greater than the first-generation peak, they also were not much lower.

Overall

Kudzu bugs were detected at higher overall densities in 2017 compared to 2016 with the exception of fourth instars (Table 2). No significant differences were seen between counties for first- or third-instar nymphs (Table 3). While some seemingly significant differences were observed between densities of second and fifth instar nymphs at sites in Blount and Knox Counties, and fourth instars at the Blount County site, compared to Polk County, the intercept estimate for these life stages was not significant (Table 2). The only life stage that showed a statistically significant estimate of the intercept was the adult. In adults, significantly lower

densities were observed in Knox County than in Polk County (estimate = -0.6620, $p = 0.0031$), while densities were significantly higher in Monroe County (estimate = 0.7201, $p = 0.0109$) (Table 3). Week-wise differences were not significant for nymphal instars I-IV (data not shown). Fifth-instar nymphs had significant week-wise differences at weeks 1, 3, 5, and weeks 11-30 (Table 4). Adults had significant week-wise differences at all weeks except for week 5 (Table 5).

Lower average densities of kudzu bug adults were observed in 2016 both overall (Table 2) and within each County (Figures 4-10). The year 2016 was both the year following the first observation of *B. bassiana* in east Tennessee and a drought year throughout our sampling range (see Ch. III). One or both of these factors could have reduced the number of kudzu bugs reaching adulthood. However, because our observations were conducted only in drought-stricken kudzu patches and not in irrigated soybean fields, it is possible that this change may be influenced by emigration in addition to mortality. The impact of abiotic factors (Ch. III) and natural enemies (Ch. IV, V) are explored in detail in the remaining chapters.

CHAPTER III

CLIMATIC INFLUENCES ON KUDZU BUG POPULATIONS

Introduction

The kudzu bug, *Megacopta cribraria*, is a non-native true bug that was first observed in northeastern Georgia in 2009. Its primary host is the invasive kudzu vine *Pueraria montana* var. *lobata*, which is most abundant in the southeastern U.S. (Figure 1) (EDDMapS 2018). Kudzu bugs can also complete development on soybean with no apparent fitness costs (Medal et al. 2016). Following initial detection, there were concerns that kudzu bug could move beyond the range of kudzu into major soybean production areas in the midwestern U.S. (Del Pozo-Valdivia and Reisig 2013). However, rates of new county reports of *M. cribraria* have decreased drastically since 2014, and it remains within a range smaller than that of kudzu itself (Figure 2) (Megacopta Working Group 2017). This decreased spread suggests that kudzu bugs may have a more restricted possible invasive range compared to kudzu.

Abiotic factors certainly affect population densities of *M. cribraria* in its invasive range. Ambient temperature is often the primary factor influencing development time, fecundity, and survival of insects (Ratte 1984), while tolerance to seasonal temperature regimes often confine the distribution of insect species (Bale et al. 2002). Additional factors such as precipitation, drought, host plant availability and host plant quality can also play a role in the success of a species in a given environment (Bale et al. 2002, Huberty and Denno 2004). Observing population-level responses to these abiotic factors can be informative for predicting the potential establishment range and when pest population explosions are expected to occur.

Kudzu bugs are typically active from mid-April to November in eastern Tennessee, and overwinter as adults for the remainder of the year (Britt 2016). Previous research has been conducted in laboratory settings to determine how temperature and humidity impacts kudzu bug development, survival, and fecundity while kudzu bugs are active (Shi et al. 2014), and how temperature impacts the reproductive biology of overwintering kudzu bugs (Golec and Hu 2015). Shi et al. (2014) determined that nymphal kudzu bug development can occur above 9.9°C (49.8°F) and is optimized at 25°C (77°F). They estimated that kudzu bugs require 849.6 degree-days to develop from egg to adult. However, little has been done thus far to determine the impacts of abiotic factors on invasive populations of *M. cribraria* in the field during their active period. Low kudzu bug populations observed in 2016 and 2017 (see Ch. II) prompted investigation into the impact that abiotic factors may have on kudzu bug population densities.

Questions to be answered by this study include: Which abiotic factors have the greatest impact on kudzu bug population densities? Which factors are associated with higher or lower population densities? The ultimate goal of this study is to provide insight into the factors that help predict higher or lower kudzu bug densities.

Materials and Methods

Collection of Environmental Data Factors

For this study, we investigated the impact of temperature, precipitation, drought, and time on total numbers of kudzu bugs observed in Knox County, Tennessee. Total numbers of kudzu bugs were recorded for sweep-net collections made in Ch. II. Temperature was further separated into average temperature on the date of collection as well as the maximum, minimum, and average temperatures during the week prior to collection. Accumulated degree-days were

calculated using 10°C (50°F) as the lower developmental threshold, as approximately described by Shi et al. (2014), and began on 1 Jan each year. These yield a total of five temperature-related parameters. Precipitation was also separated into total precipitation during the week prior to collection and total year-to-date precipitation for a total of two factors. Drought index and week of collection were also included as numeric factors.

Temperature and precipitation data were gathered from a free database maintained by the National Oceanic and Atmospheric Administration (NOAA). For our analysis, we used data collected from the weather station at the Knoxville McGhee Tyson Airport (NOAA Station USW00013891, 35.8181°, -83.9858°, 89.37m). While this station is technically not in Knox County, but in adjacent Blount County, this station was used because airports rely on accurate weather station data, thus, no gaps were present in the data during our sampling season.

Drought index data were gathered from the freely available U.S. Drought Monitor service, a joint effort between the National Drought Mitigation Center at the University of Nebraska-Lincoln, the United States Department of Agriculture, and NOAA. This group assigns fine-scale regional drought categorizations and publishes the results as a map each week at <http://droughtmonitor.unl.edu> (Artusa et al. 2016). Maps, such as those shown in Figure 11, were visually assessed to determine the predominant drought categorization in Knox County, Tennessee during each week kudzu bugs were collected.

Statistical Analysis

Kudzu bug numbers were combined from both 2016 and 2017 and averaged for each week. Correlation analysis was conducted on all environmental factors described above to examine for multicollinearity and remove redundant predictors using the CORR procedure (SAS

Institute Inc. 2008). Remaining data were analyzed within the parsimonious model using the generalized mixed model approach with PROC GLIMMIX (SAS Institute Inc. 2008). Resulting regression coefficients were standardized to compare effect size. Data were fitted to a Poisson distribution; thus, standardized regression coefficients were exponentiated prior to interpretation.

Results and Discussion

Analysis found that five of the nine factors originally considered in the model were significantly related to kudzu bug density: drought index, accumulated degree days, sum of precipitation during the week prior to sampling, average temperature during the week prior to sampling, and week. Week had a variable effect throughout the sampling period, and thus the analyses were conducted separately for weeks 1-19 and 20-31 (Figure 4, Figure 11).

During weeks 1-19, accumulated degree days, precipitation in week prior to sampling, and average temperature all increased significantly as kudzu bug numbers increased (Table 6). Accumulated degree days were most strongly related to kudzu bug numbers, with a 4.08-fold increase of kudzu bugs with each standard deviation increase of accumulated degree days ($p < 0.0001$). Precipitation (1.16-fold, $p < 0.0001$) and average temperature (1.42-fold, $p < 0.0001$) in the week prior to collection also had a positively related to kudzu bug numbers but were not related as strong as accumulated degree days. Drought was the only factor to have a negative relationship, with a 1.57-fold decrease of kudzu bugs per each standard deviation increase of drought index ($p < 0.0001$). Kudzu bug numbers were similar during weeks 1-19, and thus week did not have a statistically significant positive or negative relationship with kudzu bug numbers during this timeframe (0.02314, $p = 0.1466$) (Table 6).

From week 20 – 31, week was significantly negatively related to kudzu bug densities, with 1.22-fold decrease of kudzu bug numbers each week ($p < 0.0001$). Average temperature during the week prior to sampling continued to be positively related to kudzu bug numbers, albeit not as strongly as in earlier weeks (1.08-fold, $p < 0.0001$). Drought continued to be negatively related to kudzu bug numbers, and in fact this relationship was slightly stronger compared to weeks 1-19 (-1.79-fold, $p < 0.0001$). Precipitation during the week prior to sampling became negatively related to kudzu bug numbers, coinciding with 1.05-fold decrease in kudzu bugs ($p < 0.0001$). Accumulated degree days were not significantly related to kudzu bug densities during this time (1.15-fold, $p = 0.5552$).

The timeframe for development of the two kudzu bug generations in eastern Tennessee has been observed from late April/early May to early September both by Britt (2016) and throughout the course of this research (see Ch. II). This timeframe aligns closely with weeks 1-19. While the largest population peak typically occurs during the remaining weeks, this peak is likely an artifact of pre-overwintering aggregation rather than development (Suiter et al. 2010, Ruberson et al. 2013) (see Ch. II). Given these observations of kudzu bug life history, it is not surprising that factors often used to predict insect development and survival (accumulated degree days, average temperature and precipitation in week prior to sampling), were all positively associated with kudzu bug numbers during this timeframe (Table 6). Temperature in particular drives insect development (Ratte 1984), so it makes sense that temperature-related factors would be significant during this time, and that accumulated degree days would have the strongest effect on kudzu bug numbers (Table 6). The observations that at week 20 accumulated degree days ceases to be significant while week begins to have a significant effect further support the idea

that, by mid-September, the second generation of kudzu bugs has completed development and has transitioned to pre-overwintering behavior (Table 7).

From these analyses, water availability also seems to be a key predictor for the success of kudzu bug populations. Kudzu bugs are phloem feeders, so it follows that the water content or general plant health of kudzu could be related to population growth (Stubbins 2016).

Precipitation during the week prior to sampling did have a positive effect on kudzu bug numbers during weeks 1-19 (Table 6), but not during weeks 20-31 (Table 7.) These observations tie short-term water availability to development of nymphs, as well as oviposition site selection by dispersing adults, but also indicate that later-season rainfall events may cause mortality to kudzu bugs. The negative impact of drought on kudzu bug numbers throughout the season further supports this observation. Increased drought severity implies reduced kudzu growth, which can also have the secondary effect of reduced availability of food and habitat to kudzu bugs. Reduced growth and soil water content may have led to a decrease in kudzu phloem, and scarcity of this food source could have acted as a limiting factor for kudzu bugs during the 2016 season. Kudzu bugs are typically found under leaves or on stems underneath the kudzu canopy and may be shielded from weather extremes with sufficient canopy cover. Canopy cover was noticeably reduced at many of the sites sampled in 2016 (personal observation, 28 Sep 2016), and seemed to worsen as the season continued and drought categorizations increased (Artusa et al. 2016). Reduced canopy cover due to drought and natural senescence of kudzu following the first frost may also contribute to the negative effect of precipitation during weeks 20-31, as plant ultrastructures used by insects to avoid rainfall may be limited and result in kudzu bug mortality.

It is also worth noting that kudzu bug populations were assessed only in kudzu in eastern Tennessee for both 2016 and 2017, and comparable surveys were not conducted within irrigated

soybean fields or in western Tennessee. However, the estimated combined cost of treatment and loss of product attributed to kudzu bug in soybeans in Tennessee climbed from \$402,103 in 2015 to \$1,551,469 in 2016 (Musser et al. 2016, Musser et al. 2017). While lower kudzu bug densities were observed in drought-stricken kudzu during the 2016 season in eastern Tennessee (see Ch. II), it is possible that as the drought worsened with the progression of the season that these bugs dispersed to irrigated soybean fields in greater numbers in eastern Tennessee. This increase in monetary loss by growers throughout the state could have been caused by greater colonization of soybeans by kudzu bug, causing more fields to meet the recommended treatment threshold of 1 nymph/sweep or 5 bugs/plant on average (Stewart and McClure 2016). The increase in cost of treatment could also have been a reflexive response to large numbers of adult kudzu bugs in soybean fields during early podfill, similar to the response homeowners have to nuisance infestations of large numbers of kudzu bugs on homes (Suiter et al. 2010). Adult kudzu bugs are less likely to cause economic losses than nymphs (Seiter et al. 2015b), which is an important economic consideration for growers considering a knockdown spray of adults. However, the arrival of these insects may tip recommendations toward treatment if other pests, particularly hemipterans, are already present in the field. It is also worth noting that the vast majority of soybeans in Tennessee are grown in the western region of the state, and that estimated treatments and losses in soybeans are thus largely driven by this region. It remains unclear if our observations in eastern Tennessee kudzu were in fact related to the economic impact of kudzu bug in western Tennessee soybeans in 2016. Ultimately, the results of our model show that drought decreases kudzu bug densities within kudzu patches and could lead to a lower burden of second-generation kudzu bugs infesting soybean fields.

CHAPTER IV

THREE YEARS LATER: *BEAUVERIA BASSIANA* IS A PERSISTENT ENTOMOPATHOGEN OF KUDZU BUG IN TENNESSEE

Introduction

The kudzu bug, *Megacopta cribraria* (F.) (Hemiptera: Plataspidae), is a true bug and occasional pest of soybeans within its native range in Asia. Following initial detection in Georgia, U.S. in 2009, kudzu bugs dispersed quickly throughout Southern states and became economic pests of soybeans (Suiter et al. 2010, Ruberson et al. 2013, Megacopta Working Group 2017). Much initial research on invasive *M. cribraria* was focused on their pest potential and chemical controls for growers of soybeans and other legumes (Del Pozo-Valdivia and Reisig 2013, Seiter et al. 2013b, Seiter et al. 2015a) to prevent yield losses of up to 59% (Seiter et al. 2013b). This work is invaluable to give growers the proper tools and education to manage kudzu bug outbreaks when they occur. However, chemical treatments are typically reactionary and remedial, do not target kudzu bugs at their source in kudzu patches, and often impact only one of two kudzu bug generations each year. Additional management strategies, such as biological control, are needed to effect long-lasting, full-season reduction of populations of *M. cribraria* to maintain them below economically damaging levels.

Naturally-occurring populations of the entomopathogenic fungus, *Beauveria bassiana* (Bals.- Criv.) Vuill (Ascomycota: Hypocreales: Cordcypitaceae), cause kudzu bug mortality in the U.S. (Seiter et al. 2014, Britt et al. 2016). This fungus was first observed to be infecting adult and nymph kudzu bugs in a Georgia kudzu patch in 2010 (Ruberson et al. 2013) and was first observed in kudzu patches in eastern Tennessee on Sep 1, 2015 (Britt et al. 2016). *B. bassiana*

usually kills kudzu bugs within 7-10 days of infection, and mortality levels seem to be density-dependent (Seiter et al. 2014).

Immediately following the discovery of *B. bassiana* in eastern Tennessee, Britt et al. (2016) launched a survey to investigate kudzu bug mortality caused by this fungus within kudzu patches in Blount and Polk Counties (15 Sep 2015 – 19 Oct 2015). Results of this study showed that level of infection rose from the initiation to the termination of the study, and that immature kudzu bugs were infected at higher rates than adults. However, further study was needed to examine the impact of this fungus throughout the region to understand its role in kudzu bug ecology.

The source of *B. bassiana* inoculation in the field is not yet known. Previous research has shown that *B. bassiana* can endophytically colonize plants including soybeans (Russo et al. 2015), and that endophytic *B. bassiana* can be virulent against herbivorous insects in tomatoes (*Solanum lycopersicum*, L.) (Powell et al. 2009). *B. bassiana* may occur endophytically in wild populations of kudzu, and this incidence could be the source of infection for kudzu bugs. If so, this observation could lead to the development of an endophytic seed treatment to protect commercial soybeans from this pest.

With the exception of the study conducted by Britt et al. (2016), relatively little is known about *B. bassiana* and its impact on kudzu bug populations in Tennessee. Questions to be answered by this study include: Does this fungus persist in nature, and is it more effective at certain times of the year? What life stages of kudzu bugs are more susceptible to mortality by *B. bassiana*? Are there particular sites that have higher rates of mortality caused by this fungus? What impact does this entomopathogenic fungus on kudzu bug populations? Does this fungus

exist endophytically within kudzu? Ultimately, this study assesses the impact of *B. bassiana* on populations of kudzu bugs in kudzu.

Materials and Methods

Assessment of Infection Levels of Kudzu Bugs

Samples containing both infected and uninfected kudzu bugs were collected from kudzu patches in five counties in eastern Tennessee and one county in northern Georgia (Figure 12, Table 8). Samples were collected once every two weeks while entomopathogenic activity was observed from (7 Sep – 31 Oct 2016 and 2017). Within each kudzu patch, one upper slope and one lower slope 50-m transect were each established. Each linear transect contained five sampling points separated by 10 m. Sampling points were delimited by PVC pipes (0.5 m) hammered into the ground one week prior to the beginning of the study. All samples were collected within a 0.5-m radius extending from the pipe. Clippings of kudzu were taken that included kudzu bugs of any developmental stage and placed into a resealable plastic bag (3.79 L). Samples were returned to the laboratory, and bags containing samples were stored in a 2°C until processing. Kudzu bugs were sorted by life stage (adult and nymph), and the number of infected and uninfected specimens were counted and translated into percent infection.

Data Analysis

Data were analyzed as a mixed effects model, with site and week included as fixed effects (dependent variables) to determine their impact on percent infection of immatures (instars I-V grouped) and adults (independent variables). Values were rank transformed to fit the assumptions of the model. Data from both years were pooled for each site to provide a multi-year

perspective. A two-way ANOVA was conducted using the SAS PROC MIXED procedure (SAS Institute Inc. 2008) to determine the impact of site, week, and the interaction of site and week on infection rates per life stage. Tukey's HSD was performed to separate means at $\alpha \leq 0.05$.

Assessment of Endophytic *Beauveria bassiana* in Kudzu

Ten clippings of kudzu each containing attached *B. bassiana* infected kudzu bugs were collected from University Park Apartments, Elevation Apartments, and the Halls Senior Center (SUP, WCO, and NHS respectively) (Table 1) within Knox County, TN on 21 Dec 2017 (n = 30). Stems were cut a minimum of 2.5 cm away from the nearest infected kudzu bug to prevent contamination. Kudzu bugs were removed with forceps prior to surface sterilization of cut stems. These clippings were submerged in a 10% Clorox bleach solution for one minute before being rinsed in sterile DI water, air-dried, and placed into Petri dishes (9 mm) on selective agarose growth medium (Doberski and Tribe 1980). Petri dishes were then sealed with parafilm and stored at $21 \pm 1^\circ\text{C}$ in a foil-lined box to prevent breakdown of selective agents. Dishes were checked for fungal growth at least twice weekly for six weeks.

Results and Discussion

Assessment of Infection Levels of Kudzu Bug

In total, 2,902 adults and 5,555 nymphs were collected throughout this study (in 2016: $n_{\text{adult}} = 1,399$, $n_{\text{nymph}} = 2,734$; in 2017: $n_{\text{adult}} = 1,503$, $n_{\text{nymph}} = 1,176$). Kudzu bugs infected with *B. bassiana* were first observed in the field on 22 Aug in both 2016 and 2017. However, rain events during this period in both years (storm system remnants of hurricanes Harvey and Irma in 2017) delayed sampling by two weeks until 7 Sep in both years.

Site and week both had a significant effect on percent infection of adults (Site: $p = 0.0078$; Week: $p = <0.0001$), but the interaction of these factors was not significant (Site*Week: $p = 0.0721$) (Table 9). Percent infection of nymphs was also significantly influenced by site and week; however, these did have significant covariance (Site: $p = <0.0001$, Week: $p = 0.0012$, Site*Week: $p = 0.0021$) (Table 9). Adult infection levels mostly increased with site regardless of week (Figure 13) while nymphal infection rates appear to be more complicated. Increased infection levels are positively associated with week in Knox, Blount, and Murray Counties, neutral in Polk County, and negatively associated with week in Monroe and McMinn Counties (Figure 14).

For adults, the only significant county-wise differences were found between Knox County and McMinn County ($\bar{x}_{\text{diff}} = 26.5423$, $p = 0.0776$), and no significant differences were found through week-wise comparisons. However, within sites, infection levels overall increased as the weeks progressed. For example, within Blount County, infection levels of adults significantly increased from week 20 to week 26 ($\bar{x}_{\text{diff}} = 46.4273$, $p = 0.0081$) and 28 ($\bar{x}_{\text{diff}} = 50.2473$, $p = 0.0004$). Differences in infection levels at different sites sampled in the same week, i. e. Blount and Murray Counties during week 24 ($\bar{x}_{\text{diff}} = -64.7125$, $p = 0.0062$), were also observed (Figure 13).

For nymphs, overall significant differences were found between Blount and McMinn Counties ($\bar{x}_{\text{diff}} = -20.9753$, $p = 0.0080$), between Knox and McMinn Counties ($\bar{x}_{\text{diff}} = -30.1543$, $p < .0001$) and between Knox and Monroe Counties ($\bar{x}_{\text{diff}} = -19.2258$, $p = 0.0157$). The only significant difference across weeks at all sites was detected between weeks 20 and 22 ($\bar{x}_{\text{diff}} = 27.2750$, $p = 0.0077$). Within Blount County, infection levels of nymphs were significantly higher in week 26 compared to week 20 ($\bar{x}_{\text{diff}} = 53.1113$, $p = 0.0011$). No significant differences

were detected between weeks within Knox, McMinn, Monroe, Murray or Polk Counties. The lowest infection level of immatures was observed in McMinn County during week 24 (17.15%) and was significantly different from infection levels in Knox County in the following weeks (Figure 14).

It is worth noting that the kudzu patches sampled in Knox, Blount, Murray, and Polk Counties are under shade for several hours more each day than those in Monroe and McMinn Counties. This microclimatic disparity could be the driving force behind the differences seen between infection levels of nymphs at these different sites (Figure 14). Increased shade, cooler temperatures, and moisture are often associated with fungal growth. Previous studies have shown that some strains of entomopathogenic *B. bassiana* can have a broad range of tolerance to both fluctuating temperature and humidity regimes, and can be virulent to hemipteran hosts even in fairly dry, hot conditions (Fargues and Luz 2000, Liu et al. 2003, Bugeme et al. 2008). These characteristics explain the presence of fungus at all sites, including those with more sun exposure and during periods of extreme drought in 2016 (see Ch. III). However, the fungus is more virulent when exposed to longer periods of high humidity – such as prolonged morning dew on kudzu leaves – and less extreme temperature fluctuations (Fargues and Luz 2000).

Immature kudzu bugs cannot fly and are largely sequestered to their kudzu patch until they reach adulthood. Ultimately, nymphs, and the *B. bassiana* conidia available to infect them, are largely susceptible to the microclimate present within their kudzu patch. These site differences could drive decreasing infection levels of nymphs at the sunlight-exposed sites. However, adults have a broader range of functional behavioral responses to microclimatic shifts that may increase their risk of infection. Winged adults may have more opportunities to be exposed to *B. bassiana* when they disperse and seek mates in different kudzu patches and

soybean fields. They may also have a greater ability to move short distances to shaded areas during hotter periods of the day, which could have a cooler, more humid environment that boosts hyphal *B. bassiana* growth.

Assessment of Endophytic *Beauveria bassiana* in Kudzu

Beauveria bassiana was detected growing endophytically from kudzu containing infected kudzu bugs on 21 Dec 2017. Twenty of the thirty cuttings collected produced visually identifiable *B. bassiana* (Table 10). In all twenty of these cases, *B. bassiana* growth arose from the point where an infected kudzu bug was attached. However, in ten of these cases, *B. bassiana* also grew out from the end of the surface-sterilized stem. The SUP and WCO sites had equal rates of endophytic growth occurring from both the attachment site (80%) and cut end (50%). Growth occurred from the attachment sites only in cuttings collected from NHS, and NHS had the lowest level of growth from all sites tested (50%) (Table 10).

This study gives critical insight to the potential source of *B. bassiana* inoculum on kudzu bugs in the field. Conidia of entomopathogenic *B. bassiana* have been recorded anywhere from 2.1 – 2.6 μm in size on average (Liu et al. 2003). Labial openings of kudzu bugs range from ~2.85 μm in first-instar nymphs to ~28.57 μm in adults, and stylet openings in adults are approximately 6.48 μm (Stubbins 2016). It is therefore feasible that, if *B. bassiana* exists endophytically within kudzu, that conidia could be taken up by the kudzu bug through probing or feeding upon the plant. If the conidia are not taken up directly into the digestive system of the insect, it is also plausible that the conidia could reside on other mouthpart microstructures until the host insect moves to a new feeding location. Endophytic *B. bassiana* could potentially be the route of infection for the kudzu bug, and a potential method of spread for the fungus.

During the time of collection, kudzu bugs were largely overwintering. However, this study gives us critical insight into the persistence of *B. bassiana* in the field. Kudzu bugs are detectable in kudzu patches for as long as late April – late November (see Ch. II), and are presumably feeding for a large portion of this time. However, infection of kudzu bugs by *B. bassiana* does not occur until much later in the season, typically late August. Future studies are needed to investigate the seasonality of *B. bassiana* within this system, particularly the seasonal nature of when this fungus may become endophytic within kudzu.

CHAPTER V

FIRST RECORD OF A PARASITOID (HYMENOPTERA: ENCYRTIDAE) ON EGGS OF *MEGACOPTA CRIBRARIA* (HEMIPTERA: PLATASPIDAE) IN TENNESSEE AND INITIAL ECOLOGICAL INSIGHTS

Introduction

The kudzu bug, *Megacopta cribraria* (F.) is a true bug native to Asia that was first observed in Georgia, U.S. in 2009 (Eger Jr. et al. 2010, Hosokawa et al. 2014). By 2017, it had spread to 14 states as well as the District of Columbia (Megacopta Working Group 2017). Kudzu bugs can successfully complete development on both kudzu (*Pueraria montana* Lour. [Merr.] var. *lobata* [Willd.]) and soybean (*Glycine max* [L.] Merr. [Fabales: Fabaceae]) and have many documented alternative hosts (Eger Jr. et al. 2010, Zhang et al. 2012, Medal et al. 2013, Medal et al. 2016). While kudzu bug is an occasional pest of soybeans in its native range (Suiter et al. 2010), it quickly became an annual economic pest of soybeans in the southeastern U.S. Yield losses can reach as high as 59% in experimental cage studies (Seiter et al. 2013b). On a practical scale, the combined cost of targeted treatment and loss of crop yield to kudzu bug across seven Southern states was estimated at over \$4 million in 2016, with over \$1.5 million in Tennessee alone (Musser et al. 2017).

Invasive populations of kudzu bugs most likely originated in Japan (Hosokawa et al. 2014), so naturally, biological control agents were heavily scouted in Japan for possible introduction to the U.S. Two species of parasitic wasps have been the focus of much research: *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) and *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygastriidae). Both *O. nezarae* and *P. saccharalis* parasitize eggs belonging to *Megacopta* spp. in wild kudzu and soybean in Japan (Takagi and Murakami 1997, Hoshino et al.

2017). However, *O. nezarae* has also been documented as a parasitoid of eggs belonging to members of several hemipteran families in addition to Plataspidae in its native range (Hirose et al. 1996, Zhang et al. 2005). These alternate hosts include several soybean pests such as the Southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae). Brazil seriously investigated *O. nezarae* for release as a biological control agent of several stink bug pests in the 1980's (Kobayashi and Cosenza 1987, Jones 1988), while U.S. quarantine specialists found its host range too broad to consider for release in kudzu bug control (Ruberson et al. 2013).

Paratelenomus saccharalis has a much more restrictive host range, and thus was considered for classical biological control in favor of *O. nezarae* in the U.S. (Ruberson et al. 2013). This species had been under investigation for potential release as a biological control agent of kudzu bugs by the USDA-ARS National Biological Control Laboratory in Mississippi before separate, incidentally introduced populations were found in Georgia and Alabama in 2013 (Gardner et al. 2013a). *P. saccharalis* has been shown to overwinter in Alabama, Georgia, and Florida (Medal et al. 2015, Ademokoya 2016, Tillman et al. 2016).

No egg parasitoids of kudzu bug were recovered from either yellow pan traps or *M. cribraria* eggs in a 2015 survey of kudzu in east Tennessee (Britt 2016), suggesting that *P. saccharalis* may only be viable in states with warmer weather farther south. However, kudzu bug dispersal levels and population densities have been declining since 2014 (Britt 2016, Megacopta Working Group 2017). Thus, a one-year research project was initiated to assess presence and distribution of parasitoids of eggs of kudzu bugs in eastern Tennessee and northern Georgia in 2017.

Materials and Methods

Collection of Kudzu Bug Egg Masses

Kudzu bug egg masses were collected from kudzu patches at 14 localities in East Tennessee and one locality in northern Georgia from 7 June 2017 – 15 Sep 2017 (Figure 3, Ch. II). Eggs were collected prior to sweep-net sampling at the same localities used in Ch. II to prevent damage to eggs. Three egg masses containing at least 10 eggs each were collected from each locality on each collection date from 7 June 2017 – 24 Aug 2017. Collections made from 29 Aug 2017 – 15 Sep 2017 were solely opportunistic due to reduced *M. cibraria* oviposition.

All egg masses from the same locality and date were placed in a Petri dish containing filter paper moistened with three drops of deionized water and sealed with Parafilm. Parasitoids present at the time of collection were removed by aspirator to prevent further oviposition and stored in vials containing 95% ethanol. Petri dishes were then stored in an open cardboard box on a bench in the lab at ambient conditions ($24 \pm 1^\circ\text{C}$). Dishes were observed a minimum of twice per week until a period of two weeks with no parasitoid or kudzu bug emergence had passed. During heavy emergence periods, dishes were monitored daily. Any parasitoids observed were removed by aspirator and stored in 95% ethanol. Total number of eggs, number of parasitoids, number of kudzu bugs, and number of nonproductive eggs were recorded.

Parasitoid Identification

Specimens were identified to family using the key of Goulet and Huber (1993). Whole specimens of recorded adult parasitoids were photographed in ethanol using a dissecting scope. One female and one male each were partially cleared using Andre's agent I before being dissected and mounted onto slides using Hoyer's medium.

One additional male and female each were photographed using Scanning Electron Microscopy (SEM) for more detailed images. Specimens and images were sent to a collaborator at the USDA for species-level identification. Voucher specimens are deposited in the Insect Museum at the University of Tennessee, Knoxville.

Results and Discussion

A total of 511 kudzu bug egg masses containing 10,282 individual eggs were collected throughout the duration of this study. *M. cribraria* egg masses were readily available for collection from 7 June 2017 – 24 Aug 2017, while numbers of both egg masses and individual eggs decreased dramatically beginning 29 Aug 2017 (Figure 15). *P. saccharalis* was not recovered. Instead, a different parasitoid species emerged from kudzu bug eggs. This newly observed parasitoid is most likely a single species of sexually dimorphic wasps. This wasp is currently being identified by Dr. Jason Mottern, an encyrtid specialist at the USDA. Specimens recovered from kudzu bug eggs in Tennessee and northern Georgia in 2017 share several morphological and behavioral characteristics with *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae) (Zhang et al. 2005, Ademokoya et al. 2018), and most likely belong to this species or a congener (Figure 16). Recent recoveries of *Ooencyrtus* wasps from kudzu bug eggs in states neighboring Tennessee further support this possibility: *O. nezarae* was recovered from kudzu bug eggs on soybeans in Auburn, AL in 2016, marking its first record in North America (Ademokoya et al. 2018), and *Ooencyrtus* spp. were recovered from kudzu bug eggs in Virginia in 2015 (Dhammi et al. 2016).

Parasitoids of this yet unidentified species were recovered from kudzu bug eggs collected in four counties in Tennessee and one county in Georgia on 7 Jun 2017, and were recovered from

eggs in the remaining county (Polk Co.) on 22 Jun 2017. To the knowledge of this author, no other species of kudzu bug egg parasitoids were recovered throughout the duration of this study. Additionally, no parasitoids of any species were recovered from limited collections of kudzu bug eggs in a small companion survey conducted in soybeans at the West Tennessee Research and Education Center in Jackson (Stewart, unpublished data). If this parasitoid is in fact *O. nezarae*, these collections will represent the first report of this parasitoid species on kudzu bug eggs in kudzu in North America, as well as a first report of this species in Georgia and Tennessee.

Overall parasitism averaged across all localities for the duration of the season was 51.97%, ranging from 11.15% - 60.85% (Figure 17). High-resolution sampling in Knox County, Tennessee showed the highest average parasitism rates near the beginning and end of the survey, from late June – early July and mid-August – late September (Figure 17). Similar patterns were observed in Blount, Polk, and Murray counties, while Monroe County had a delayed first peak on 20 July, and McMinn County had the highest parasitism rates throughout July. The parasitoid was also successfully recovered from kudzu bug eggs for several weeks while kudzu bug oviposition was in decline, with parasitism rates ranging from 8.29% – 100% across all sites during this timeframe. These later-season detections indicate that the wasp may be a sustainable, effective biological control agent even when *M. cribraria* egg densities are low.

In Knox County, kudzu bugs hatched at higher rates than parasitoids in 7 Jun as well as 10 Jul – 8 Aug. Interestingly, rates of eggs that produced neither a kudzu bug nor a parasitoid were also high from mid-July – early August (Figure 12). The increase in kudzu bug hatch rate corresponds with higher rates of first-instar kudzu bug nymphs recovered from sweep-net samples (Figure 5, Ch. II). Interestingly, rates of eggs that produced neither a kudzu bug nor a parasitoid were also high during weeks 12 – 16. The mid-June – early July and mid-August

peaks do not entirely match with studies conducted in Japanese soybean and kudzu (Hirose et al. 1996, Hoshino et al. 2017); this disparity may be due to regional climate differences, or a relative lack of competition with other natural enemies of kudzu bugs.

These data suggest that the parasitoid, like *M. cribraria*, exhibits two peaks of kudzu bug parasitism on kudzu bug eggs each year in eastern Tennessee, which coincide roughly with the timeframe that adult kudzu bugs complete development, mate, and begin to oviposit. In any case, higher parasitism rates observed toward the end of the egg laying season may reduce the number of second-generation kudzu adults, and therefore reduce the burden of adult kudzu bugs as a nuisance pest in the fall. Furthermore, the fungus *Beauveria bassiana* (Ascomycota: Hypocreales: Cordycipitaceae) has not been observed to exhibit entomopathogenicity in *M. cribraria* prior to late August in east Tennessee kudzu patches. The early June – mid September activity for this parasitoid provides biological control of kudzu bug much earlier in the season. The presence of this early-season natural enemy could help reduce and maintain kudzu bug densities below levels that are economically damaging to soybean producers.

Some concerns about this parasitoid, however, have not yet been explored. The 2016-2017 winter was relatively mild compared to the 2017-2018 winter in our sampling range. The average minimum temperature 1 Nov 2016 – 31 Mar 2017 was about 3.78°C, with only 41 days where the minimum temperature was below 0°C (NOAA 2018). The 2017-2018 winter had an average minimum temperature of approximately 1.88°C and 55 days with a minimum temperature below 0°C. *P. saccharalis* has been recovered at fairly low rates and only in states to the south of Tennessee since its initial discovery, which may be due to a lack of cold-tolerance in the invasive population (Ruberson et al. 2013, Hoshino et al. 2017). Established populations of kudzu bug, however, have become established much farther north (Grant et al. 2014). Additional

surveillance will be needed in subsequent years to determine if this parasitoid can overwinter in Tennessee and provide biological control beyond the extreme southeastern range of this invasive soybean pest.

Furthermore, *O. nezarae* was first found in a location (Alabama) where *P. saccharalis* has been consistently found. While it is yet unknown how either parasitoid was introduced to the U.S., it is important to understand how these populations may interact. *O. nezarae* and *P. saccharalis* do coexist in their native range. Hoshino et al. (2017) have found some evidence of interspecific competition between these wasps, the most worrisome of which is that *O. nezarae* can hyperparasitize final-instar *P. saccharalis* within kudzu bug eggs. While the resulting egg parasitoid that emerges can offer control of *M. cribraria* regardless, this may be an economic consideration for those planning to release *P. saccharalis* as a part of their pest management program. If the parasitoid recovered in Tennessee does not belong to either species, further research will be needed to determine how the recovered species interacts with *O. nezarae* and *P. saccharalis*.

CHAPTER VI CONCLUSIONS

The kudzu bug, *Megacopta cribraria* (F.) (Hemiptera: Plataspidae), is an invasive insect first found in the U.S. in Georgia in 2009, and in Tennessee in 2012. This insect feeds primarily on the invasive kudzu vine, *Pueraria montana* Lour. (Merr.) var. *lobata* (Willd.) (Fabales: Fabaceae), but can complete development on closely related soybean (*Glycine max* (L.) Merr. (Fabales: Fabaceae)) with no apparent fitness costs. Severe nuisance infestations of kudzu bug adults can also occur on white and tan structures in the fall, before adults overwinter. Kudzu bugs quickly dispersed throughout the southeastern U.S. and became established as a severe economic pest of soybeans. Much initial research focused on pest management within soybeans and for homeowners, but little research was done on kudzu bug populations within kudzu. In Tennessee, kudzu bugs generally do not disperse to soybeans until the first generation completes its development on kudzu. Research in soybeans alone would ignore the first and founding generation of this pest. The first investigation of kudzu bug populations on kudzu in Tennessee was conducted by Britt (2016) and led to more questions. Thus, a two-year research project was initiated to further examine kudzu bugs and factors influencing their populations in the region.

Populations of kudzu bugs were studied in 2016 and 2017 using the same sites and methodologies employed during 2014-2015 by Britt (2016) in order to observe longer-term trends or changes throughout the region. Expected results of this continued study were that kudzu bug densities would persist at similar levels to those observed in previous years, and that three population peaks would be observed coinciding with the first generation, second generation, and population maturation into pre-overwintering adults. However, several differences were observed. Kudzu bug populations have declined overall since the beginning of

population monitoring in 2014. The lowest population densities of adults were observed in 2016 at all sites and no peaks at the end of the first generation or pre-overwintering aggregations were observed. During the one noticeable population peak in Knox County, densities of late instar nymphs were higher than adult densities for the first time. Densities of older nymphs also exceeded adult densities prior to the second-generation peak in 2017. Three expected adult peaks were observed in Knox, Blount, Monroe, and Murray Counties in 2017, with higher adult population densities following the end of the first generation. Similarly, higher adult population densities were observed following the end of the first generation in McMinn and Polk Counties; however, large peaks later in the season were not observed in either county. Adult densities at the end of the first generation and during pre-overwintering aggregations were similar in Monroe and Murray Counties. The highest adult densities late in the season were found in Knox and Blount Counties. These counties are the two northernmost, as well as the most heavily urbanized, of any counties sampled. The remaining four counties are more rural and have more soybean production; these factors could drive the differences seen between the sites.

Several abiotic environmental factors were investigated to determine their impact on densities of kudzu bug populations. Accumulated degree days, drought index, week of collection, and temperature and precipitation during the week prior to collection had significant relationships with kudzu bug population densities. However, the relationships of these factors with kudzu bug populations differed throughout the season. Accumulated degree days had the strongest positive relationship with kudzu bug densities of any factor during the developmental timeframe (Apr – Aug) but ceased to have a statistically significant relationship after this point. Drought index had a strong negative relationship with kudzu bug densities throughout the season. Week of collection was not related to kudzu bug numbers during the developmental

timeframe (Apr – Aug) but had a significant negative relationship with population density during the pre-overwintering phase (Sep – Oct). Precipitation during the week prior to collection had a significant positive relationship with kudzu bug density during population development but had a significant negative relationship beginning in September. Average temperature in the week before collection had a significant positive relationship with kudzu bug density throughout the season.

Degree days accumulation is a common method used to predict pest emergence and pest outbreaks. However, accumulated degree days were not a significant predictor for densities of pre-overwintering kudzu bugs, which cause the greatest nuisance infestations of homes. This model shows that several other factors are at play for both kudzu bug development and densities. Phloem-feeding kudzu bugs also appear to be limited by water availability, as shown by the impact of recent precipitation and drought. However, because our observations were conducted only in drought-stricken kudzu patches and not in irrigated soybean fields, it is possible that the negative impact of drought on kudzu bugs may be influenced by emigration in addition to mortality.

The presence of natural enemies of kudzu bug may also be driving the reduction in kudzu bug densities. *Beauveria bassiana* (Ascomycota: Hypocreales: Cordycipitaceae) has been recovered as an entomopathogen of kudzu bugs on kudzu patches in eastern Tennessee from late August – late October each year since its initial detection in kudzu bugs. Individual site characteristics may lead to localized differences in infection rates of kudzu bugs, but the extent of these differences seem to vary by life stage. Adults appear to be infected at higher rates as the weeks progress following detection of *B. bassiana* each year at every site sampled. Infection levels of nymphs seem to be more complicated when site differences are considered. In Blount,

Knox, and Murray Counties, percent infection of nymphs largely increased from the beginning of sampling until its conclusion 10 weeks later. In Polk County, infection levels were fairly consistent throughout sampling. In McMinn and Monroe Counties, nymph infection levels decreased over time. These counties receive the greatest amount of time in full sun each day, which could be related to these observed differences.

Endophytic *B. bassiana* was also recovered from wild kudzu collected at three sites within Knox County in December 2017. Kudzu bugs are largely overwintering during this time, but this survey gives us some insight into the potential mechanism by which kudzu bugs are infected by this fungus. Further research is needed to determine if *B. bassiana* colonizes kudzu tissue at certain times of the year, and if endophytic activity overlaps with the seasonal infections of kudzu bug by this fungus.

The exotic egg parasitoid *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygasteridae) had not been detected in Tennessee since it was initially found in Georgia in 2013 and was not found throughout the course of this study. However, another egg parasitoid species (Hymenoptera: Encyrtidae) was detected. This study represents the first known occurrence of any egg parasitoid of kudzu bug in Tennessee and the first record of an encyrtid egg parasitoid of kudzu bug in Georgia. This insect is currently being identified to species by a specialist at the USDA. These specimens share many morphological and behavioral characteristics with *Ooencyrtus nezarae* Ishii (Hymenoptera: Encyrtidae), and if they do belong to this species, this study would represent the first record of this insect in kudzu in North America. This parasitoid was recovered from all sites surveyed and emerged from kudzu bug eggs collected 7 Jun – 15 Sep 2017. Average parasitism across all sites throughout the season was 51.97%. Further surveillance is needed to determine if this parasitoid can successfully

overwinter in Tennessee. If so, this parasitoid may be able to exert early-season control on kudzu bug populations during their first and second generations in Tennessee.

Declines in populations of kudzu bugs during the last four years illustrate the influence of abiotic and biotic factors on population dynamics of this invasive pest. In fact, the combination of egg parasitism from early June – mid-September and infection by *B. bassiana* from late August – late October could result in sustainable, near season-long control of kudzu bug in Tennessee. These natural enemies may be able to exert sufficient control to keep kudzu bugs from reaching levels that are economically damaging to soybean growers, while still allowing kudzu bugs to persist at levels that reduce biomass of kudzu. Nearly 150 years after the introduction of “the vine that ate the South,” the kudzu bug may be a sustainable and natural kudzu management tool.

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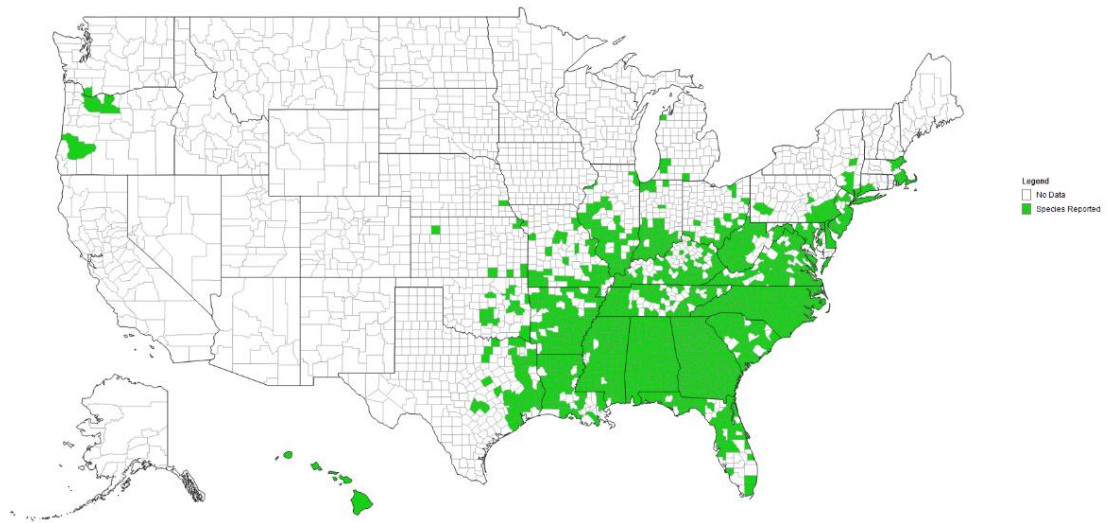
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APPENDIX

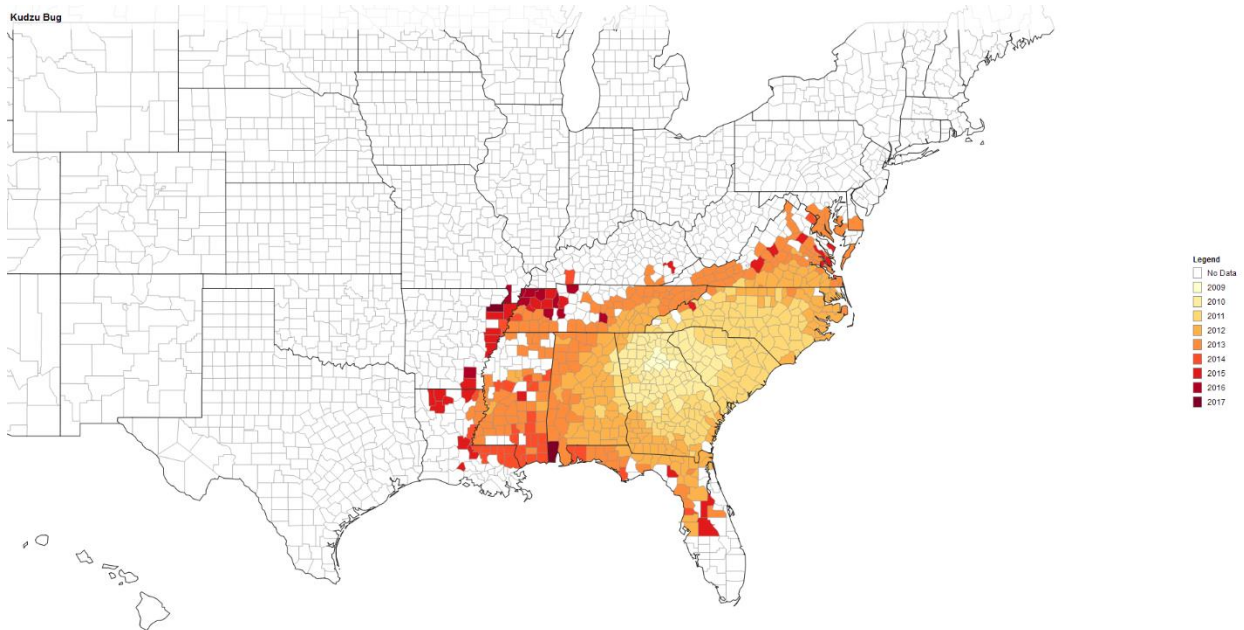
kudzu (*Pueraria montana* var. *lobata*)



Map generated on Jan 21, 2018

EDDMapS
EPA's Environmental Data Management System

Figure 1. Distribution of Kudzu in the United States (EDDMapS 2018).



Map generated on Dec 14, 2017

EDDMapS
EPA's Environmental Data Management System

Figure 2. Distribution of Kudzu Bug in the United States (Megacopta Working Group 2017).

Table 1. Localities sampled for kudzu bug, Tennessee and Georgia, 2016 and 2017.

County‡	Site Code	Latitude / Longitude	Years Sampled
Knox County	SRT (South, River Towne Apartments)	N 35.942995 W 83.926313	2016*
Knox County	SUP (South, University Park Apartments)	N 35.949290 W 83.910237	2017*
Knox County	SKB (South, Kern's Bakery)	N 35.95197 W 83.91433	2016 2017
Knox County	SBB (South, Bayou Bay Restaurant)	N 35.96883 W 83.82430	2016 2017
Knox County	WAU (West, Aubrey's Restaurant)	N 35.942584 W 84.095780	2016 2017
Knox County	WBC (West, Ball Camp Baptist Church)	N 35.95657 W 84.12556	2016 2017
Knox County	WCO (West, Country Oaks Apartments)	N 35.951081 W 83.991177	2016 2017
Knox County	NLA (North, Lambert Family Property)	N 36.00985 W 84.03224	2016 2017
Knox County	NUT (North, UT Medical Plaza Halls)	N 36.069874 W 83.926533	2016 2017
Knox County	NHS (North, Halls Senior Center)	N 36.076025 W 83.918947	2016 2017
Blount County	Blount	N 35.74912 W 83.96170	2016 2017
Monroe County	Monroe	N 35.56232 W 84.29740	2016 2017
McMinn County	McMinn	N 35.28909 W 84.54333	2016 2017
Polk County	Polk	N 35.16464 W 84.68258	2016 2017
Murray County (GA)	Murray	N 34.95133 W 84.73602	2016† 2017

* The SRT site was destroyed and fenced following the end of the 2016 sampling season. The SUP site was selected in 2017 as a replacement.

† Vegetative kudzu growth at the Murray Co. site was destroyed after 24 Oct 2016. Sampling was not conducted following that date in 2016. The kudzu regenerated in 2017 and was sampled for the full season.

‡ All counties were located in Tennessee with the exception of Murray County (GA).

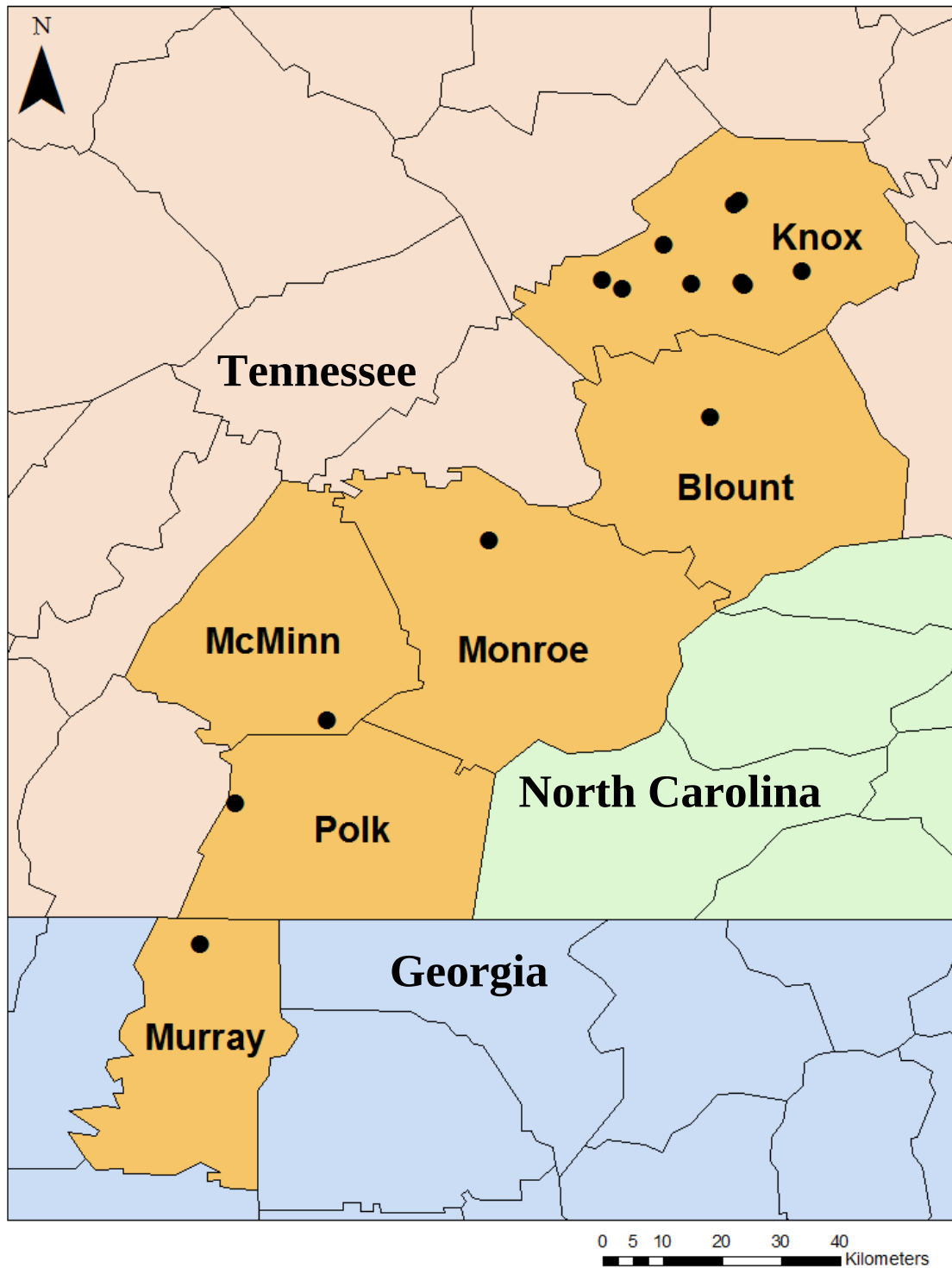


Figure 3. Map of localities visited to assess kudzu bug populations, 2016 and 2017.

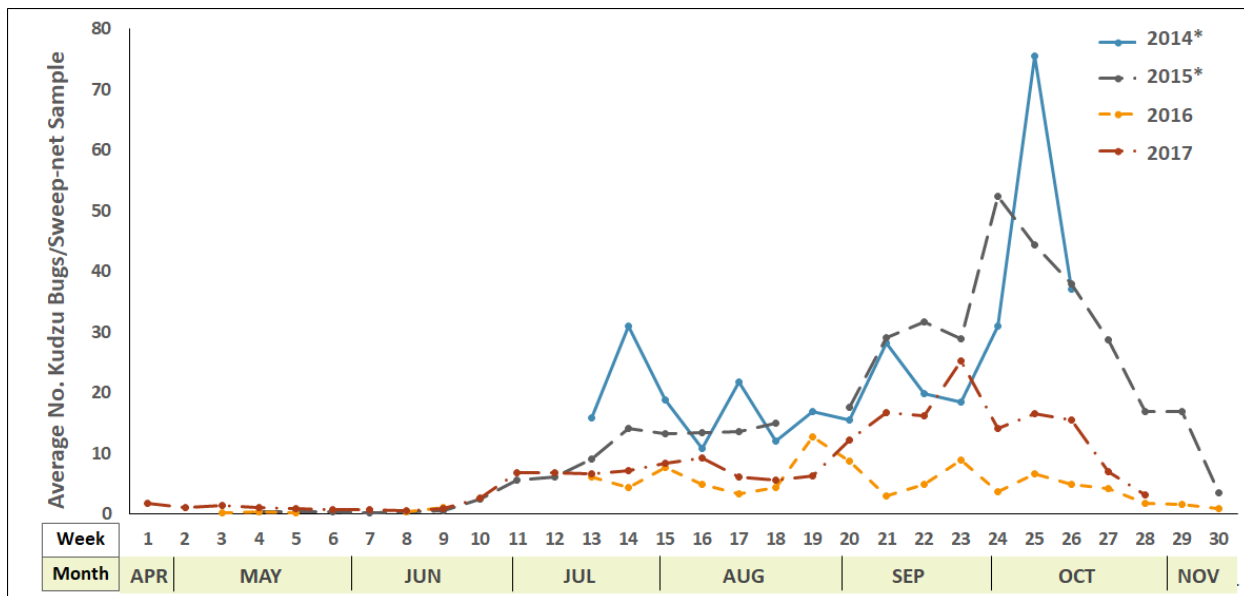


Figure 4. Adult kudzu bug population densities on kudzu in Knox County, TN over 4 years. *Data from 2014 and 2015 provided by Kadie Britt (2016).

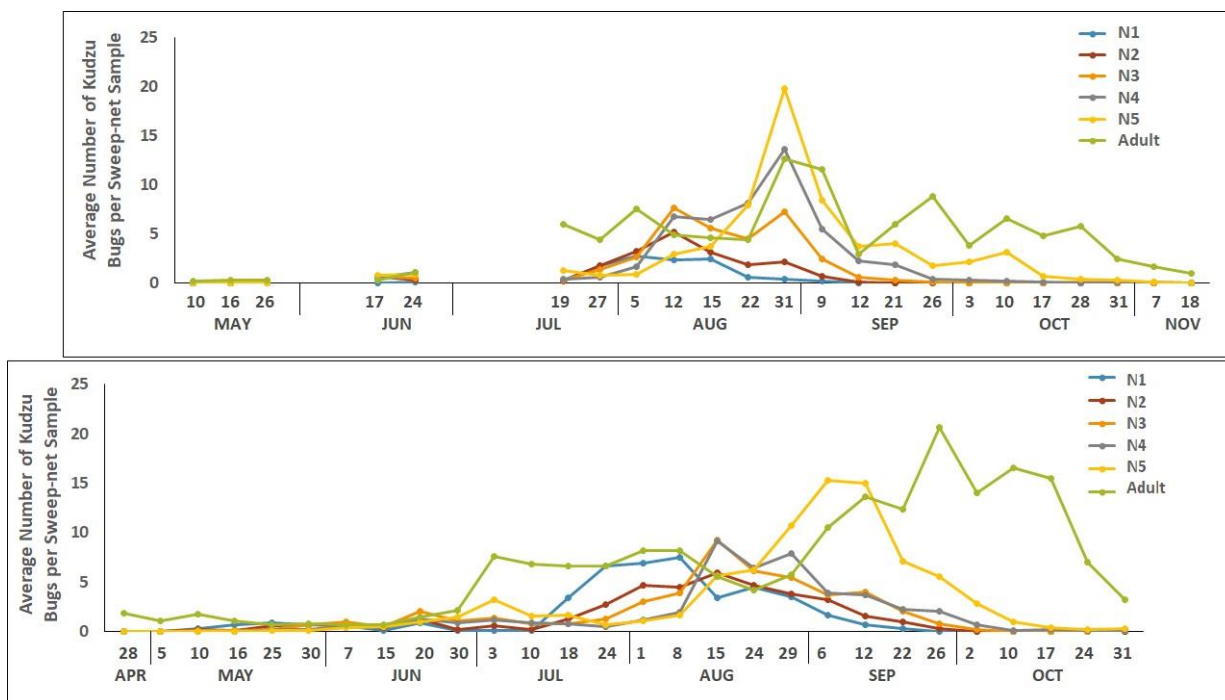


Figure 5. Estimated densities of kudzu bugs on kudzu by life stage in Knox County, TN in 2016 (A) and 2017 (B).

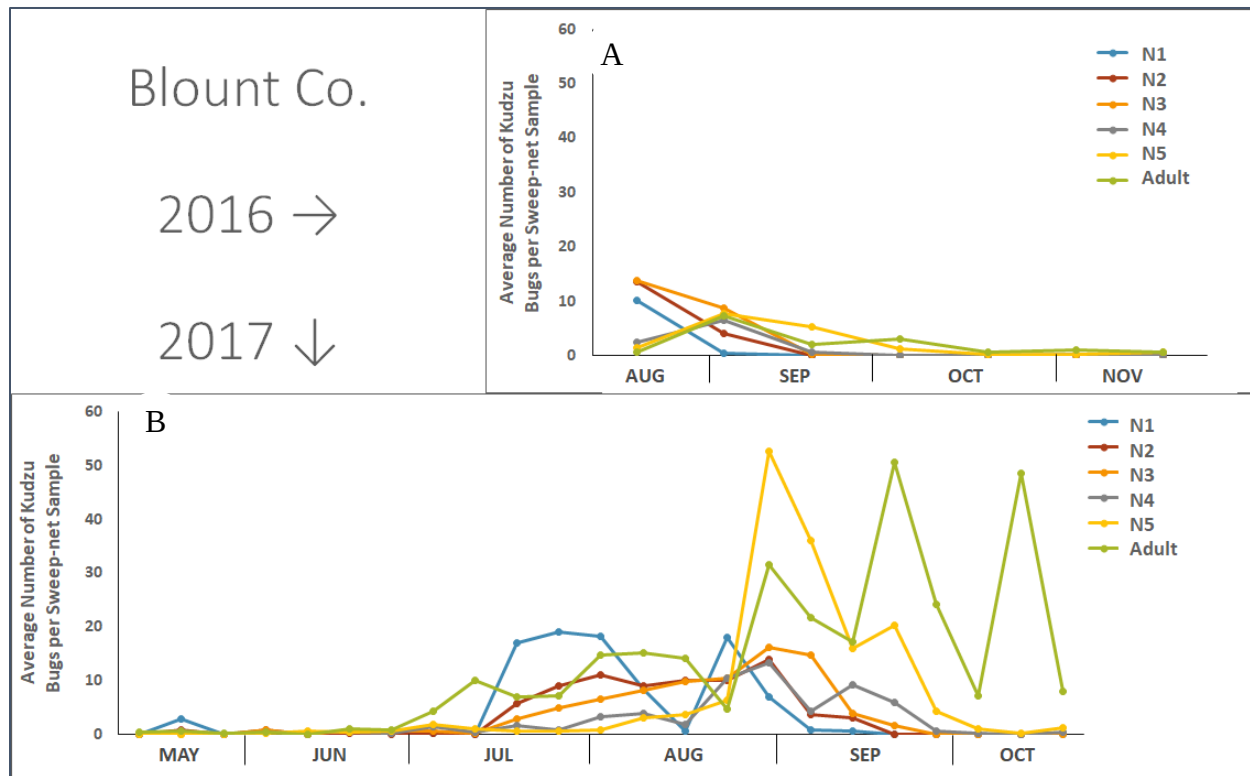


Figure 6. Estimated densities of kudzu bugs on kudzu by life stage in Blount County, TN in 2016 (A) and 2017 (B).

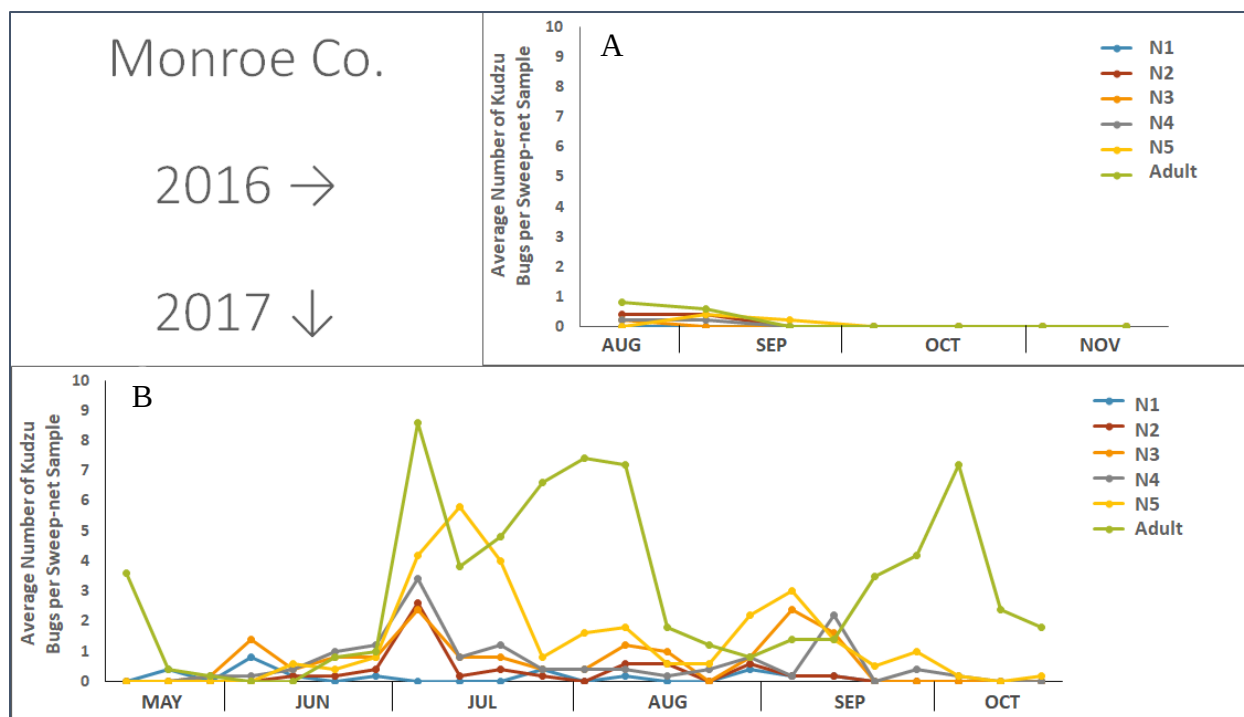


Figure 7. Estimated densities of kudzu bugs on kudzu by life stage in Monroe County, TN in 2016 (A) and 2017 (B).

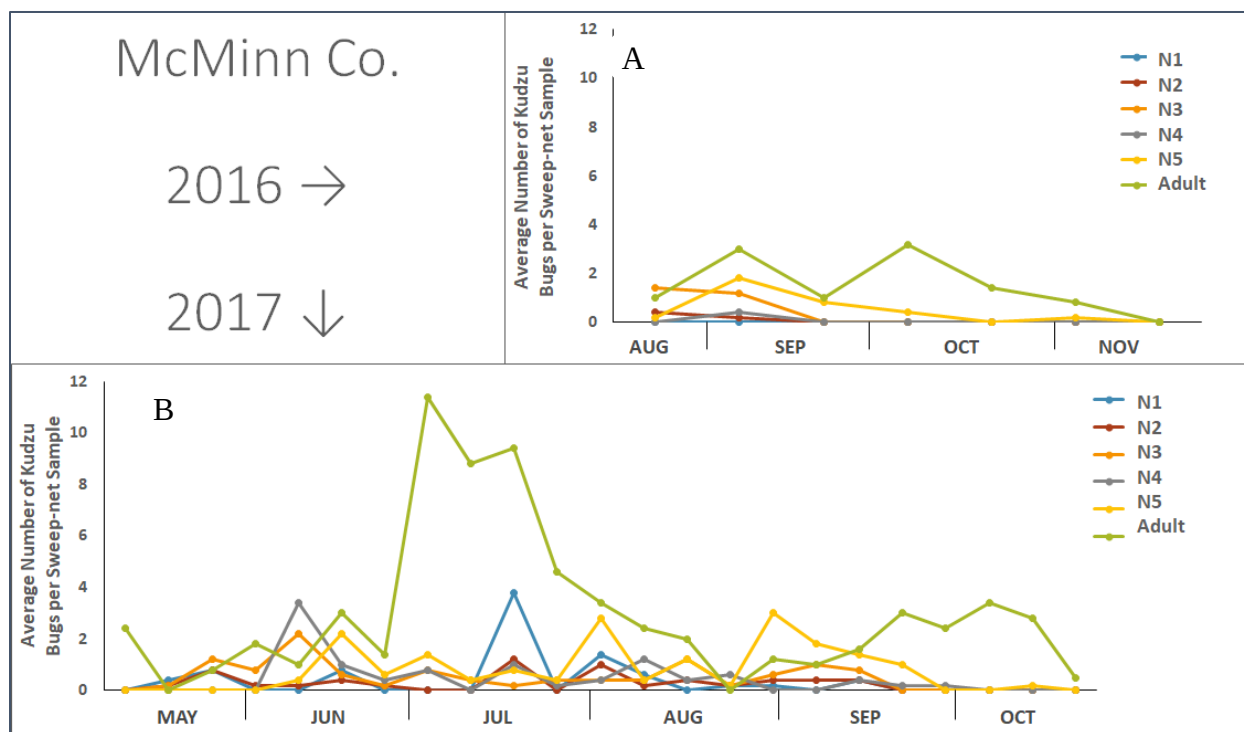


Figure 8. Estimated densities of kudzu bugs on kudzu by life stage in McMinn County, TN in 2016 (A) and 2017 (B).

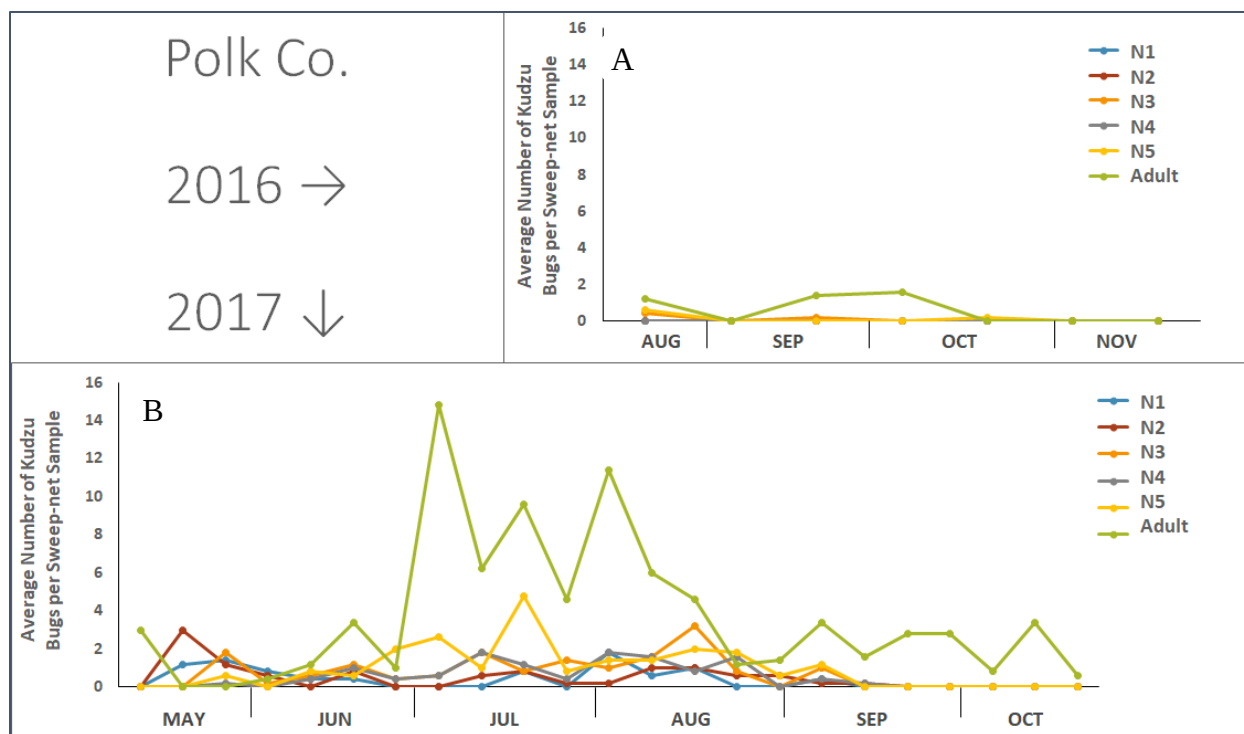


Figure 9. Estimated densities of kudzu bugs by life stage in Polk County, TN in 2016 (A) and 2017 (B).

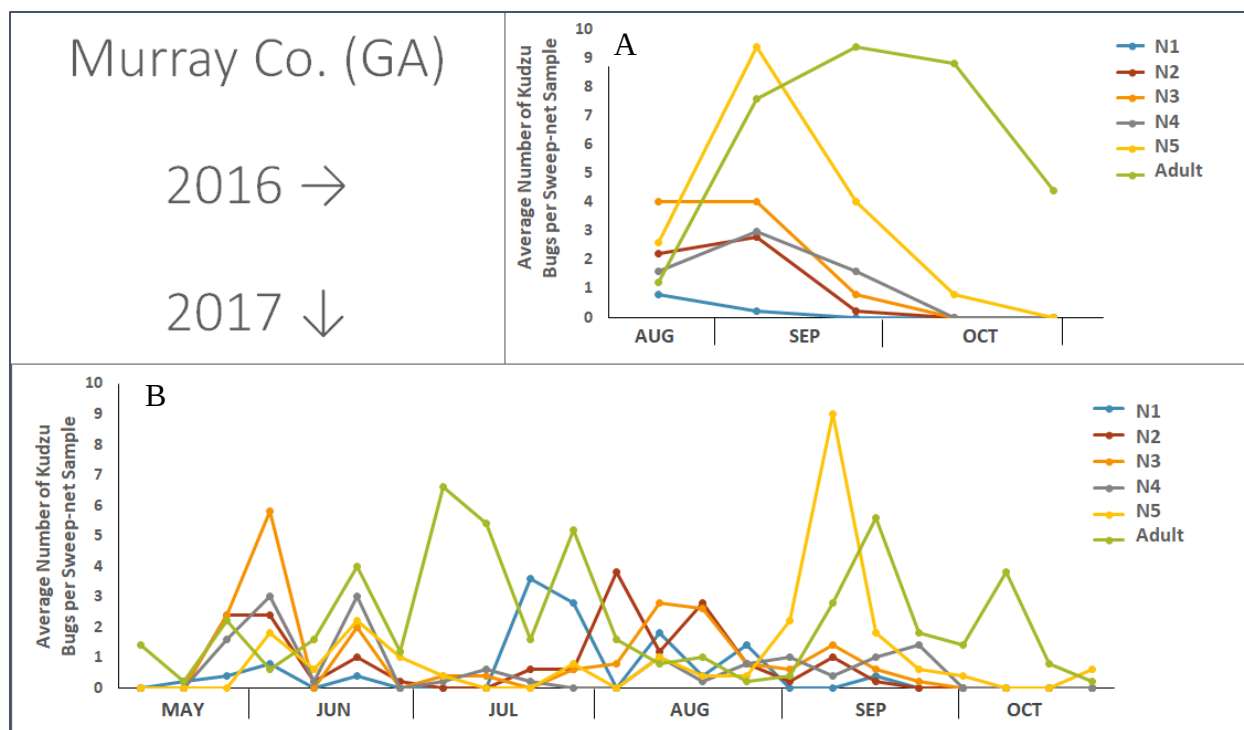


Figure 10. Estimated densities of kudzu bugs on kudzu by life stage in Murray County, GA in 2016 (A) and 2017 (B).

Table 2. Numbers of kudzu bugs per life stage per year across 14 sites, 2016 and 2017.

Life Stage	Total	2016	Total	2017
		Average/ Sweep $\pm$ SE		Average/ Sweep $\pm$ SE
N1	506	0.43 ± 0.05	2,841	1.55 ± 0.14
N2	944	0.80 ± 0.07	2,564	1.40 ± 0.09
N3	1,662	1.41 ± 0.13	3,203	1.75 ± 0.11
N4	2,307	1.95 ± 0.17	3,052	1.67 ± 0.12
N5	3,055	2.59 ± 0.19	5,542	3.03 ± 0.19
Adults	4,828	4.09 ± 0.19	12,393	6.76 ± 0.30
Adult ♀	2,323	1.97 ± 0.09	5,648	3.09 ± 0.13
Adult ♂	2,505	2.12 ± 0.11	6,745	3.69 ± 0.18

Table 3. Relative differences in kudzu bug densities between counties.

Analysis of Maximum Likelihood Zero Inflation Parameter Estimates								
Life Stage	County	df	Estimate	SE	Wald 95% Confidence Limits		Wald χ^2	P
N1	(Intercept)	1	0.4810	0.2902	-0.0878	1.0497	2.75	0.0975
	Blount	1	-0.5458	0.3666	-1.2643	0.1727	2.22	0.1365
	Knox	1	-0.1267	0.2972	-0.7093	0.4559	0.18	0.6699
	McMinn	1	0.0751	0.4238	-0.7556	0.9057	0.03	0.8594
	Monroe	1	0.6863	0.5319	-0.3561	1.7287	1.66	0.1969
	Murray	1	-0.2114	0.4130	-1.0209	0.5981	0.26	0.6088
	Polk*	0	0.0000	0.0000	0.0000	0.0000	.	.
N2	(Intercept)	1	0.3236	0.2844	-0.2338	0.8810	1.29	0.2552
	Blount	1	-0.9960	0.3761	-1.7332	-0.2588	7.01	0.0081
	Knox	1	-0.5855	0.2920	-1.1577	-0.0132	4.02	0.0449
	McMinn	1	-0.5551	0.6270	-1.7840	0.6739	0.78	0.3760
	Monroe	1	0.2230	0.4502	-0.6594	1.1055	0.25	0.6203
	Murray	1	-0.6879	0.3951	-1.4623	0.0864	3.03	0.0817
	Polk*	0	0.0000	0.0000	0.0000	0.0000	.	.
N3	(Intercept)	1	-0.3038	0.2945	-0.8810	0.2734	1.06	0.3023
	Blount	1	-0.4352	0.3755	-1.1712	0.3009	1.34	0.2465
	Knox	1	-0.0755	0.3018	-0.6670	0.5160	0.06	0.8024
	McMinn	1	-0.2447	0.4636	-1.1533	0.6639	0.28	0.5976
	Monroe	1	0.2577	0.3919	-0.5104	1.0259	0.43	0.5108
	Murray	1	-0.3398	0.4146	-1.1524	0.4727	0.67	0.4124
	Polk*	0	0.0000	0.0000	0.0000	0.0000	.	.
N4	(Intercept)	1	-0.1284	0.3567	-0.8276	0.5708	0.13	0.7190
	Blount	1	-1.1167	0.4711	-2.0401	-0.1933	5.62	0.0178
	Knox	1	-0.2950	0.3621	-1.0048	0.4148	0.66	0.4153
	McMinn	1	0.4629	0.4720	-0.4622	1.3881	0.96	0.3267
	Monroe	1	0.2022	0.4409	-0.6620	1.0664	0.21	0.6465
	Murray	1	0.2529	0.4318	-0.5934	1.0992	0.34	0.5581

Table 3. Continued.

Analysis of Maximum Likelihood Zero Inflation Parameter Estimates								
Life Stage	County	df	Estimate	SE	Wald 95% Confidence Limits		Wald χ^2	P
N4	Polk*	0	0.0000	0.0000	0.0000		0.0000	.
	(Intercept)	1	-0.2315	0.2435	-0.7087	0.2456	0.90	0.3416
	Blount	1	-1.1630	0.3579	-1.8645	-0.4615	10.56	0.0012
	Knox	1	-0.6328	0.2521	-1.1269	-0.1387	6.30	0.0121
N5	McMinn	1	-0.6551	0.4175	-1.4734	0.1633	2.46	0.1167
	Monroe	1	0.0524	0.3332	-0.6007	0.7054	0.02	0.8751
	Murray	1	-0.0891	0.3330	-0.7418	0.5637	0.07	0.7891
	Polk*	0	0.0000	0.0000	0.0000	0.0000	.	.
Adult	(Intercept)	1	-1.0311	0.2115	-1.4457	-0.6166	23.76	<.0001
	Blount	1	-0.5186	0.3211	-1.1480	0.1107	2.61	0.1063
	Knox	1	-0.6620	0.2236	-1.1001	-0.2238	8.77	0.0031
	McMinn	1	-0.1919	0.3154	-0.8102	0.4263	0.37	0.5429
	Monroe	1	0.7201	0.2829	0.1655	1.2746	6.48	0.0109
	Murray	1	-0.5877	0.3527	-1.2790	0.1036	2.78	0.0957
	Polk*	0	0.0000	0.0000	0.0000	0.0000	.	.

* The Polk County site was used as a reference for all comparisons.

Table 4. Week-wise differences in fifth-instar nymph densities, collected from kudzu in 2016 and 2017.

Analysis of Maximum Likelihood Parameter Estimates							
Week	df	Estimate	SE	Wald 95% Confidence Limits		Wald χ^2	P
(Intercept)	1	-1.6160	0.3962	-2.3924	-0.8395	16.64	<.0001
1	1	1.6126	0.4504	0.7299	2.4953	12.82	0.0003
2	1	0.7735	0.4575	-0.1232	1.6701	2.86	0.0909
3	1	1.2548	0.4238	0.4241	2.0855	8.77	0.0031
4	1	0.1817	0.4443	-0.6891	1.0525	0.17	0.6826
5	1	-1.9616	0.8107	-3.5504	-0.3727	5.86	0.0155
6	1	0.5817	0.4643	-0.3284	1.4917	1.57	0.2103
7	1	0.7682	0.4394	-0.0931	1.6294	3.06	0.0805
8	1	-0.0528	0.4459	-0.9268	0.8212	0.01	0.9057
9	1	0.5932	0.4230	-0.2358	1.4223	1.97	0.1608
10	1	1.2821	0.4302	0.4389	2.1254	8.88	0.0029
11	1	1.8755	0.4225	1.0475	2.7035	19.71	<.0001
12	1	2.7902	0.3957	2.0146	3.5659	49.71	<.0001
13	1	2.5294	0.3944	1.7564	3.3023	41.13	<.0001
14	1	2.4913	0.3953	1.7166	3.2660	39.73	<.0001
15	1	2.6957	0.3945	1.9224	3.4689	46.69	<.0001
16	1	2.6212	0.3924	1.8521	3.3903	44.62	<.0001
17	1	2.5676	0.3940	1.7955	3.3398	42.48	<.0001
18	1	2.7876	0.3934	2.0164	3.5587	50.20	<.0001
19	1	2.8656	0.3933	2.0948	3.6365	53.08	<.0001
20	1	2.9682	0.3932	2.1975	3.7389	56.98	<.0001
21	1	3.2277	0.3924	2.4587	3.9967	67.68	<.0001
22	1	3.1686	0.3924	2.3994	3.9377	65.20	<.0001
23	1	3.5375	0.3919	2.7694	4.3055	81.49	<.0001
24	1	3.4050	0.3928	2.6352	4.1748	75.16	<.0001
25	1	3.3981	0.3920	2.6298	4.1665	75.13	<.0001
26	1	3.3613	0.3921	2.5928	4.1298	73.49	<.0001

Table 4. Continued.

Analysis of Maximum Likelihood Parameter Estimates							
Life Stage	df	Estimate	SE	Wald 95% Confidence Limits		Wald χ^2	P
27	1	2.8280	0.3942	2.0554	3.6006	51.47	<.0001
28	1	2.3493	0.3976	1.5699	3.1286	34.91	<.0001
29	1	1.5371	0.4316	0.6912	2.3829	12.68	0.0004
30	1	1.3002	0.4305	0.4565	2.1439	9.12	0.0025
31*	0	0.0000	0.0000	0.0000	0.0000	.	.

* Week 31 was used as a reference for all comparisons.

Table 5. Week-wise differences in adult densities, collected from kudzu in 2016 and 2017.

Analysis of Maximum Likelihood Parameter Estimates							
Week	df	Estimate	SE	Wald 95% Confidence Limits		Wald χ^2	P
	1	-1.7458	0.3654	-2.4620	-1.0296	22.82	<.0001
1	1	2.3365	0.3912	1.5698	3.1032	35.68	<.0001
2	1	1.5437	0.4019	0.7559	2.3314	14.75	0.0001
3	1	2.6521	0.3819	1.9035	3.4007	48.21	<.0001
4	1	1.0090	0.3882	0.2481	1.7699	6.76	0.0093
5	1	0.1126	0.4357	-0.7415	0.9666	0.07	0.7961
6	1	1.3210	0.4077	0.5219	2.1200	10.50	0.0012
7	1	1.2508	0.3941	0.4783	2.0233	10.07	0.0015
8	1	0.8226	0.3872	0.0637	1.5815	4.51	0.0336
9	1	1.2881	0.3802	0.5429	2.0333	11.48	0.0007
10	1	1.9768	0.3812	1.2296	2.7239	26.89	<.0001
11	1	2.2713	0.3729	1.5404	3.0021	37.10	<.0001
12	1	3.3321	0.3648	2.6171	4.0471	83.44	<.0001
13	1	3.1210	0.3640	2.4075	3.8344	73.51	<.0001
14	1	3.0678	0.3643	2.3539	3.7818	70.93	<.0001
15	1	3.2915	0.3642	2.5777	4.0053	81.69	<.0001
16	1	3.1481	0.3631	2.4364	3.8599	75.15	<.0001
17	1	3.0304	0.3640	2.3171	3.7438	69.32	<.0001
18	1	3.3420	0.3638	2.6290	4.0550	84.40	<.0001
19	1	3.3615	0.3637	2.6487	4.0744	85.43	<.0001
20	1	3.4207	0.3638	2.7077	4.1336	88.43	<.0001
21	1	3.6065	0.3633	2.8944	4.3185	98.54	<.0001
22	1	3.7057	0.3633	2.9938	4.4177	104.07	<.0001
23	1	3.9383	0.3630	3.2267	4.6498	117.67	<.0001
24	1	3.8753	0.3635	3.1628	4.5878	113.65	<.0001
25	1	3.8366	0.3631	3.1249	4.5483	111.63	<.0001
26	1	3.7347	0.3632	3.0228	4.4466	105.73	<.0001
27	1	3.1939	0.3645	2.4794	3.9084	76.76	<.0001

Table 5. Continued.

Analysis of Maximum Likelihood Parameter Estimates							
Week	df	Estimate	SE	Wald 95% Confidence Limits		Wald χ^2	P
28	1	2.6085	0.3670	1.8892	3.3277	50.52	<.0001
29	1	1.8702	0.3911	1.1037	2.6366	22.87	<.0001
30	1	1.6644	0.3879	0.9041	2.4246	18.41	<.0001
31	0	0.0000	0.0000	0.0000	0.0000	.	.

* Week 31 was used as a reference for all comparisons.

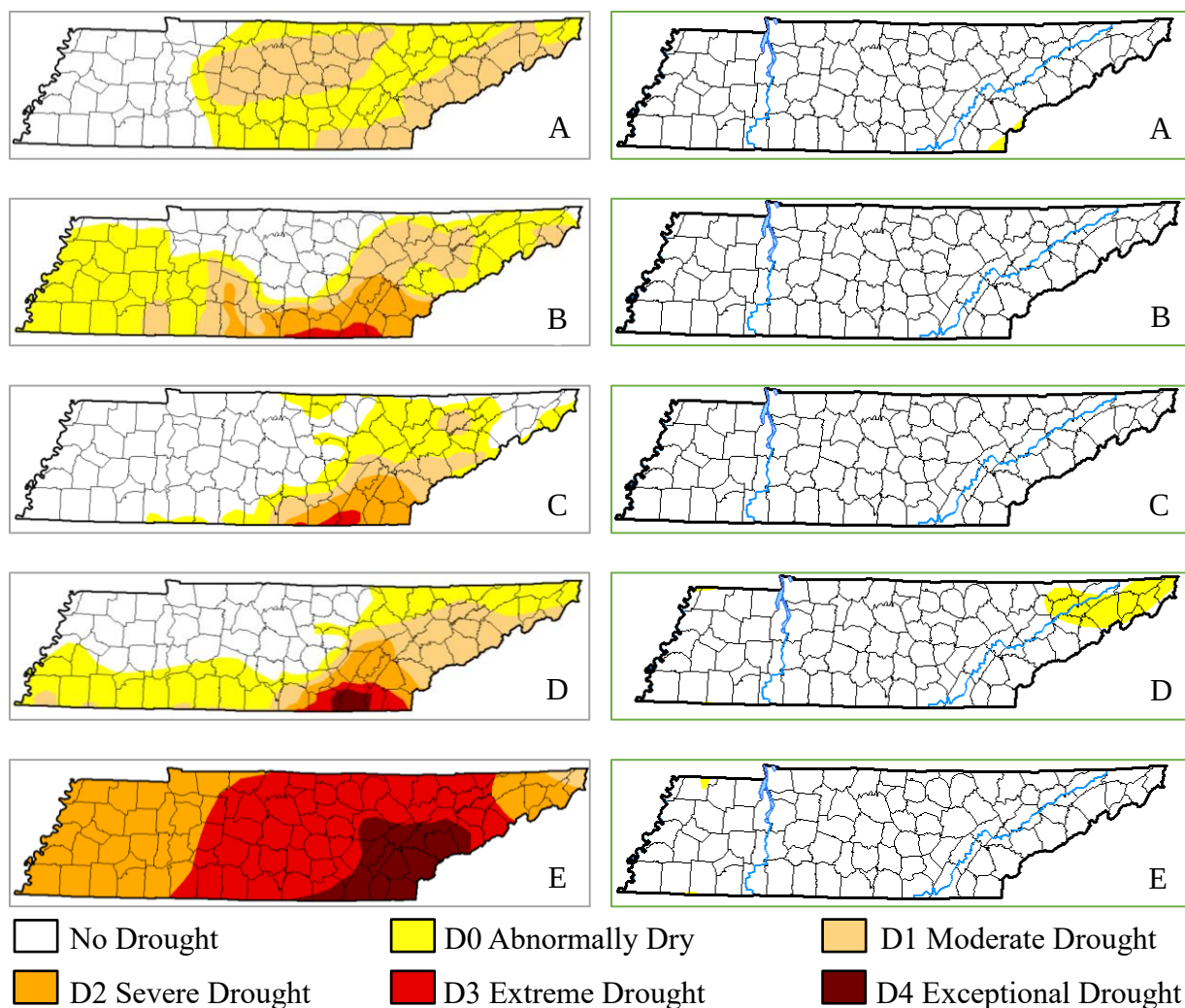


Figure 11. Drought conditions in Tennessee throughout 2016 (left) and 2017 (right) (Artusa et al. 2016). (A) Beginning of kudzu bug sampling [early May] (B) First generation of kudzu bug reaches adulthood [late Jun] (C) Week 20 [early Sep] (D) During the typical pre-overwintering peak [early Oct] (E) Kudzu bugs largely overwintering; end of sampling [mid-Nov 2016, late Oct 2017].

Table 6. Impact of factors on total kudzu bug numbers, weeks 1-19.

Effect	Regression coefficient [†]	e ^{Reg. coef.} [‡]	SE	df	t	Pr > t
Intercept	2.3224		0.3192	10	7.28	<.0001
Week*	0.0231	1.0234	0.0159	1330	1.45	0.1466
Drought Index	-0.4480	-1.5652	0.0200	1330	-22.43	<.0001
Accumulated Degree Days	1.4067	4.0825	0.1077	1330	13.06	<.0001
Precipitation WP*	0.1520	1.1642	0.0073	1330	20.83	<.0001
Average Temperature WP*	0.3520	1.4219	0.0253	1330	13.89	<.0001

* Weeks 1-19 correspond with 25 Apr – 31 Aug 2016 and 28 Apr – 29 Aug 2017.

[†] Regression coefficients with the exception of week have been standardized.

[‡] All regression coefficients have been exponentiated for interpretation.

* Week prior to sampling.

Table 7. Impact of factors on total kudzu bug numbers, weeks 20-31.

Effect	Regression coefficient [†]	e ^{Reg. coef.} [‡]	SE	df	t	Pr > t
Intercept	7.2431		0.3601	9	20.12	<.0001
Week*	-0.2016	-1.2234	0.0216	914	-9.32	<.0001
Drought Index	-0.5836	-1.7925	0.0294	914	-19.87	<.0001
Accumulated Degree Days	0.1403	1.1506	0.2377	914	0.59	0.5552
Precipitation WP*	-0.0544	-1.0559	0.0078	914	-6.94	<.0001
Average Temperature WP*	0.0766	1.0796	0.0159	914	4.83	<.0001

* Weeks 20-31 correspond with 9 Aug – 21 Nov 2016 and 6 Sep – 31 Oct 2017.

[†] Regression coefficients with the exception of week have been standardized.

[‡] All regression coefficients have been exponentiated for interpretation.

* Week prior to sampling.

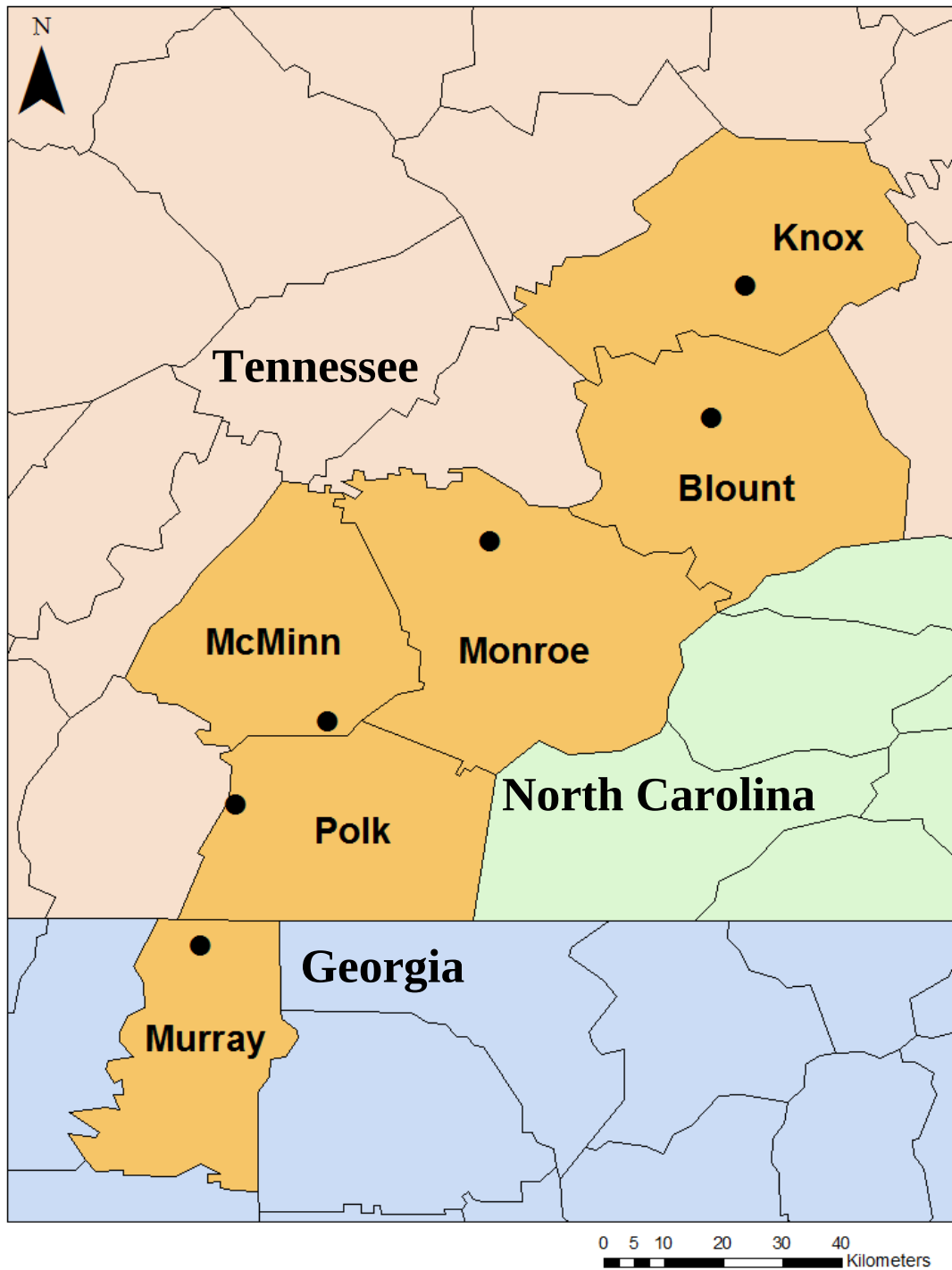


Figure 12. Sites surveyed for *Beauveria bassiana*, 2016 and 2017.

Table 8. Sites sampled for *Beauveria bassiana* in eastern Tennessee and Northern Georgia, 2016 and 2017.

County	Site Code	Latitude / Longitude	Years Sampled
Knox County	SRT (South, River Towne Apartments)	N 35.942995 W 83.926313	2016*
Knox County	SUP (South, University Park Apartments)	N 35.949290 W 83.910237	2017*
Blount County	Blount	N 35.74912 W 83.96170	2016 2017
Monroe County	Monroe	N 35.56232 W 84.29740	2016 2017
McMinn County	McMinn	N 35.28909 W 84.54333	2016 2017
Polk County	Polk	N 35.16464 W 84.68258	2016 2017
Murray County (GA)	Murray	N 34.95133 W 84.73602	2016† 2017

* The SRT site was destroyed and fenced in following the end of the 2016 sampling season. The SUP site was selected in 2017 as a replacement.

† Vegetative kudzu growth at the Murray Co. site was destroyed after 24 Oct 2016. Sampling was not conducted following that date in 2016. The kudzu regenerated in 2017 and was sampled for the full season. Thus, Murray Co. data could not be included in the week-wise analysis for weeks 26 and 28.

Table 9. Analysis of variance results for influence of site and week on percent infection with *B. bassiana* of kudzu bug by life stage.

Life Stage	Response Variable	Source of Variation	df	F	p
Nymph (I-V)	Percent Infection	Site	5, 361	5.80	<0.0001
		Week	4, 361	4.62	0.0012
		Site x Week	18, 361	2.30	0.0021
Adult	Percent Infection	Site	5, 243	3.22	0.0078
		Week	4, 243	15.98	<0.0001
		Site x Week	18, 243	1.56	0.0721

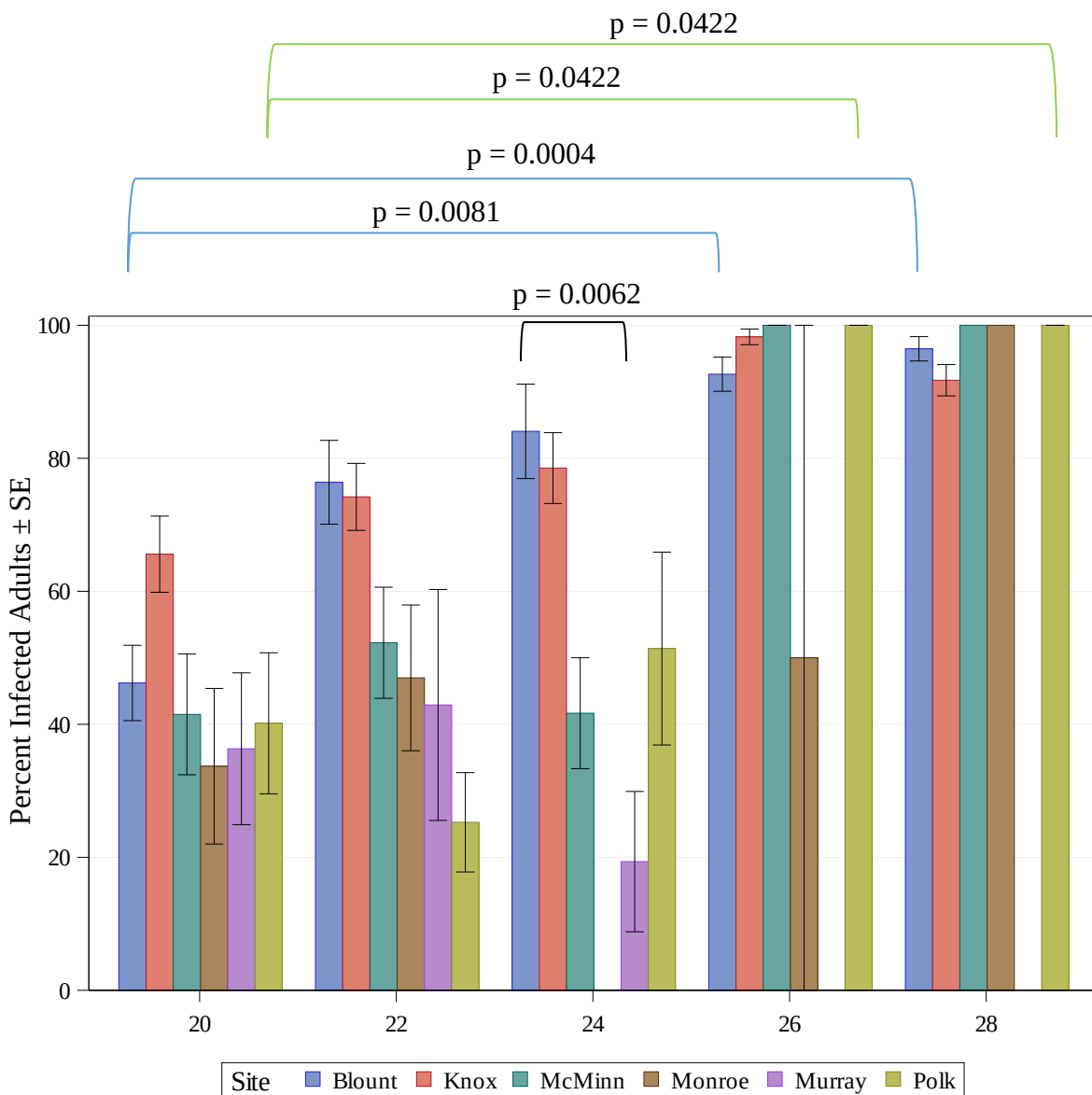


Figure 13. Percent infection of adult kudzu bugs with *B. bassiana* by site and week, 2016 and 2017. Bars represent average percent infection $\pm$ SE at each collection location and horizontal brackets represent significant differences between means (Tukey's HSD, $\alpha = 0.05$).

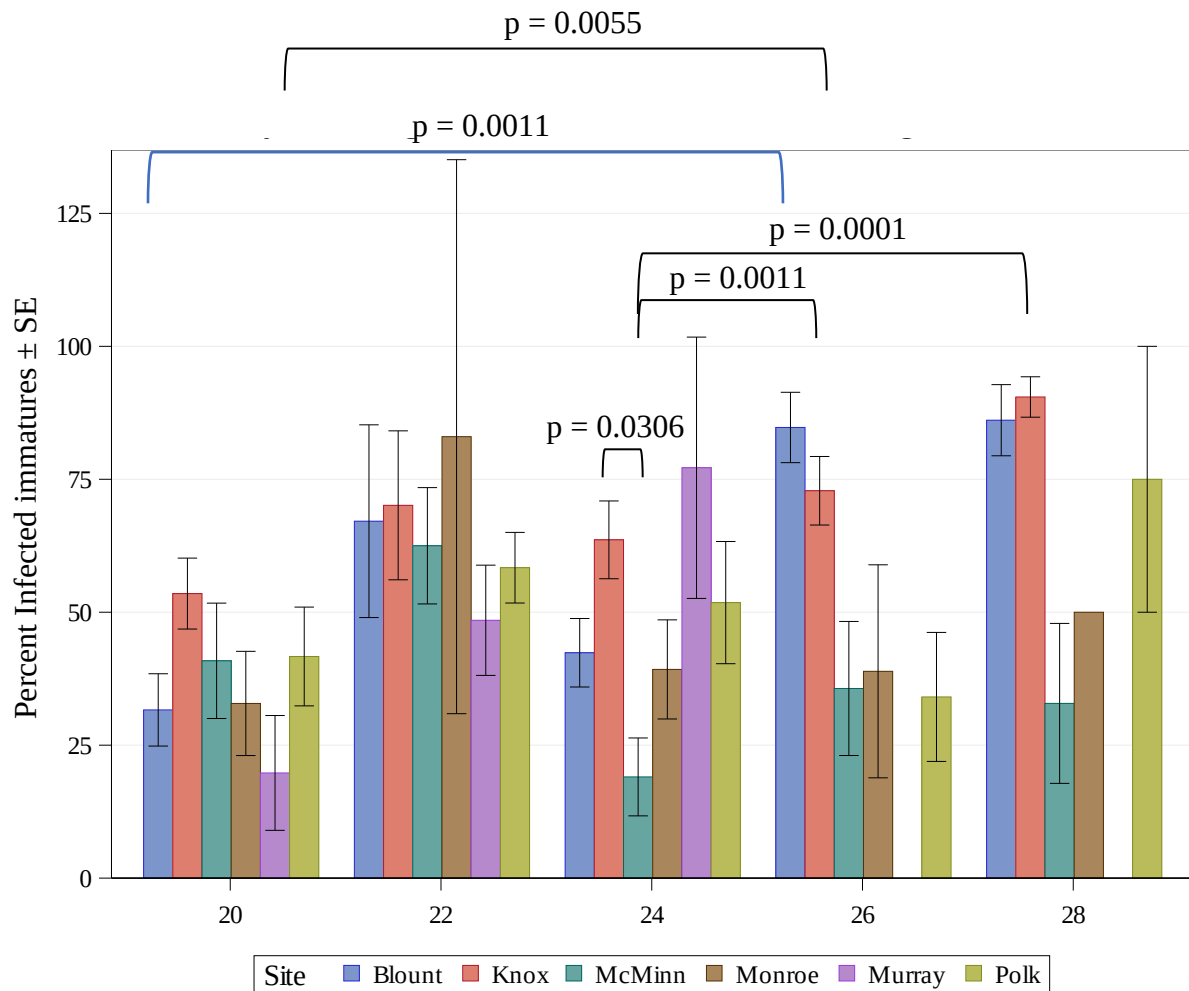


Figure 14. Percent infection of kudzu bug nymphs with *B. bassiana* by site and week, 2016 and 2017. Bars represent average percent infection $\pm$ SE at each collection location and horizontal brackets represent significant differences between means (Tukey's HSD, $\alpha = 0.05$).

Table 10. Results of screening for endophytic *Beauveria bassiana* in kudzu, 21 Dec 2017.

Site*	Sample # †	Middle	End
SUP	U1	✓	✓
	U2	✓	✓
	U3	✓	✓
	U4	✓	
	U5		
	L1	✓	
	L2	✓	✓
	L3	✓	✓
	L4		
	L5	✓	✓
WCO	1	✓	✓
	2	✓	
	3	✓	
	4	✓	✓
	5	✓	
	6	✓	✓
	7	✓	
	8	✓	✓
	9		
	10		
NHS	1		
	2	✓	
	3	✓	
	4	✓	
	5		
	6	✓	
	7		
	8	✓	
	9		
	10		

Check marks indicate samples which produced *B. bassiana* grew from surface-sterilized stems. “Middle” indicates growth from the site where kudzu bugs were attached and “End” indicates growth from the cut end of the stem.

* Site abbreviations for University Park Apartments (SUP), Elevation Apartments (WCO), and Halls Senior Center (NHS).

† Collections at the SUP site were made within pre-existing upper (U) and lower (L) transects used for assessment of infection levels (see Ch. II).

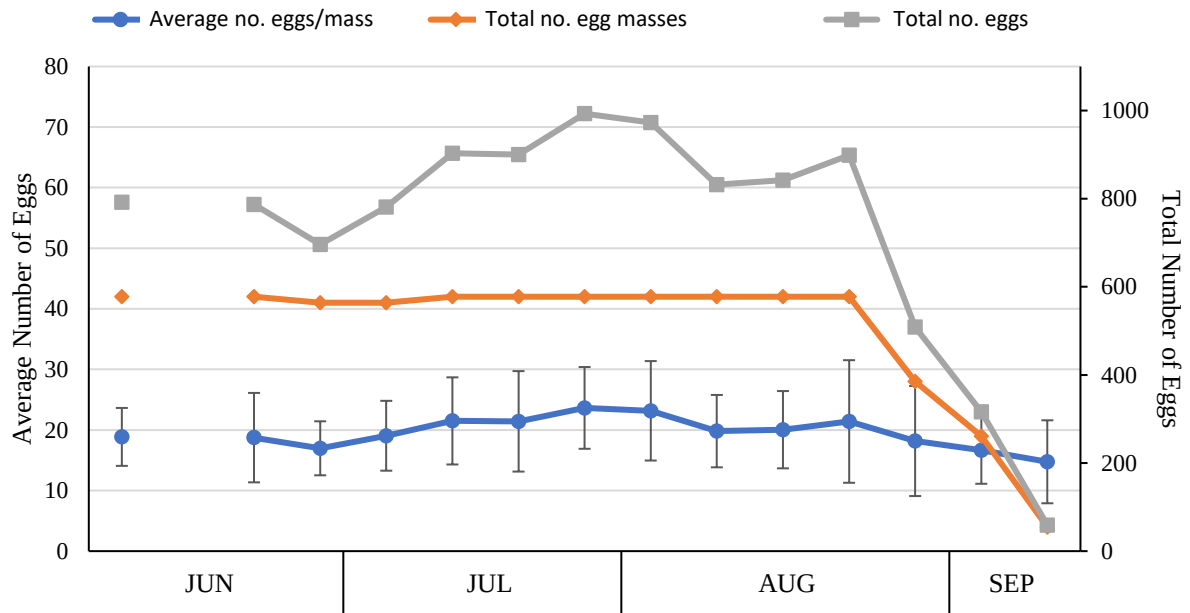


Figure 15. Number of kudzu bug eggs and egg masses collected per week across all localities in eastern Tennessee and northern Georgia, 2017.

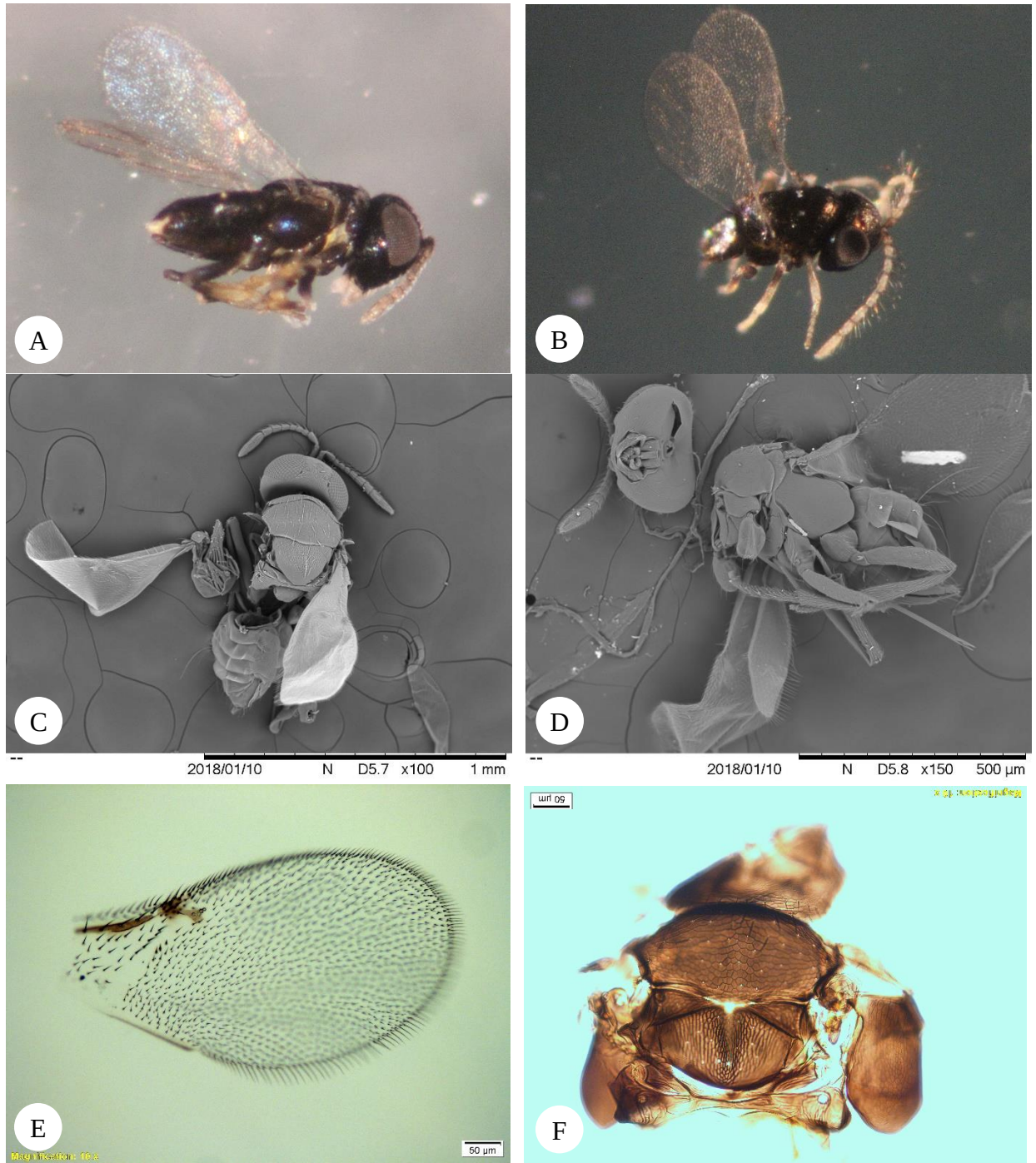


Figure 16. Images of parasitoid recovered from eggs of *M. cribraria* in Tennessee and Georgia, 2017. (A) Female habitus. (B) Male habitus. Note the dimorphic antennal morphology. (C, D) SEM image detailing the female dorsal (C) and lateral (D) sclerites. (E) Female forewing. (F) Female dorsal mesosoma and detail of ultrastructure.

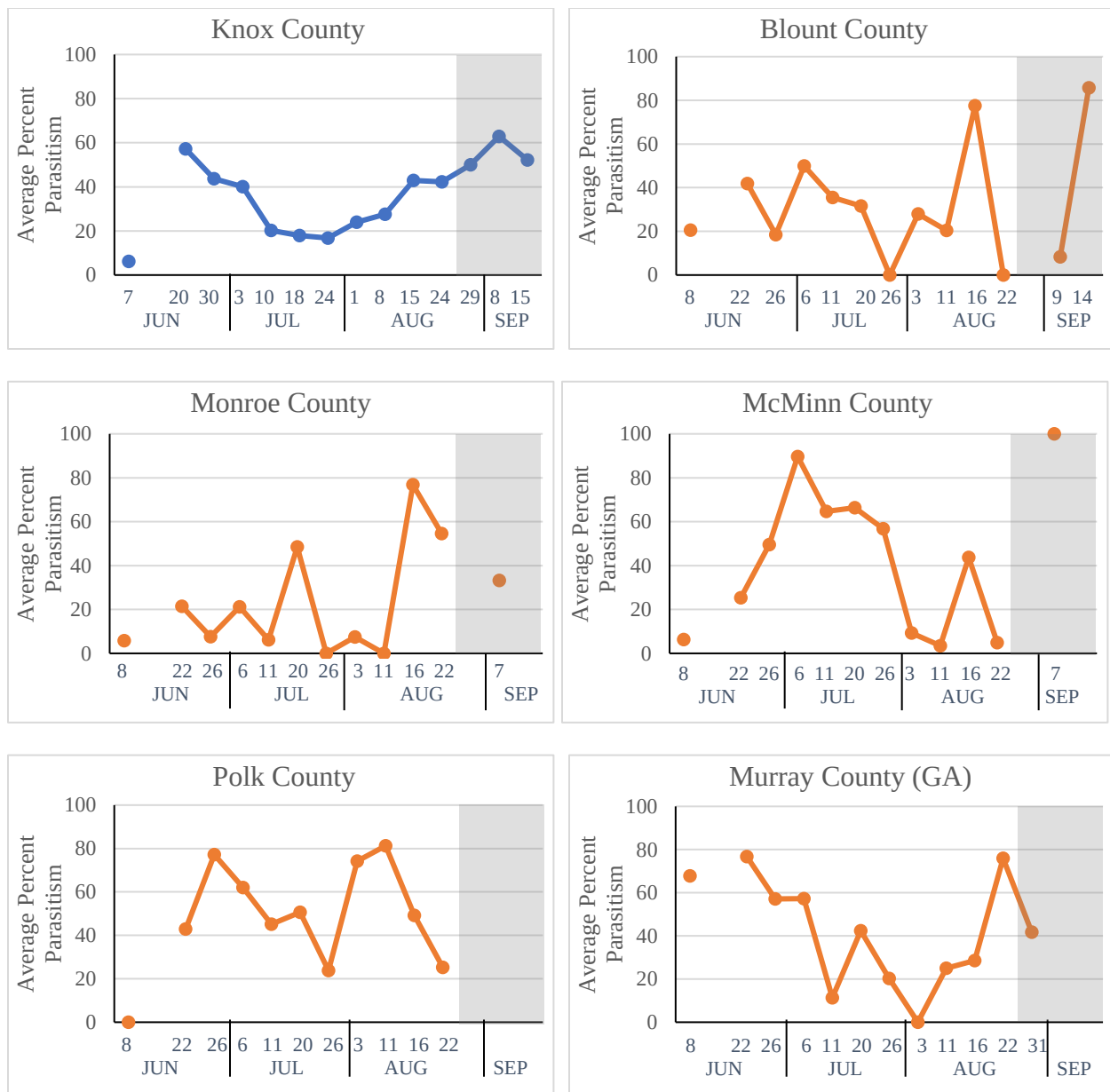


Figure 17. Average parasitism rates in all counties, 2017. Shaded portion of graphs indicate opportunistic collections due to lower *M. cribraria* oviposition rates and are presented for conceptual purposes only.

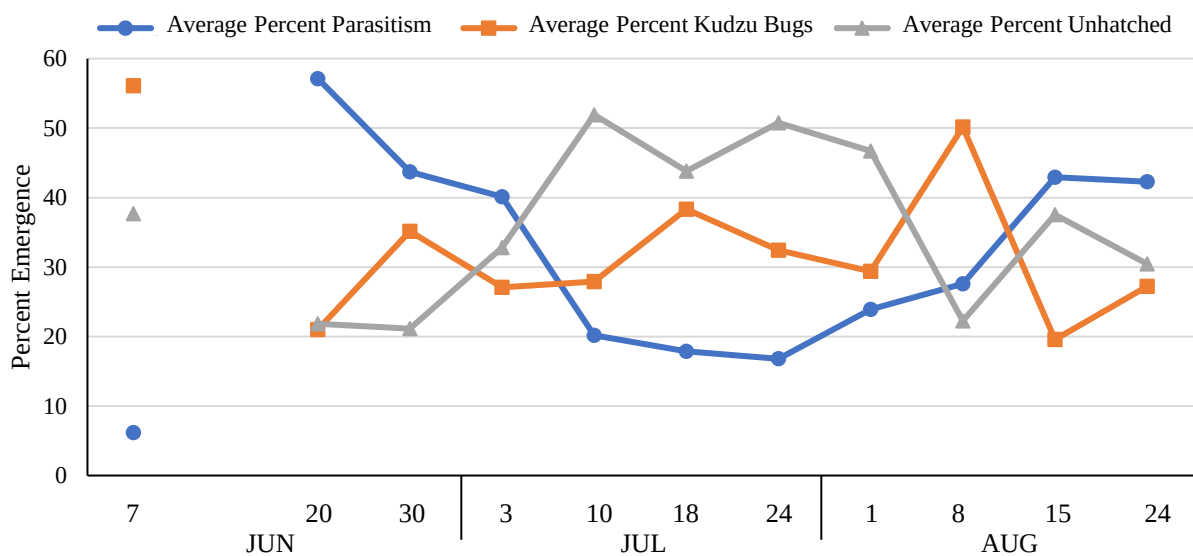


Figure 18. Seasonality of parasitoid and *M. cribraria* emergence in Knox County, TN, 2017.

VITA

Amy Marie Michael was born in Cincinnati, Ohio in 1991 to Dan and Linda Michael. She grew up exploring nature in Lebanon, Ohio. She moved to California to pursue her undergraduate studies and became interested in the role that entomology plays in food security after taking an introductory course. She obtained her Bachelor of Science degree in Entomology at the University of California, Riverside in 2013. While pursuing her studies, she conducted research both in a systematics laboratory as well as an extension laboratory. She then worked with Cooperative Agricultural Support Services in conjunction with the California Department of Food and Agriculture as a part of the Asian citrus psyllid biological control program.

Determined to further her ability to apply entomology the greater good, Amy began her Master of Science degree program in the Department of Entomology and Plant Pathology at the University of Tennessee in August of 2016 and completed her program in May 2018.

Amy loves all things related to food, science, and nature. She loves spending time with her large extended family, friends, her fiancée, David Theuret, and their two ferrets, Buddy and Flower.