

ABSTRACT

Title of Thesis: PHENOLOGY AND COLD TOLERANCE OF
MEGACOPTA CRIBRARIA: AN INVASIVE
SOYBEAN PEST AT ITS NORTHERN LIMIT

Jessica Irene Grant, Master of Entomology 2016

Thesis Directed By: Professor William Lamp, Department of
Entomology

Megacopta cribraria, kudzu bug, is an invasive pest of soybeans in the Southeast United States, with a northern distribution limit in Maryland. Soybeans, however, are grown north of Maryland, which places producers at risk of future range shifts. Additionally, producers in the current distribution need a development model to aid management. To address both concerns, I studied the cold tolerance of the kudzu bug and its phenology. I examined the supercooling point (SCP) and lethal temperature to kill 50% (LLT₅₀) of three populations over the fall of 2015 to determine the cold tolerance. I found the kudzu bug is a freeze avoidant species with an average SCP of -12.6°C and LLT₅₀ of -5.1°C. I also determined that the kudzu bug moves to thermally protected leaf litter in late November to overwinter. To address phenology, I determined the kudzu bug requires 625 degree-days at a minimum temperature of 16°C to complete egg and nymphal development. Field validation fit the model well with a biofix of May 1st and 30 days for a preoviposition period. The phenological degree-day model indicates two generations per year and allows producers evaluate and time pest management strategies within a year. Cold tolerance enables producers to predict the surviving population each year as well as the potential for northward range expansion.

PHENOLOGY AND COLD TOLERANCE OF *MEGACOPTA CRIBRARIA*: AN
INVASIVE SOYBEAN PEST AT ITS NORTHERN LIMIT

By:

Jessica Irene Grant

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Entomology
2016

Advisory Committee:
Professor William Lamp, Chair
Associate Professor Cerruti Hooks
Associate Professor Paula Shrewsbury

© Copyright by
Jessica Irene Grant
2016

Dedication

To my family: Thank you for the countless times you have come to visit me when I was not able to leave my bugs.

To Colin: I cannot imagine having finished this work without you as my pillar. Thank you for all your patience and support.

Acknowledgments

I would like to thank my advisor, Dr. Bill Lamp. Bill brought me on to work with the kudzu bug but did not dictate what I had to study. He allowed me to form my own questions and build a project from them. The trust he had in me to develop my own ideas taught me how capable I am of rising to any challenge.

I would also like to thank those serving on my advisory committee, Dr. Paula Shrewsbury and Dr. Cerruti Hooks. I appreciate you giving me your time and advice.

The Lamp lab has been a constant network of support and help. I would like to thank all the past and present members who have helped me by giving advise, listening to presentations, problem solving, or spending hours of their time in a car with me: Alan Leslie, Elanor Spadafora, Ryan Gott, Rebecca Eckert, Rebecca Wilson, Jennifer Jones, Morgan Thompson, Alison Post, Claire Hirt, Chloe Garfinkel, Raina Kaji, Lauren Leffer, and Cullen McAskill.

I would also like to acknowledge the funding for my work: Department of Entomology Gahan Fellowship, Maryland Soybean Board, and Northeastern Sustainable Agriculture Research and Education.

Table of Contents

Dedication	II
Acknowledgments.....	III
Table of Contents	IV
Chapter 1: Temperature and Kudzu Bugs: A Review	1
Introduction	1
Kudzu Bug.....	2
Cold Tolerance	7
Phenology.....	13
Conclusions	19
Chapter 2: Cold Tolerance of the Kudzu Bug	21
Abstract	21
Introduction	22
Methods.....	26
Measurement of SCP	26
Measurement of LLT ₅₀	28
Fall Movement Observations.....	29
Microhabitat Temperature Measurements	30
Application of Temperature to Range Expansion.....	31
Results	31
Measurement of SCP	31
Measurement of LLT ₅₀	33
Fall Movement Observations.....	34
Microhabitat Temperature Measurements	36
Application of Temperature to Range Expansion.....	37
Discussion	40
Chapter 3: Degree-Day Model and Validation of Kudzu Bug Phenology	48
Abstract	48
Introduction	49
Methods.....	51
Determining Degree-Day Development	51
Microhabitat Temperature Measurements	53
Observations of Phenology and Persistence	54
Results	55
Determining Degree-Day Development	55
Microhabitat Temperature Measurements	57
Observations of Phenology and Persistence	60
Discussion	63
Literature Cited.....	70

List of Figures

Figure 1: Kudzu bug distribution from 2009 to 2016 in the United States.....	3
Figure 2: Pictures of kudzu bug nymphs and adults	4
Figure 3: Map of Maryland and Virginia with locations of cold tolerance studies.	28
Figure 4: Super-cooling points for month and population during the fall of 2015.....	32
Figure 5: Probability of surviving at the tested temperatures for month and population combinations.	33
Figure 6: Caged study depicting median score of bugs observed on plants and on cage walls over the observation time.	35
Figure 7: Percentages of total bugs found in each area when the cage was destroyed.	36
Figure 8: Percentage of field samples with kudzu bugs found in the respective layers (vine, leaf litter, or soil) over the fall.	36
Figure 9: The average difference of day to nighttime temperatures per month.....	38
Figure 10: The average nighttime difference between ambient, leaf litter, and vine microhabitat temperatures.....	38
Figure 11: Ambient and leaf litter hours which exceeded the LLT ₅₀ threshold of -4.9°C	39
Figure 12: LLT-50, SCP, and future SCP lines mapped against the current 2016 distribution of the kudzu bug.	40
Figure 13: Average temperature difference (Ambient-Vine) for a kudzu patch in Calvert County, Maryland over 2015 and 2016.	58
Figure 14: Average temperature difference (Ambient-Soybean canopy) of a soybean field in Prince George's County, Maryland over 2015 and 2016.	58
Figure 15: Persistence sampling in Maryland in 2016.....	61
Figure 16: Accumulated degree-days for nymph development calculated from temperatures recorded in Oxon Hill, Maryland over 2014 to 2016.....	62
Figure 17: Percentage of kudzu bug nymphs caught in 2016 in Oxon Hill, Maryland. ..	62
Figure 18: Predicted phenology of the kudzu bug in Maryland based on degree-days and field observations.....	68

List of Tables

Table 1: Model parameters and effect likelihood ratio test results of the LLT ₅₀ logit model.....	34
Table 2: Predicted lethal temperature to kill 50% (LLT ₅₀) of the population based on when the probability of surviving was 0.5 in the logit model.	34
Table 3: Occurrence of hours below the November Calvert County LLT ₅₀ threshold of -4.9°C and below the mean Calvert County SCP threshold of -11.5°C.	39
Table 4: Number of egg masses, number of individuals for each chamber, percent of hatched nymphs to adulthood, adult longevity	59
Table 5: Days between instars of egg mass cohorts per chamber temperature.	59
Table 6: Days between egg laying and hatch, percent of total eggs laid that hatched per temperature, number of egg masses per female, total number of egg per female, preoviposition period, oviposition period	59
Table 7: 2014 phenology counts for each location by month are broken into eggs, nymphs, and adults.....	60

Chapter 1: Temperature and Kudzu Bugs: A Review

Introduction

Kudzu bug, *Megacopta cribraria*, is a recent invader to the United States (Suiter et al. 2010). Rapid range expansion from detection in Georgia in 2009 up to the current northern limit in Maryland occurred in only four years (Leslie et al. 2014). Since detection in Maryland in 2013, the distribution has not progressed northward (Grant et al. 2014). As an economically damaging pest of soybeans (Seiter, Greene, et al. 2013), growing concern from producers centers on management. Through this thesis, I focus on determining the relationship between temperature and the kudzu bug.

Insects are poikilothermic and are often highly regulated by temperature. The ability of an insect to survive the winter can be predicted by the temperature at which the insect dies (Bale and Hayward 2010, Sinclair et al. 2015). Predicting winter survival has implications on pest pressure the following year. As more insects are able to survive the winter, the potential for damaging populations the spring throughout the growing season increases (Hallman and Denlinger 1998). Winter survival can also predict areas of concern for range expansion (Crozier 2003, Lehmann et al. 2015). All insects have a physiological limit to which they can withstand cold temperatures. The temperatures experienced by an insect can therefore in part limit range. Soybeans are grown well beyond the current kudzu bug distribution to the Northeast and Mid-West. Predicting range shifts based on winter temperatures can alert producers to future pest populations. To date few studies have focused on the cold tolerance of the kudzu bug.

Temperature also determines and can predict the development of an insect. Phenology of the kudzu bug, described for the Southeast, has not been determined in Maryland. The use of degree-days can predict the timing of life stages, phenology, based on temperatures (Hartley et al. 2010). The phenology is crucial to understanding voltinism, or the number of generations per year, and timing of pest management. Furthermore, in combination with phenology, life history such as fecundity can relate to the outbreak potential of an area. Currently, a degree-day model exists for the kudzu bug based on populations in China (Shi et al. 2014) but no model has been developed for North American populations.

A background framework of the literature and general theory provides the context for the work of my thesis. This review focuses on the following three topics (1) an introduction to the kudzu bug, (2) factors of cold tolerance and how it can be used to understand range expansion and population dynamics, and (3) using development to understand phenology, voltinism, and outbreak potentials.

Kudzu Bug

The kudzu bug was introduced to the United States from a single female lineage (Jenkins et al. 2010) originating in the Kyushu region of Japan (Hosokawa et al. 2014). The bug was first reported in October 2009 by homeowners in Northeast Georgia who noticed large aggregations on their homes (Eger Jr et al. 2010, Suiter et al. 2010). Populations rapidly expanded from northern central Georgia up to Maryland by 2013 (Leslie et al. 2014). The current range encompasses twelve states primarily in the southeast region (Figure 1). Since the initial rapid range expansion from 2009 to 2013, the

kudzu bug has had little extension particularly at the northernmost edge in Maryland (Grant et al. 2014).

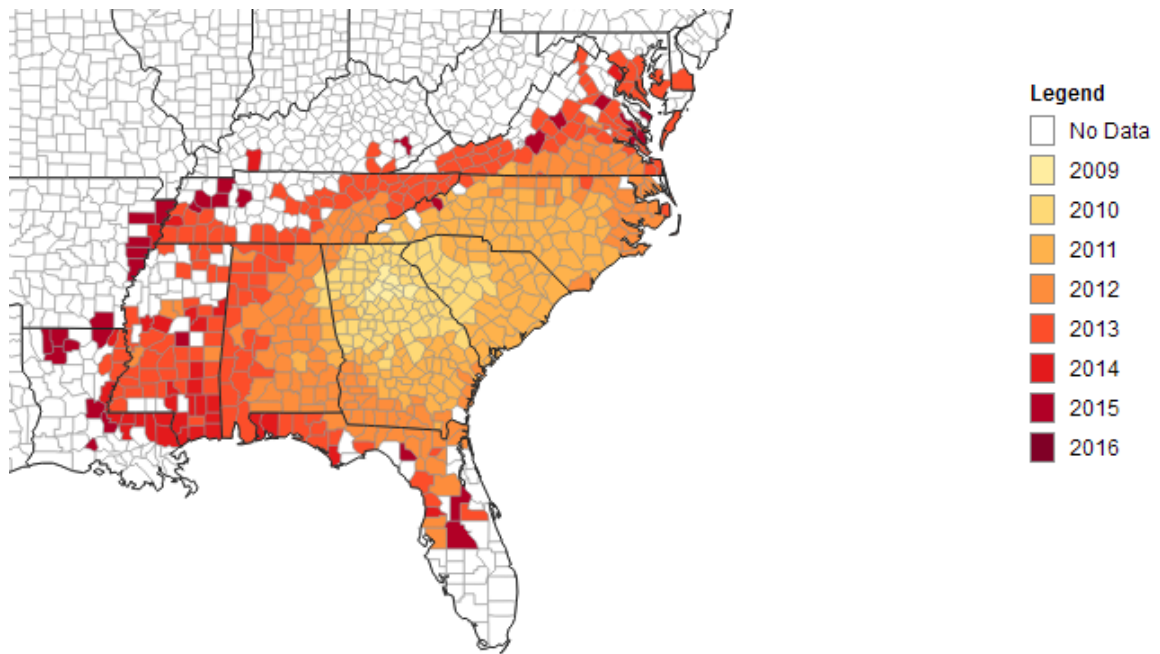


Figure 1: Kudzu bug distribution from 2009 to 2016 in the United States. Map generated from the Early Detection and Distribution Mapping System (EDDMapS 2016).

Kudzu bug, the only member of the family Plataspidae in North America, differs from related native families such as Pentatomidae. Adult males and females are 3.5-6.0 mm in length, square in outline, and dorsally convex giving them a globular appearance (Figure 2). Coloration varies from a yellowish brown to greenish brown with brown stippling. Distinctive red eyes and odor emitted via a spray make the kudzu bug easily identifiable. Eggs are laid in two parallel rows with symbiont rich globules (Leslie et al. 2014). The γ -proteobacterial symbiont '*Candidatus Ishikawaella capsulata*' is taken up upon nymph hatching and is necessary for normal development and dictating host plant preference (Fukatsu and Hosokawa 2002, Hosokawa et al. 2007, Jenkins et al. 2010). Nymphs progress through five instars before adult eclosion. Nymphs are hairy and range

in color from tan to green with dark wing pads.



Figure 2: Left, nymphs after hatching out of an egg mass; middle, late instar nymphs; right, adults. Photo credit: Jessica Grant.

Kudzu bug undergoes two generations per year in Georgia and up to three in Asia (Zhang et al. 2012). Phenological timing in the Southeast United States, based on observations, indicates that eggs are laid in April-May and again in July-August. Corresponding peaks of adults occur at the time of egg laying and subsequently in the fall (Zhang et al. 2012). An experimental temperature based development model from Zhejiang, China, reports that the kudzu bug requires 849 degree-days with a minimum threshold temperature of 14.25°C for eggs to develop into adults. Eggs require 62 degree days with a minimum threshold temperature of 14.68°C (Shi et al. 2014). However, the model from China may not accurately represent U.S. populations impart because of climatic differences between the populations. Application of the model should be cautious of potential discrepancies in the United States. In the fall, adults will aggregate on the sides of houses and other light colored surfaces. Adults cluster under tree bark and leaf litter during the winter but will also sun themselves on warm winter days (Lahiri et al. 2015). An average of 15% of females mate before the winter, which may enhance oviposition in the spring (Golec and Hu 2015).

A number of legumes, family Fabaceae, are reported as hosts of the kudzu bug; but soybean, *Glycine max*, and kudzu, *Pueraria montana*, are the most documented (Srinivasaperumal et al. 1992, Eger Jr et al. 2010, Zhang et al. 2012, Blount et al. 2015, Medal et al. 2016). Concern for commercial beans grown in the United States has prompted studies to confirm suitable developmental hosts. Of the legumes only kudzu, soybean, edamame, and pigeon pea are robust developmental hosts (Medal et al. 2013, Blount et al. 2015). Additionally, one study found two non-legume hosts, firecracker plant *Crossandra infundibuliformis* and cotton *Gossypium hirsutum*; however, development was slower than normal (Eger Jr et al. 2010). While the nymphs may be limited in host plant diet, an examination of adult gut DNA showed a wide diet of legumes as well as other flowering plants and conifers (Lovejoy and Johnson 2014). The wide adult diet breadth has been suggested to improve adult survival while moving long distances between nymphal hosts (Lovejoy and Johnson 2014).

Concern for the kudzu bug has focused on soybeans, as the United States is a top producer of soybean oil in the world with 3.93 billion bushels of beans produced in 2015 (USDA-NASS 2016). Feeding by high densities of the kudzu bug can reduce number of seeds per pod and seed weight which if untreated can cause up to 59% yield loss (Seiter, Greene, et al. 2013). Pod development stages are the most at risk from feeding damage that results in yield loss (Seiter et al. 2016). Spatially, adults and nymphs are often found together and are typically more dense at field edges (Seiter, Reay-Jones, et al. 2013). Kudzu was believed to be an obligate host to the first generation of the kudzu bug each year; however, a caged greenhouse study demonstrated that the first generation can develop on soybean (Del Pozo-Valdivia and Reisig 2013). The kudzu bug can therefore

affect early-planted soybeans as well as late season beans. Additionally, a study found more kudzu bugs in early untreated soybeans than late thus further distinguishing the risk to early soybeans (Del Pozo-Valdivia et al. 2016).

Pesticides are the only current commercial form of control for producers. Action thresholds are one nymph per sweep and generally one application of insecticide is enough to prevent yield losses (Seiter et al. 2015). Current research into alternatives focuses on parasitoids, natural enemies, entomopathogenic fungi, and variety selection. There are currently two potential control parasitoids: one adult and one egg. The adult parasitizing fly, *Strongygaster triangulifera*, is widespread across North America and was found to infect around 9.34% of females resulting in a disruption of oocyte development (Golec et al. 2013). The emerging egg parasitoid, *Paratelenomus saccharalis*, shows a high rate of parasitism but is currently restricted to the southern extent of the kudzu bug range (Gardner et al. 2013). Natural enemies found to have fed on kudzu bug by an examination of DNA in the gut are generalist predator insects including *Geocoris punctipes*, *Geocoris uliginosus*, *Orius insidiosus*, *Podisus maculiventris*, *Hippodamia convergens*, and *Zelus renardii*, and spiders including *Oxyopes salticus* and *Peucetia viridans* (Greenstone et al. 2014). The entomopathogenic fungi, *Beauveria bassiana*, has been highly effective at killing adults at low concentrations of the fungus in under a week; however, success in the field may required a high density of the kudzu bug (Seiter et al. 2014, Portilla et al. 2016). Finally, varietal selection shows the fewest adults and oviposition on narrow leafed small seed varieties such as ‘Vance’ and ‘N7103’. One additional cultivar, ‘Nitrasoy’, has also shown a reduced adult and nymph presence (Fritz et al. 2016).

Cold Tolerance

This section of the review focuses on the northern latitudinal limit of insect species bounded by cold tolerance limits. Among insects, cold tolerance is comprised of different strategies involved in surviving cold temperatures. The terminology and breakdown of these strategies is not consistent within the field. Some authors break cold tolerance into freeze tolerant and freeze susceptible/avoidance (Salt 1961, Bale and Hayward 2010). Others divide these two categories into narrower groups including opportunistic, chill susceptible, chill tolerant, freeze avoiding, and freeze tolerant (Bale 1993, Sinclair 1999). Opportunistic species, generally found in the tropics, do not exhibit a dormant state and die when temperature dips below those required for normal metabolism. Chill susceptible species are those that die after a short chilling period at moderate to high sub-zero temperatures. Chill tolerant refers to insects that die after long chilling periods at moderate to low sub-zero temperatures. Chill susceptible and chill tolerant insects generally occur in regions that are more temperate. Freeze avoiding are those insects that can survive extensive periods in a supercooled state but die with the onset of freezing. Freeze tolerant, typically reserved for high alpine and arctic species, are insects that can survive the formation of ice in their body tissue (Bale 1993, Sinclair 1999). Normally, ice nucleation is fatal to insects as it causes cells to rupture (Salt 1961). Very few insects are truly freeze tolerant and are able to survive ice nucleation.

Many abiotic, behavioral and physiological factors influence the cold tolerance of a species. Insects usually overwinter in one life stage (Salt 1961), in which they might experience constant fluctuations in daily temperatures. Insects respond to environmental fluctuations with physiological changes such as the production of glycerol that lowers the

freezing point of the hemolymph or other cryoprotectant compounds (Salt 1961, Košťál et al. 2001, Luna et al. 2013). Overwintering life stages tend to be non-feeding not only as result of a lack of food source, but also because food in the gut increases their susceptibility to freezing. Most insects go through a period of cold hardening in which they undergo processes like evacuating the digestive tract, dissolving flight muscles and up-regulating a number of genes and cryoprotectants in order to prepare for the winter (Salt 1961, Teets and Denlinger 2013). Insects also often change behavior in preparation for winter such as moving to sheltered locations. A sheltered location may be as simple as a depression in the ground to utilize the geothermic buffering of the soil (Bale and Hayward 2010). Many species undergo diapause, a physiological state of dormancy, which in the preparation and initiation phases can include the previously described cold hardening strategies for overwintering (M J Tauber and Tauber 1976, Bemani et al. 2012).

During early cold periods, insects may improve their cold tolerance by both rapid cold hardening and long-term cold accumulation (Waagner et al. 2013). Rapid cold hardening is associated with diurnal temperature fluctuations where as cold accumulation is the seasonal change in temperature. Timing of cold temperatures can be crucial to the process of cold accumulation. Early season frost that occurs before cold accumulation has occurred can be extremely dangerous to the survival of an insect (Salt 1961, Luna et al. 2013). Therefore, seasonal variability can have significant impacts on the cold hardening and cold accumulation of insects preparing for the winter. Duration of cold temperatures during the winter is also a predominate factor in the survival of insects. Many suggest the length of time at a cold temperature is equally ecologically relevant as the lethal

temperature (Luna et al. 2013, Andersen et al. 2015). Therefore, the survival of an insect is dependent upon the frequency of cold stress in addition to minimum temperature and duration of the minimum temperature (Marshall and Sinclair 2012, 2015).

The latitudinal distribution of an insect species may be constrained by host plant distribution, cold tolerance, temperature limits on developmental rates, or inter-species interactions such as competition or natural enemies (Ungerer et al. 1999). Which factor plays the largest role is highly dependent on the life history as well as the geographical direction of expansion of a species. However, the realized range limit of a species often correlates with minimum temperature. As cold tolerance correlates strongly with the latitudinal distribution, the tolerance of a species may help predict the distribution expansion both under current and future climate conditions (Andersen et al. 2015). Whether or not winter mortality controls the northern limit of an insect species has to be determined before using cold tolerance in modeling. One approach to determine this relationship is to measure the occurrence of lethal temperatures near the northern boundary of the distribution. Another method is to examine variability of cold tolerance within a species throughout its range.

If the cold tolerance of a species determines the northern edge of the distribution, frequent lower lethal temperatures should occur near the edge of the distribution. In a study performed by Ungerer et al. (Ungerer et al. 1999), the relationship between distribution edge and occurrence of lower lethal temperatures was determined for *Dendroctonus frontalis*, the southern pine beetle. The study found a yearly probability of 90 percent of temperatures reaching below the lower lethal limit at the northern edge of the beetle species. Thus, the authors concluded that the temperature variation exhibited

on a landscape scale within the range of *D. frontalis* was a critical determinant of the geographical distribution.

A species that has a limited ability to respond to cold temperatures is also likely to be restricted in range by cold tolerance (Kimura 2004). If genotypic plasticity for cold tolerance exists within a population, individuals would be able to respond to a decrease in temperatures thus increase their probability of surviving (Andersen et al. 2015). Lacking plasticity limits the distribution of a species or population by cold tolerance. Kimura (Kimura 2004) examined evidence of plasticity in drosophilid flies. The study assessed cold tolerance variation within species across a geographical gradient. He found that geographical variation of cold tolerance in populations was small to negligible and thus lacked the ability to increase cold tolerance. He concluded that cold tolerance bound the northern distributions of the species examined due to the lack of variation.

The incapability of a population to increase cold tolerance on a genetic level could result from limited heritable genetic variation or gene flow from other populations that neutralizes local adaptations (Crozier 2003). Cold tolerance evolves slowly and the lack of increased tolerance could also result from either the genes being tightly co-adapted or the gene being pleiotropic (Kimura 2004). In the case of invasive species or isolated populations perhaps the founding individuals lacked the plasticity of cold tolerance needed to adapt to local conditions (Crozier 2003). In some cases, plasticity across generations may have a negative impact. Blow flies that experience a mild fall during the initiation of diapause produced larvae with lessened cold hardiness (Coleman et al. 2014). If followed by a cold winter, the next generation of blowflies could have a higher probability of death due to cold temperature. Consequently, genetic ability through

either plasticity or local adaptation to respond to winter severity is a key factor in range restrictions due to physiological cold tolerance limits.

If an insect species can exploit anthropogenic changes in the environment, such as overwintering in a house, then it would be possible for the distribution to expand past natural boundaries (Kimura 2004). Other species with high dispersal capabilities may simply reestablish populations every spring past their cold tolerance limit. This mechanism of annual spring expansion and winter contraction of range around a northern limit only works with insects that can disperse well and quickly (Kimura 2004).

Species distributions limited by cold tolerance are more likely to shift their ranges during climatic warming periods (Crozier 2003). Temperatures resulting from global warming are likely to exceed a 1.5°C increase by the end of the 21st century (IPCC 5th Report 2013). Furthermore, there will be fewer extreme cold events (IPCC 5th Report 2013). For species distributions, edges bound by cold thermal limits may expand more than edges bound by maximum temperatures (Sunday et al. 2012). In the case of extreme events, populations at the edge of their distributions may be most at risk (Cannon 1998). Long colder than average events could temper range expansion made possible by warming winter temperatures. Even if these events do not occur on a regular annual basis, populations along the edge of ranges may still be in danger. Depending on the severity of the cold event, surviving populations may be sufficiently small as to take several generations or years to rebound in population size. Presumably this time lag effect would be greater on univoltine species rather than multivoltine species, which would be able to recover population size faster. Aphid population size, for example, varies following the winter season. Depending on temperature, frequency, and duration of exposure, the size

of the resulting post-winter populations can range from very high (mild winter) to very low (severe winter). The moderating influence of cold temperature on population size affects the timing of spring migration and the number of migrating aphids (Bale and Hayward 2010). Therefore, a change in winter severity can have drastic impacts on population dynamics (Porter et al. 1991, Cannon 1998).

Species that are currently limited by cold tolerance, capable of dispersal, and use a wide range of hosts or habitats are likely to lead cascading environmental changes under a warming climate (Crozier 2003). In terms of agriculture, environmental and economic impact of an expanding pest species warrants attention. Being able to predict potential distributions and population sizes will help identify regions most at risk and allow for the creation of management strategies aimed at minimizing possible impacts (Baker et al. 2000). Outbreaks of pest species may increase with increased winter temperatures as a result of reduced winter mortality (Cannon 1998). In aphids, decreased winter mortality increases the post-winter migratory population. With a larger initial population, there is an increase in potential for aphid populations to grow to damaging levels by summer (Cannon 1998). Higher densities could also lead to production of more alate aphids during the growing season thus increasing aphid damage by movement of aphids within the landscape (Bale et al. 2002). In general, an increase in surviving winter populations could have a strong negative impact on crop systems. Larger populations throughout the growing season, as well as early season crop damage from higher initial populations, may cause crop yield loss or increased use of pesticides (Luedeling et al. 2011). Reliable models including climatic relationships need to be created for pest

species to determine range expansion and threat potential to agricultural systems (Ladany and Horvath 2010).

In summary, cold tolerance limits range expansion and can be used to predict the pressure of pest populations. Cold tolerance, though classified in many ways, in essence represents a relationship between survivorship and winter temperatures. Factors such as physiology, behavior, and abiotic influences can alter the cold tolerance of an insect. Ultimately, genetic variation and plasticity in a population regulates the minimum temperature threshold. Plasticity to respond to temperature appears to be limited for most insects (Gunderson and Stillman 2015). Without a mechanism to adapt to a colder climate, the northern distributions of many species are limited by their cold tolerance. Evidence of lethal temperatures at the distribution edge and little genotypic variation in cold tolerance give credence to the correlation between northern limits and cold tolerance. Range shifts due to global warming will increase within the next century. The rapid increase of winter temperatures allows species to expand given no other limits to their current distribution and thus pest species of agricultural crops will shift northward into available farmland. The potential negative impacts of such a shift are twofold: an increase in early crop damage and an ability to build up large populations during the summer. Having accurate winter survival models could help in the creation of pest management strategies for predicted crop threats such as the threat of the kudzu bug to soybeans.

Phenology

The relationship between temperature and development is often used in predicting phenology, seasonal timing of life stages, and population growth of an insect (Damos and

Savopoulou-Soultani 2011). Management of agricultural pest species often utilizes such relationships. Insect developmental rates are predominantly determined by temperature though it may also be affected by host plant quality, humidity, and even competition (Soufbaf et al. 2010, Holmes et al. 2012, Couret et al. 2014). Effects on development have cascading consequences on phenology, voltinism, population growth, and range distribution of an insect. Changes in phenology due to climate change scenarios are predicted to occur at least in shorter time scales (Hodgson et al. 2011). Predictions with respect to climate change for phenology as well as voltinism and population growth can be used in integrated pest management such as informing the timing of biocontrol agents (Moerkens et al. 2011). On a larger scale, integrating mapping and phenology could identify possible distributional changes based on developmental thresholds especially for invasive insects (Hartley et al. 2010).

Understanding phenology, voltinism, and population dynamics depends on having an accurate model of development. Degree-days offer a near linear relationship between temperature and development (Cesaraccio et al. 2001). A degree-day is quantified by the amount of development that occurs per unit of heat and is often used for agricultural pests (Cesaraccio et al. 2001, Damos and Savopoulou-Soultani 2011). Converting the amount of time at a given temperature for development to its reciprocal creates the degree-day relationship. This transformation linearizes the relationship between temperature and development. A linear relationship allows for the determination of thermal parameters including the lower threshold for development and the thermal constant. The thermal constant reflects the degree-days for completion of development (Damos and Savopoulou-Soultani 2011). Accumulation of degree-days by the rectangular method

averages the daily temperature using maximum and minimum temperatures and subtracts the lower developmental threshold (McMaster and Wilhelm 1997). Methods for adjusting degree-day accumulations based on both minimum and maximum thresholds for development may be added to improve model accuracy (Higley et al. 1986). For example, if the maximum threshold exceeds the maximum temperature for that day then the maximum is set equal to the threshold.

The rectangular method of calculation is one of the most widely used methods of calculation (Higley and Haskell 2001). A study comparing multiple ways of calculating degree-days, including the rectangular method, found the estimations were more similar to each other in the late spring and summer months as compared to the fall and winter months (Roltsch et al. 1999). Most often producers are concerned with the growing season, which may negate model deviations in fall and winter. In addition to the method of calculation, arguments have been made for the use of hourly temperatures instead of minimum and maximum temperatures to reduce model error (Roltsch et al. 1999). Simplified models such as the rectangular method, using only a minimum and maximum temperature, assume the range and pattern of temperatures is the same throughout time. Even when the mean temperature does not change, changes in the temperature range can lead to changes in life history. Though these differences are small, they could accumulate (Chen et al. 2015). Adjusting calculating degree-days by hourly temperature over an average temperature improves the accuracy of the models but may not yet be practical for producers (Tu et al. 2014). It is possible to convert minimum and maximum temperatures to hourly by use of a sine wave function (Cesaraccio et al. 2001).

A final consideration in using degree-days is to insure the accuracy of the temperatures used in calculations. Microclimates found within vegetation can differ from surrounding temperature (Rosenberg et al. 1983). Moreover, using a local weather station may not accurately represent the site-specific conditions (Archer 2004). Measured temperature may need to be site-specific or adjusted from weather station data. Even small differences in temperature can affect insects; therefore, prediction accuracy is highly dependent on correct temperature input into degree-day models.

Degree-days provide an evaluation of phenology not biased by calendar date. Photoperiod cues may also regulate phenology especially inducting and breaking diapause in many insects. Variation in phenology between years is often due to changes in temperature whereas variation between geographic locations may be interplay of both temperature and photoperiod. Changes in phenology due to temperature are the focus of this review. Long term studies have provided evidence for a general shift to earlier springtime phenology in temperate and polar regions (Ellwood et al. 2011, Forrest 2016). For example, *Operophtera brumata* eggs have been shown to hatch earlier in correspondence with warmer springs (Bale et al. 2002). The most compelling temperature driven phenological relationships tend to be species who overwinter as adults and are active in early spring (Forrest 2016). Herbivores are at risk of a mismatched timing with host plant phenology. If insects emerge too early, they could starve and those that emerge too late often miss the vulnerable tissue stages such as bud break in trees. Generalist species are therefore the most resilient to mismatches as they can feed on alternative hosts.

Voltinism is a key aspect of managing a pest species. Under climate change, many multivoltine organisms will be able to add an additional generation. Degree-days can predict the number of potential generations. Boreal and European Lepidoptera species have been increasing in voltinism often becoming multivoltine (Altermatt 2010, Pöyry et al. 2011). Increasing voltinism could increase population growth, which for agricultural or forestry pests could cause major economic damage. For example, a mean surface temperature increase of two degrees will allow enough degree days for the grape berry moth to complete an additional generation (Tobin et al. 2008). It is important to note that for the grape berry moth, it is not simply the overall quantity of increased degree-days but the timing of the increase. The moth diapauses as pupae and only those degree-days prior to the diapause induction cue will impact the number of generations in that year (Tobin et al. 2008). Other work has similarly shown that a greater than two degree C increase in temperature will likely result in additional generations in multivoltine species (Musolin 2007).

Not all insects with a longer growing season will increase in the number of generations they produce. Species that undergo a chilling period may require more heat for emergence in a year following a warm winter thus negating the spring warming (Forrest 2016). In some, a long season relaxes selection for increased developmental rate often leading to larger insects at maturity (Forrest 2016). Rapid development is not always favorable for an insect. Fast development is often associated with smaller sizes of mature adults leading to decreased fecundity in many cases (Forrest 2016). Another potential downfall is the ‘developmental trap’ in which the organisms continue with direct development but are unable to complete another generation before winter (Van

Dyck et al. 2015). Most often, this phenomenon occurs with insects that do not undergo a diapause state or those whose photoperiod and temperature cues have been confused. Population growth rate can be lower in years following a warm season due to the last generation not reaching the cold tolerant stage and dying.

Outbreak frequency and severity could increase in addition to greater population growth per season (Boggs 2016). A sawfly, *Diprion pini*, showed an increase in frequency of outbreaks in warm summers due to a tight correlation between voltinism and temperature (Haynes et al. 2014). Similarly for a bark beetle, *Ips typographus*, summer temperatures were the main driving factor in increased outbreak intensity (Marini et al. 2012). Temperature can thus affect large regional scales of outbreaks and resulting intensity of infestations.

Development and phenology can aid in predicting suitable areas for insects on regional scales. Management can use predictions either to identify areas available to currently invading species or to look at potential shifts in distribution to new hospitable areas under climate change. A study on Argentine ants used degree-days to highlight areas of concern within Haleakala National Park (Hartley et al. 2010). Rates of expansion showed a linear relationship with degree-days and thus could aid in mapping landscape level movement. Phenology and physiological developmental thresholds can be powerful additions to mapping potential insect ranges.

Development, regulated by temperature, can have profound effects on phenology, voltinism, population dynamics, and even range expansion. Understanding the temperature and development relationship for a species can enhance prediction and timing of integrated pest management options. The temperature relationship must be

evaluated across geographic ranges especially those of an invasive population. Founding populations of an introduced species may respond differently to temperature in the introduced range than populations across the native range.

Conclusions

Highlighted throughout this chapter, understanding temperature in relation to the kudzu bug is of importance to understanding and predicting kudzu bug populations. Cold tolerance has yet to be characterized for the kudzu bug and could play a major role in the extent of its current and future range. The phenology, though observed in the Southeast, has to be determined at its northern edge. Additionally, there has not been a development model created for the American populations. Thus, my thesis has two main goals: (1) to determine the cold tolerance of the kudzu bug in Maryland, and (2) to determine the degree-day model to predict the phenology of kudzu bug development in Maryland.

My cold tolerance research describes the physiological temperature thresholds, the seasonal timing of movement to overwintering locations, overwintering location of the kudzu bug in Maryland, and any thermal buffering their refugia might create. Phenological observations at field sites assessed the seasonal patterns of life stages within Maryland. A degree-day model with field validation provides a deterministic relationship between temperature and development. Finally, an assessment of microhabitat use by kudzu bugs and its temperature indicates any adjustments needed between measured ambient and realized temperatures by the kudzu bugs.

Combining the work of cold tolerance and phenology allows for a more comprehensive temperature based model for winter survival, range expansion, and population dynamics of the kudzu bug. Producers can use predictions to assess the threat

of economic loss due to the kudzu bug in its current and future range as well as to determine timing of pest management options. Furthermore, this work can help establish a methodology applicable to modeling other invasive pest species of interest to the Northeast, especially in light of climate change.

Chapter 2: Cold Tolerance of the Kudzu Bug

Abstract

Kudzu bug, *Megacopta cribraria*, first discovered in the United States in 2009, is an invasive pest of soybeans. Since 2013, Maryland has been the northern limit of its distribution in the United States. I sought to determine the physiological cold temperature limits and movement timing of the kudzu bug to overwintering locations. In addition, I characterized the temperature of leaf litter between 2014 and 2016 at a kudzu site in Calvert County, Maryland. I measured the supercooling point (SCP), or point at which the insect freezes, on populations from three USDA plant hardiness zones between October and December of 2015. SCP did not show a clear trend based on month or population. The average SCP across all sample months and populations was -12.6°C . Additionally, I assessed the lower lethal temperature to kill 50% of the population (LLT_{50}) at the same population locations in October and November 2015. Again, there was no clear trend based on population but I did find a modest depression in the LLT_{50} values between October and November. The average LLT_{50} over both months and all three populations was -5.1°C . The kudzu bug overwinters in leaf litter and begins to move into the litter in late November to early December. Leaf litter moderates diurnal temperature differences in the winter creating a more constant temperature. Furthermore, leaf litter temperature was warmer than ambient temperature by an average of 1.2°C . Evidence suggests that the cold tolerance of the kudzu bug limits its distribution north of Maryland.

Introduction

Kudzu bug, *Megacopta cribraria*, is a recent invader to the United States arriving in Georgia in 2009 (Suiter et al. 2010). Its distribution expanded to Maryland by 2013 but has since not moved any farther northward (Grant et al. 2014, Leslie et al. 2014). Kudzu bug can be extremely damaging at high densities to soybeans. Yield reductions in untreated plots with high densities ranged up to 59% (Seiter, Greene, et al. 2013). A lack of observations of kudzu bug populations north of its 2013 range even though host plants are available suggests that they have reached their northern limit. Therefore, physiological temperature limits may be the determining factor of their northern limit.

Insects are poikilothermic meaning temperature affects development and survival. Cold tolerance, the ability to withstand winter temperatures, can be a powerful tool in assessing winter mortality rates as well as possible geographical range limits (Sinclair et al. 2015). Many insects face a physiological barrier along the northern extent of their range. Mapping the physiological lower lethal limits can elucidate the potential range expansion of a new invasive or the shift of a range under climate change (Crozier 2003). Lower lethal limits can also aid in predictions of winter mortality, which may aid in predicting fluctuations in population levels (Cannon 1998). Thus, knowledge of the kudzu bug lethal temperature limits would inform the prediction of potential colonization areas as well as annual density within the current distribution. Kudzu bug overwinters as adults in leaf litter and under tree bark but little else is known about their behavior (Lahiri et al. 2015). To date, no studies have quantified their lower physiological temperature limits in the United States.

Cold tolerance is often divided into two strategies: freeze tolerant and freeze avoidant (Bale and Hayward 2010). Freeze tolerant insects can withstand ice nucleation in the body cavity whereas freeze avoidant cannot. For freeze avoidant species, ice nucleation is fatal and often their lower lethal temperature is well above their freezing point (Sinclair et al. 2003, Bale and Hayward 2010). The cold tolerance of an insect can change throughout the fall season into winter. During cold accumulation, the seasonal change in cold tolerance, insects may evacuate their bowels or up-regulate cryoprotectant proteins (Salt 1961, Denlinger and Lee 2010). Timing of cold accumulation is critical because if an early frost occurs, insects may face unusually high mortality rates (Ungerer et al. 1999). It is important to look at the cold tolerance of an insect over the fall into the winter and not just at one time to assess potential cold accumulation.

A common method to assess cold tolerance is to quantify the supercooling point (SCP). SCP is the lowest temperature possible before the onset of ice crystallization in the body. SCP has long been used as a measure of cold tolerance ability though its ecological relevance has been debated (Renault et al. 2002). Often SCP is used to determine the broad difference between freeze tolerant and freeze avoidant species (Bale and Hayward 2010, Denlinger and Lee 2010). Those that are freeze tolerant can withstand ice nucleation and subsequently temperatures far below their SCP. Insects that are freeze avoidant cannot withstand ice nucleation and often die at temperatures above their SCP (Bale and Hayward 2010). Two major limitations exist for using the SCP method. First, the constant cooling rates often used are often much faster than would be experienced naturally. Faster cooling rates can cause an artificially higher SCP as they do not allow for the rapid cold hardening response (Kelty and Lee 1999, Sinclair et al.

2003). The second limitation is that the SCP of an individual is plastic and depends on the seasonal cold accumulation, physiological state, and rapid cold hardening (Danks 2005). Nevertheless, SCP may be used in freeze avoidant species to compare among life stages as well as among other species even though it may not be able to inform survival rate (Renault et al. 2002). For predictive purposes, the SCP can be an absolute geographic range boundary where such minimum temperatures occur.

Ecologically, lower lethal temperature (LLT) limits can more accurately represent winter mortality in freeze avoidant species than the SCP. The lethal temperature to kill 50% of the population (LLT_{50}) commonly determines survival rates. A study of cold tolerance parameters including LLT_{50} and SCP on drosophilid flies found a stronger correlation with LLT_{50} than with the SCP to the estimated coldest annual minimum temperature among populations (Andersen et al. 2015). In biocontrol agents, LLT_{50} values have been shown to correspond with range edges where establishment is patchy because of the winter knockdown effect (Byrne et al. 2004). Though the LLT_{50} is a measure of mortality, it does not account for long-term effects on those surviving individuals. Non-lethal temperature damage may affect longevity or fecundity especially with repeated cold stress (Hallman and Denlinger 1998, Marshall and Sinclair 2012). Effects of repeated cold stress can be difficult to ascertain from confounding variation among individuals or other causes of change in longevity or fecundity than by temperature.

Further complications to evaluating cold tolerance include the variation across latitudinal gradients and physical factors such as body size and sex. Sex and body size have long been understood to have effects on cold temperature physiology, with larger

bodies sizes increasing the SCP of an individual (David and Vannier 1996, Salin et al. 2000, Renault et al. 2002, Hahn et al. 2008). Geographically, SCP and LLT tend to increase moving equatorially (Addo-Bediako et al. 2000). Therefore, latitudinal gradients near the northern range edge need evaluation. For invasive insects, thermal plasticity is often limited due to founder effects (Colautti et al. 2010). However, given time, local adaptation may cause a shift in thermal tolerance or behavior. The brown marmorated stink bug, *Halyomorpha halys*, has a significantly different year long mean SCP between Minnesota and Virginia. When looking seasonally, the Minnesota population acclimates to cold weather sooner but the mean SCP during winter is not statistically different between the two locations (Cira et al. 2016). In Colorado potato beetle, geographically distinct populations did not show a difference in SCP but did show an adapted behavior to avoid cold temperatures (Izzo et al. 2013). These cases highlight the importance of behavior and timing of induction cues along with physiological response as factors of cold tolerance.

Microhabitats are an understudied aspect of cold tolerance in insects. Though often mentioned, few actually evaluate the difference between microhabitat temperatures and ambient temperatures. Many insects take advantage of burrowing or moving closer to the soil for the geothermal buffering of the earth (Sinclair et al. 2003, Bale and Hayward 2010). Snow cover can provide an excellent buffer against ambient temperatures and even helps moderate diurnal fluctuations in temperature. For insects near urban developments, seeking shelter in homes or underground pipes can provide a refuge from cold temperatures (Labrie et al. 2008, Inkley 2012). Given moderating overwintering

refugia, models may exaggerate winter mortality or realized distributions by not accounting for difference in refugia temperatures from measured ambient temperatures.

As the kudzu bug has great economic damage potential to soybean growers, I determined the cold tolerance limit to predict future populations densities and range shifts. First, I determined the SCP and LLT₅₀ physiological response across sex, population, and fall timing. This information informs the geographic pattern and variation of the populations along the northern range limit. Second, I observed the timing of movement to and location of overwintering to clarify behavioral activities of bugs during fall and into winter. Third, I measured microhabitat temperature of overwintering sites relative to ambient temperature to aid in predictive modeling of microclimatic temperatures experienced by the bugs. Finally, I evaluated the physiological limits for synchrony with the current distribution and future distributional shifts under climate change scenarios. My hypotheses included that the kudzu bug is (1) a freeze avoidant species with (2) little thermal plasticity showing minor changes through the fall and over a latitudinal gradient, and (3) that their current distribution limit in Maryland aligns with their ability to tolerate minimum temperatures. I further predict that with climate change their northern distribution will extend northward under warming winter temperatures.

Methods

Measurement of SCP

Adult kudzu bugs were collected once a month from October to December of 2015 at three population locations: Prince George's County 38.780685, -76.99818; Calvert County 38.432125, -76.55215; and Suffolk County 36.781478, -76.57712). Each population location corresponded to a different USDA Plant Hardiness Zone (7A, 7B, 8A

respectively) (Figure 3). Adults were captured on kudzu, *Pueraria* sp., at each site with a sweep net and brought back to the University of Maryland in a 34x34x60cm mesh popup cage (Bioquip, Rancho Dominguez, CA). Cages were held at room temperature with an edamame (*Glycine max* var. 'Midori Giant') plant.

Twenty bugs, ten male and ten female, were used to determine SCP from each population each month. To measure the SCP, an adult was placed in a 10ml glass vial with half of a standard cotton ball at the bottom. A coiled thermocouple wire (Model TT-K-24-SLE, Omega, Norwalk, CT) was depressed against the dorsal side of the insect. Thermocouple wires were attached to a thermocouple hub (Model USB-TC, Measurement Computing, Norton, MA) and temperature was recorded through a software program (Tracer Daq, Measurement Computing, Norwalk, CT). Vials were placed into a refrigerated recirculating water bath (Model AP07R-40-A11B, PolyScience, Niles, IL) filled with a cooling bath fluid (Model Polycool HC -50, PolyScience, Niles, IL). The bath was set to run from room temperature to -25°C at a cooling rate of $0.277^{\circ}\text{C}/\text{min}$. SCP was determined as the temperature immediately preceding the exothermic temperature increase from the onset of ice nucleation (Sinclair et al. 2015). After assessing the SCP, each bug was dried for 24hrs in an oven (Model Symphony E191047, VWR, Randor, PA) and weighed on a fine scale (Model XS105, Mettler Toledo, Columbia, MD).

Population, month, and sex for SCP were analyzed with an ANOVA and post hoc tukey on significant terms. The effect of weight on SCP was run for each sex and population by linear regression (JMP Pro version 11, SAS Institute, Cary, NC).

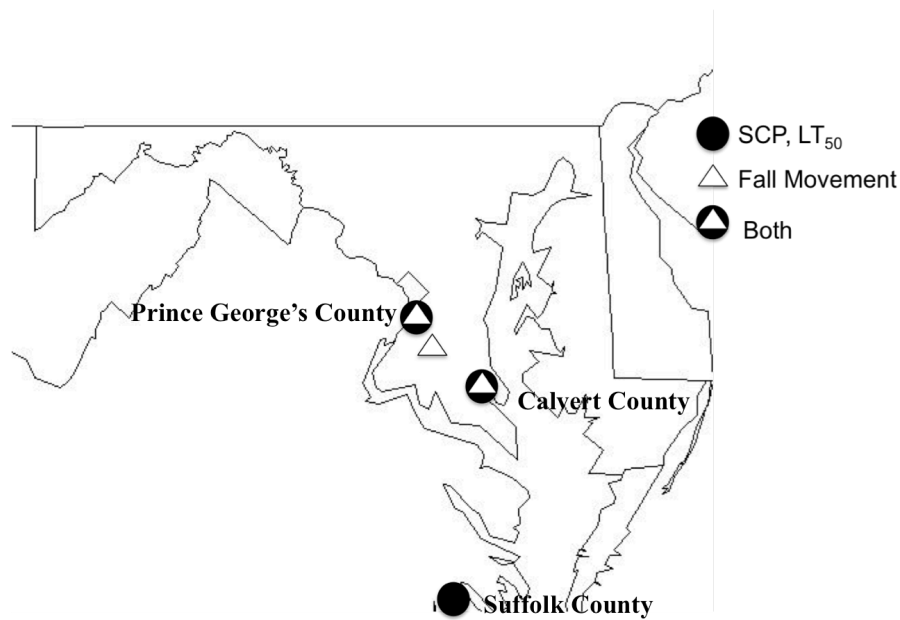


Figure 3: Map of Maryland and Virginia with locations of SCP, LLT₅₀ and fall movement studies.

Measurement of LLT₅₀

Adult kudzu bugs were collected and kept in the same manner as the SCP experiment. LLT₅₀ was determined for the three populations in October and November of 2015. December was excluded due to low population densities. To determine the LLT₅₀, each population and month was tested using eight replicates of five adults for five minimum temperatures. Minimum temperatures used were -10, -6, -4, -2, +2°C to achieve a range of 100% to 0% death. Each group of five adults was placed in a 10ml glass vial and topped with half of a standard cotton ball before capping. All vials, eight per population, were placed into a refrigerated water bath (Model AP07R-40-A11B, PolyScience, Niles, IL) filled with a low temperature bath fluid (Model Polycool HC -50, PolyScience, Niles, IL). One additional vial was placed into the bath with a thermocouple wire (Model TT-K-24-SLE, Omega, Norwalk, CT) to monitor temperature. Each run was cooled from room temperature to one of the minimum temperatures (-10, -6, -4, -2, +2°C)

at a rate of 0.15°C per min. The run was held at the minimum temperature for 12 hours, which corresponds to the longest natural cold duration in Maryland (Grant unpublished). After the 12hour period, the run was warmed to room temperature at a rate of 0.15°C per min. Upon reaching room temperature each vial of bugs was transferred to a petri dish with a ventilated lid containing an edamame leaflet (*Glycine max* var. ‘Midori Giant’) wrapped on the cut end with a moistened cotton ball. Twenty-four hours after the end of the run, insects were checked for mortality. Death was defined as a lack of movement when provoked with an artist brush. Counts of dead individuals in each petri dish were recorded.

A logit model of the death counts was run with variables for month, population, minimum temperature, and all interactions (month x population, month x minimum temperature, population x minimum temperature, and month x population x minimum temperature). Parameter significance was checked with the effect likelihood ratio test. The LLT_{50} with upper and lower 95% confidence interval was calculated for each month and population combination from the modeled 50% probability of death (JMP Pro version 11, SAS Institute, Cary, NC).

Fall Movement Observations

To determine timing of fall movement to overwintering locations, a field cage was stocked with kudzu bug and observed over the fall of 2015. The mesh 1.8x3.6x1.8m cage was erected outside the greenhouse complex at the University of Maryland. The bottom of the cage was covered with a fine weed cloth and edges sealed with a combination of tape and bricks. Inside the cage was an arrangement of eight grey 45x35x12cm bins with drainage holes in the bottom. Four bins were filled with

approximately 8cm of potting soil and the other four were filled similarly with soil and topped with 8cm of leaf litter collected from Maryland kudzu sites. A large holed mesh was used to cover and secure the leaf litter. Additionally, eight kudzu plants in large pots were placed in the cage, four supported on 1.5 meter plant stakes and the other four left to naturally twine. The kudzu plants were propagated at the University of Maryland greenhouse complex from vine cuttings taken from a nearby kudzu patch. Released into the cage were 2,661 kudzu bug adults from Calvert County, Maryland and Suffolk County, Virginia. The cage was checked twice a week from October 22, 2015 to December 19, 2015 for location of bugs. A score of 3=30+, 2=10-30, 1<10, 0=no bugs visible was recorded for each plant and the cage sides. On January 14, 2016, the cage was destructively sampled and total number of bugs in each bin, plant, and cage floor was counted.

In addition to the caged study, field habitats were observed during the fall of 2015. Once a month from October to December, three sites (Figure 3) were searched for the location of bugs within the kudzu patch. At each site, five random points were chosen and a 0.5x0.5m section of vine, leaf litter, and topsoil were collected. Counts of nymphs and adults were assessed in the lab from collected samples.

Microhabitat Temperature Measurements

In a kudzu patch in Calvert County Maryland, five stakes were deployed with two temperature monitors (Model DS1921G iButton, Maxim Integrated, San Jose, CA) on each. One monitor was within the leaf canopy of the kudzu vine and the other in the leaf litter. Also, an additional monitor measuring ambient temperature was suspended on the

north side of a tree within the kudzu patch. Monitors recorded temperature once an hour from December 2014 to April 2016.

Application of Temperature to Future Range Expansion

Average minimum winter temperature was obtained from the USDA plant hardiness zone map. A hardiness zone's temperature range was adjusted for the average winter difference in microhabitat temperature. Isolines were created at the top edge of the corresponding zone in which the LLT_{50} and SCP values fall. This was then compared to the current kudzu bug distribution from EDDMaps (EDDMaps 2016). Predicted climate RCP 4.5 from the IPCC (IPCC 5th Report 2013) was applied to determine to the change in plant hardiness zones. New temperature isolines were added to represent the possible change in geographic range for the kudzu bug.

Results

Measurement of SCP

The overall SCP across populations, month, and gender was $-12.6 \pm 3.5^\circ\text{C}$. SCP was not significantly affected by gender but was affected by population and month ($F_{1,154}=2.77$, $p=0.0984$; $F_{2,154}=6.54$, $p=0.0019$; $F_{2,154}=19.19$, $p<0.0001$ respectively). Mean SCP \pm standard error of the Prince George's population was $-12.2 \pm 0.4^\circ\text{C}$, the Calvert population was $-11.5 \pm 0.4^\circ\text{C}$, and the Suffolk population was $-13.7 \pm 0.5^\circ\text{C}$. I expected a latitudinal gradient, however only the Suffolk population was significantly different among the sites by a Tukey HSD ($p<0.05$). Mean SCP in October was $-13.0 \pm 0.4^\circ\text{C}$, November was $-14.0 \pm 0.4^\circ\text{C}$, and December was $-10.2 \pm 0.5^\circ\text{C}$. I expected a

change in SCP throughout the fall but only found December to be significantly different among the months by a Tukey HSD ($P < 0.05$) (Figure 4).

Dry weight of the bugs was affected by sex and population but not month ($F_{1,154}=10.46$, $p=0.0015$; $F_{2,154}=6.88$, $p=0.0014$; $F_{2,154}=1.44$, $p=0.2410$ respectively). Females weighed on average 10.3 ± 0.4 mg, which was significantly heavier than males that weighed on average 8.7 ± 0.4 mg ($t_{=154}=3.2349$, $p < 0.05$). The Suffolk population, weighing on average 10.1 ± 0.5 mg, was the heaviest population followed by the Prince George's population weighing on average 9.8 ± 0.4 mg. The Calvert population significantly differed by Tukey HSD ($p < 0.05$) from the other two populations and weighed least with an average of 8.3 ± 0.4 mg. Weight did not correlate with SCP for Prince George's ($F_{1,59}=0.5405$, $p=0.4652$) and Suffolk ($F_{1,39}=1.1666$, $p=0.2869$) populations; however, there was a significant negative correlation in the Calvert population ($F_{1,59}=5.51$, $p=0.0223$). Male and female weight were significantly negatively correlated with SCP ($F_{1,81}=7.56$, $p=0.0074$; $F_{1,77}=4.20$, $p=0.0437$ respectively).

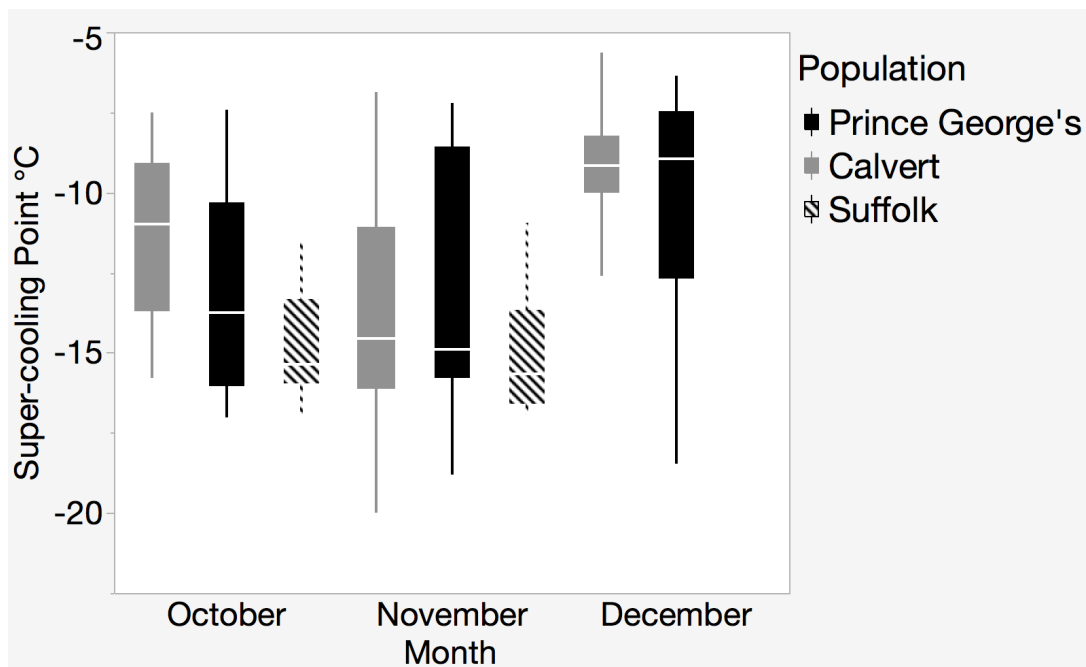


Figure 4: Supercooling points for month and population during the fall of 2015.

Measurement of LLT₅₀

The Suffolk population in November was dropped from analysis due to a high incidence of death due to a fungal infection of *Beauveria bassiana* in the population. Model parameters for month, temperature, population, and population by temperature significantly affect kudzu bug survival (Table 1). Probability of surviving increased in November suggestive of seasonal cold acclimation (Figure 5). Populations showed differences but did not consistently follow a latitudinal trend (Figure 5). Predicted LLT₅₀ values ranged from -4.1°C from the Calvert population in October to -6.7°C from the Prince George's population in November. The 95% confidence interval of the Prince George's population in November did not overlap with any population in October but did overlap with the Calvert population in November (Table 2). Overall, LLT₅₀ across populations and months averaged -5.1°C with a lower and upper confidence interval of -6.0°C and -4.4°C respectively.

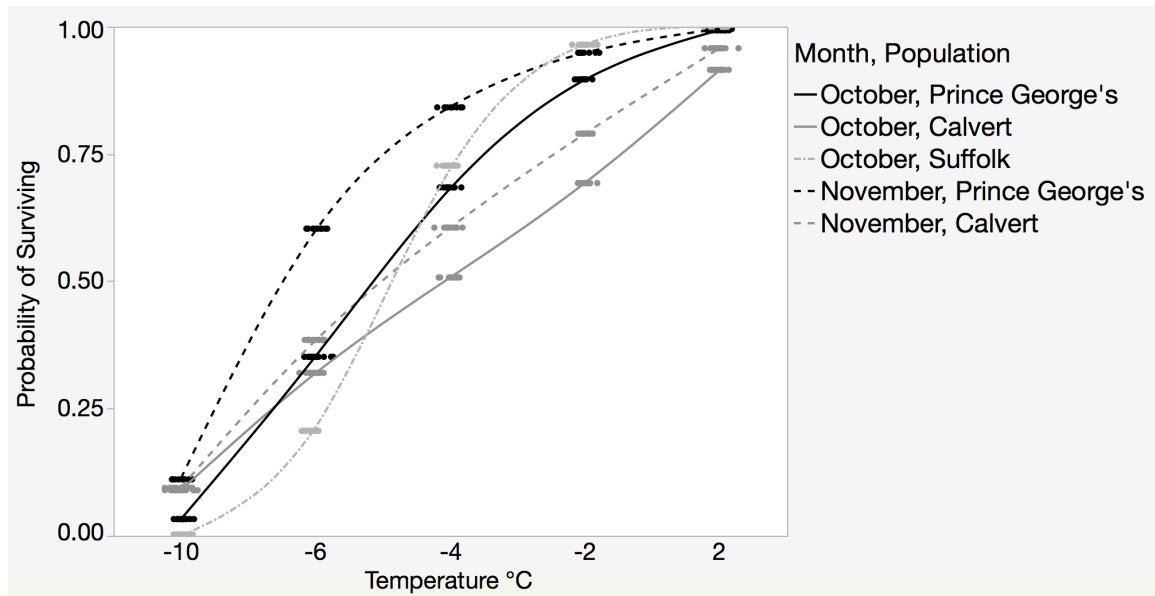


Figure 5: Probability of surviving at the tested temperatures from month and population combinations. Probability of surviving reflects the logit modeled of survivorship data at each temperature.

Table 1: Model parameters and effect likelihood ratio test results including degrees of freedom, chi squared, and p value of the LLT₅₀ logit model. All bolded p values are considered significant ($\alpha=0.05$).

Model Term	Degrees of Freedom	Chi Squared	P value
Month	1	5.31	0.0212
Temperature	1	127.06	<0.0001
Month*Temperature	1	0.21	0.6448
Population	2	21.06	<0.0001
Month*Population	1	1.08	0.2983
Temperature*Population	2	13.67	0.0011
Month*Temperature*Population	1	0.50	0.4753

Table 2: Predicted lethal temperature to kill 50% (LLT₅₀) of the population based on when the probability of surviving was 0.5 in the logit model. The 95% confidence interval, upper and lower, is given for each combination of month and population.

		LLT ₅₀ (°C)	Lower (°C)	Upper (°C)
October	Prince George's	-5.1	-5.8	-4.5
	Calvert	-4.1	-5.0	-3.2
	Suffolk	-4.8	-5.3	-4.4
November	Prince George's	-6.7	-7.7	-5.9
	Calvert	-5.0	-6.0	-4.0
Overall		-5.1	-6.0	-4.4

Fall Movement Observations

In the caged study, median rating of bugs was higher throughout October and November on the plants than on the cage. A general decline in median rating of bugs was observed from late October through the middle of December (Figure 6). By December 12th, bugs were no longer visibly observed on the plants or cage walls. Most of the movement of bugs to protected overwintering sites occurred in November (Figure 6). At the time of the destruction of the cage, 58% of the insects were recovered. Of the recovered, 35% were

found alive. Percentages of bugs observed in the different sampled areas of the destroyed cage were: 45% on plants, 10% in soil, 14% in leaf litter, and 31% on the cage. For all areas sampled more dead insects were found than alive. Plants and the leaf litter showed the highest survival for insects (Figure 7).

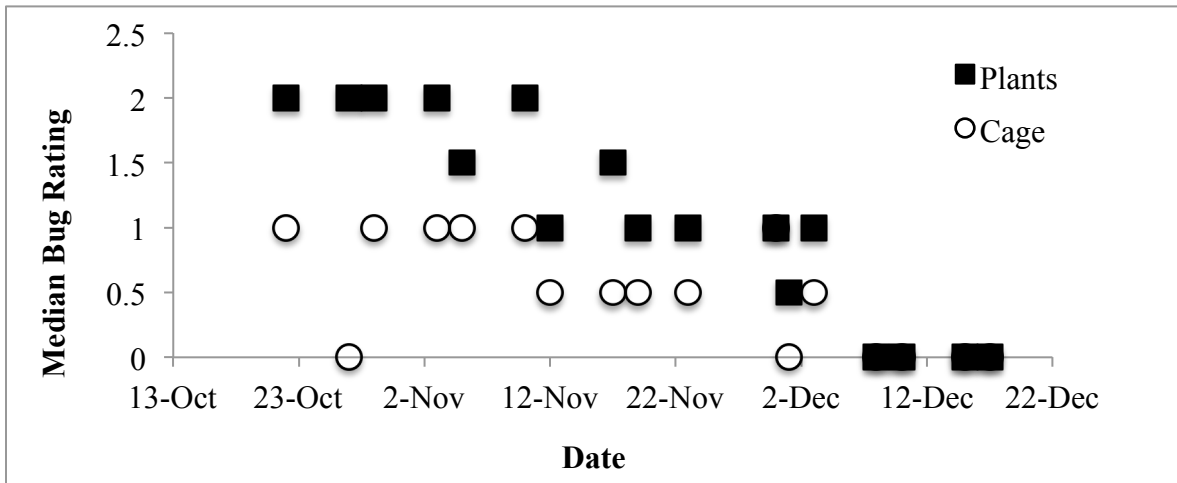


Figure 6: A cage was visited between October 22 and December 19, 2015. Median score of bugs observed on plants and on cage walls is depicted over the observation time.

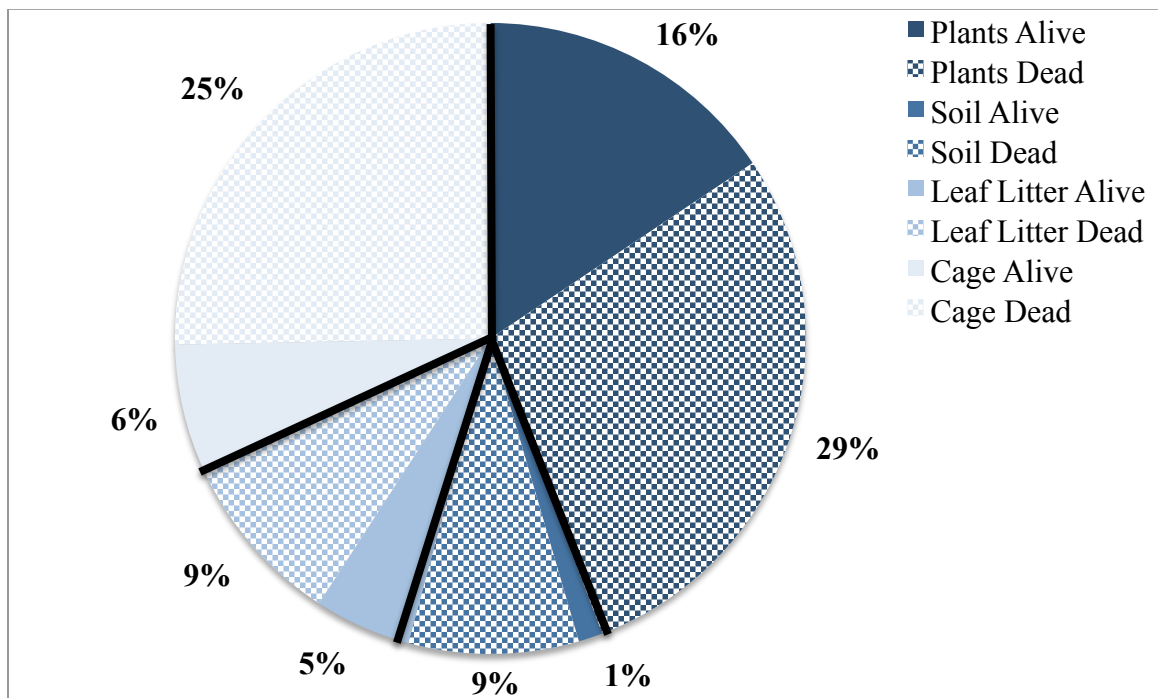


Figure 7: Percentages of total bugs found in each area when the cage was destructively sampled in January, 2016. Areas in the cage include: plants, soil, leaf litter, and cage. Each area is subdivided into dead (hashed) and alive (solid) insects.

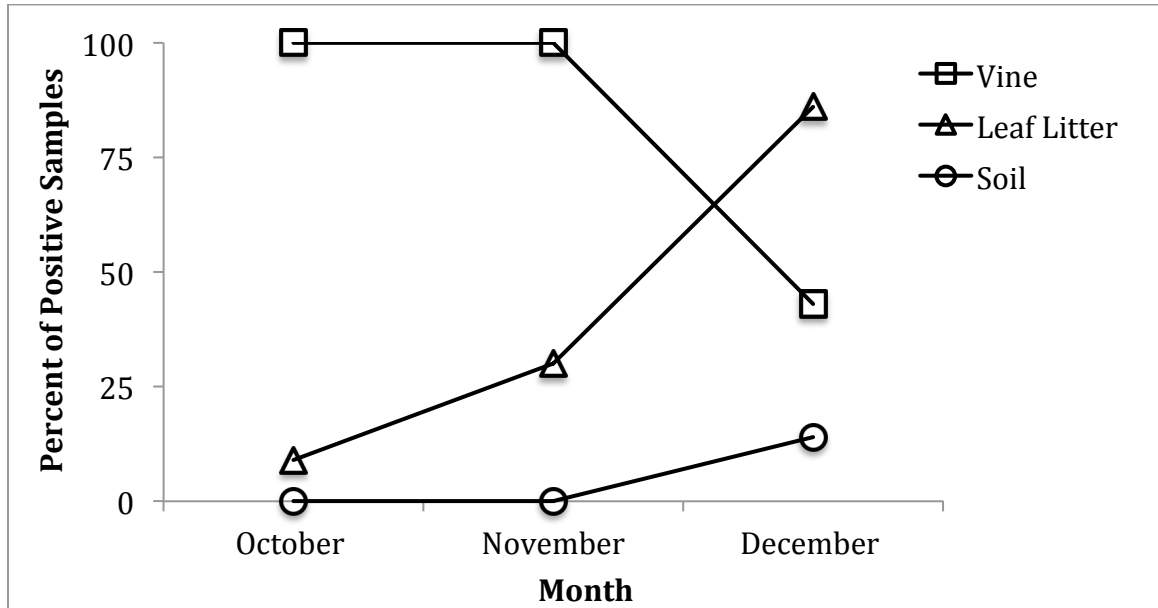


Figure 8: Percent of samples with kudzu bugs in which at least one insect was found in the respective layer (vine, leaf litter, or soil) over the fall sampled once a month.

In the field study, a decline in bugs was observed from October to December in the vine samples and an increase in bugs found in the leaf litter samples. October showed nearly all bugs in the kudzu vines (Figure 8). By November, bugs were still mostly found in the vines; however, bugs began to appear more in the leaf litter as well. December saw a reversal in which leaf litter was the dominant location of bugs. Vines though still harboring a few bugs was greatly reduced from the previous month. December was the first observance of an insect in the soil.

Microhabitat Temperature Measurements

Temperature changes between day and night were most variable in the vine and ambient monitors. Leaf litter showed some thermal buffering to changes in temperature (Figure 9). Mean difference between average day and night temperature over October to

April for ambient was 5.5°C with a minimum of 4.4°C and maximum of 6.7°C. In the vines the mean difference was 5.7°C with a minimum of 3.8°C and maximum of 8.8°C. In the leaf litter the mean difference was 2.8°C with a minimum of 1.8°C and a maximum of 4.15°C. On average vines were colder than the ambient temperature whereas on average leaf litter was warmer (Figure 10). Over October to April the average difference between ambient and vines was -0.5°C with a minimum of -1.6°C and a maximum of 0.4°C. In leaf litter the mean was 1.2°C with a minimum of -0.0°C and a maximum of 2.9°C. The greatest buffering differences in the leaf litter occurred in January and February (Figure 10).

Recorded hourly ambient temperature at the kudzu site exceeded the November LLT₅₀ value of -4.9°C a total of 534 times between December 2014 and April 2016 (Table 3). The leaf litter exceeded the LLT₅₀ threshold 43 times and only occurred between January and March of 2016 (Figure 11). Temperatures varied in duration of consecutive hours below LLT₅₀ threshold and often equaled or exceed 12 consecutive hours (Figure 11). The mean Calvert population SCP of -11.5°C was exceed 62 times in the ambient temperature with 48 of those occurrences happening in February of 2015 (Table 3).

Application of Temperature to Range Expansion

An average temperature difference of 1.2°C between ambient and leaf litter microhabitats was applied to the minimum temperature ranges in the USDA plant hardiness zone map. For the future SCP, a 3°C increase was applied to the minimum temperature after microhabitat adjustment. The mean SCP of -12.6°C and mean LLT₅₀ of -5.1°C was used for creating the temperature threshold isolines. Based on these assumptions, the kudzu bug will be able to advance one plant hardiness zone by the end of the century (Figure 12).

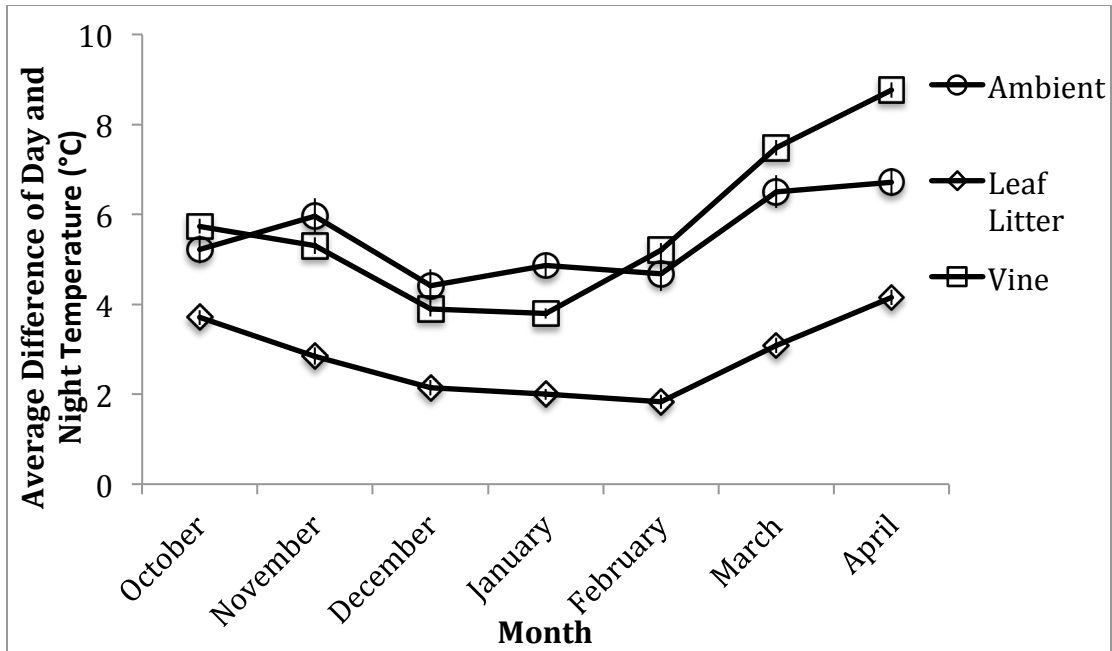


Figure 9: The average difference of day to nighttime temperatures per month. Error bars are shown but for most points are too small to visualize on the graph.

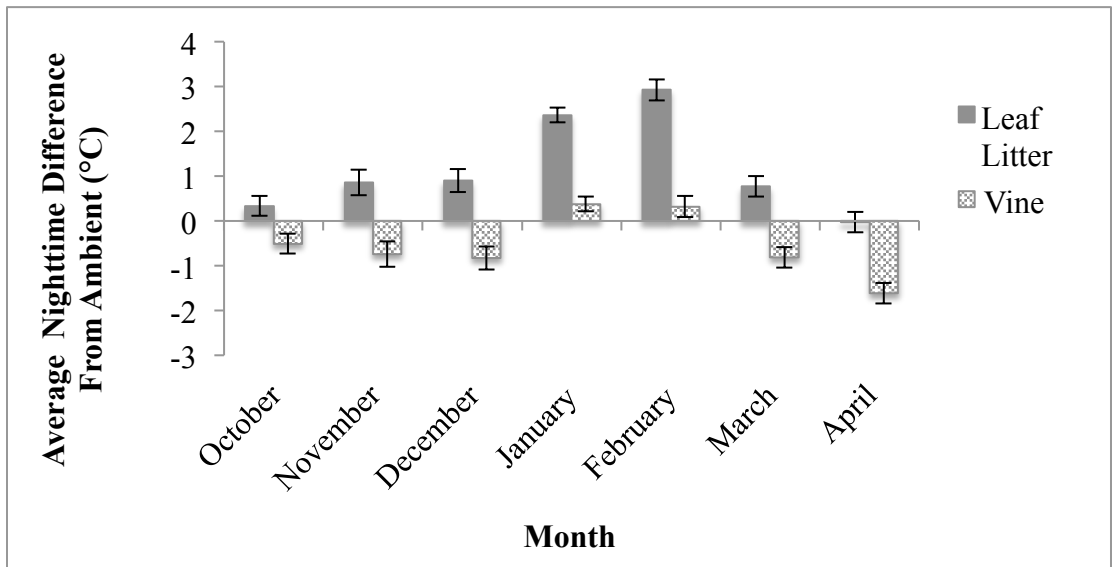


Figure 10: The average nighttime difference between ambient, leaf litter, and vine microhabitat temperatures. If positive the temperature was warmer than ambient. Standard error bars are shown.

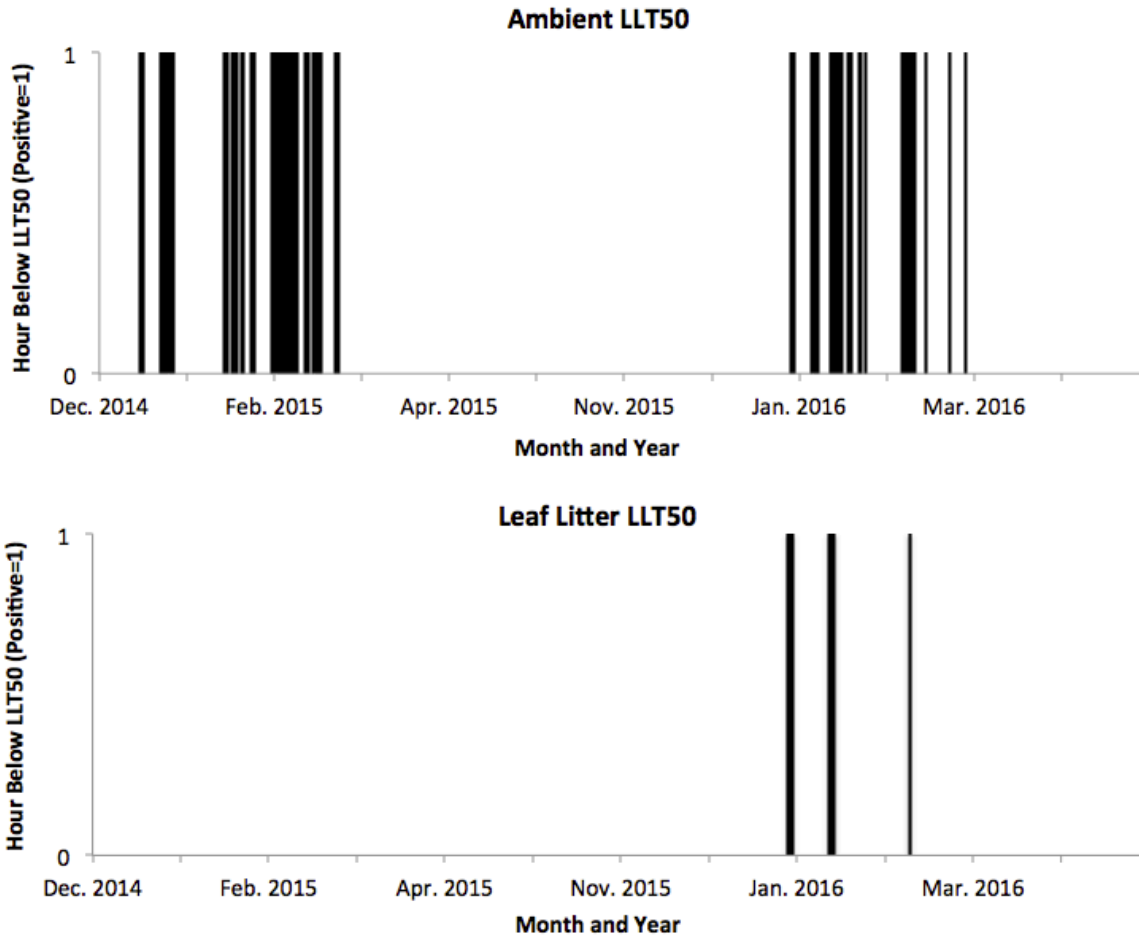


Figure 11: Ambient (top) and leaf litter (bottom) temperature was recorded every hour between December 2014 and April 2016 in Calvert County, Maryland kudzu patch. Each hour in which the temperature was below the November LLT_{50} threshold of $-4.9^{\circ}C$ was scored as 1 and if above was scored as a zero. Thicker bars indicate consecutive hours below the threshold.

Table 3: Ambient and leaf litter temperature in a kudzu patch located in Calvert County, Maryland was recorded every hour from December 2014 to April 2016. Occurrence of hours below the November Calvert County LLT_{50} threshold of $-4.9^{\circ}C$ and below the mean Calvert County SCP threshold of $-11.5^{\circ}C$ were counted.

	SCP		LLT_{50}	
	2014-2015	2015-2016	2014-2015	2015-2016
Ambient	62	0	363	171
Leaf Litter	0	0	0	43

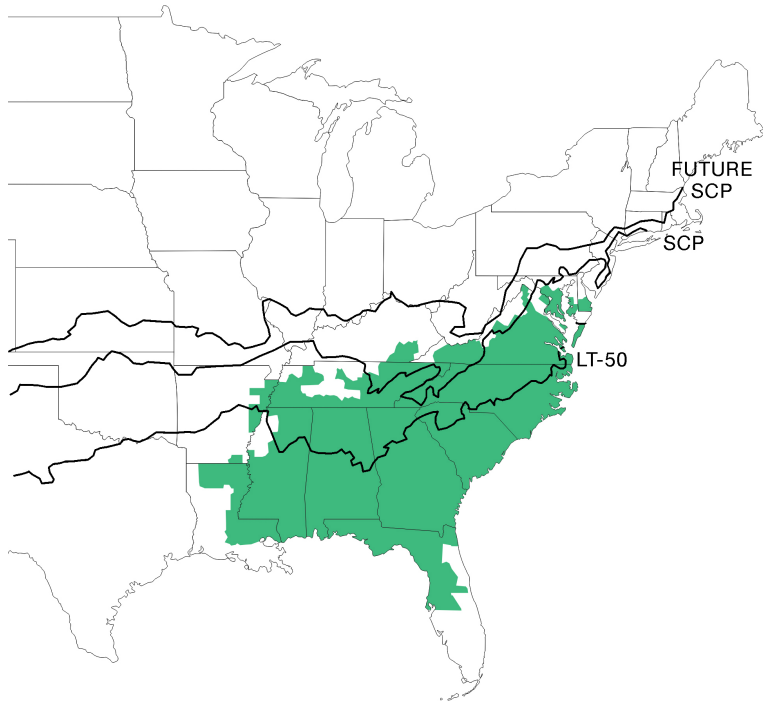


Figure 12: LLT-50, SCP, and future SCP lines are based on the USDA plant hardiness zone boundaries. Green shaded areas show the current 2016 distribution of the kudzu bug.

Discussion

The objective of this study was to determine the cold tolerance of the kudzu bug and its relation to the current and future distribution. My hypotheses were: (1) that the kudzu bug is a freeze avoidant species with (2) slight changes to its cold temperature tolerance through the fall and over a latitudinal gradient, and (3) that its current local distribution limit aligns with its ability to tolerate minimum temperatures. Furthermore, I predicted that with climate change the kudzu bug northern distribution would extend northward under warming winter temperatures. I found that the kudzu bug is a freeze avoidant species with an average SCP of $-12.6 \pm 3.5^\circ\text{C}$ and LLT_{50} of -5.1°C with a lower and upper confidence interval of -6.0°C and -4.4°C respectively. Furthermore, the kudzu bug has limited thermal plasticity and no apparent local adaptation between populations. The leaf litter, which the kudzu bug moves into between late November and early

December to overwinter, provides thermal buffering. The microclimate created by the leaf litter is not only on average warmer than the ambient temperature but also is a more constant temperature between day and night. Given the cold temperature thresholds, which match the current northern distribution limit, the kudzu bug is expected to expand into the 6B USDA plant hardiness zone by the end of the century.

SCP values showed no effect of sex and unexpected trends in the effects of month and county. Differences in the response to cooling between sexes can cause a bimodal SCP (Salin et al. 2000, Renault et al. 2002). For example in the lesser mealworm, males exhibited a significantly lower supercooling point than females (Salin et al. 2000). Though sex should be evaluated, there is a precedence of sex not affecting the cold tolerance of an insect (Khani and Moharramipour 2010, Morey et al. 2012). My finding of no effect of sex is therefore not surprising even in light of the difference in weight between the sexes.

The effect of month on the SCP of populations does not show a consistent trend over the fall. December had a warmer SCP than November and October. In some insects, the SCP becomes more cold tolerant over the fall. For example, in the oriental corn borer larvae, *Ostrinia furnacalis*, the SCP decreases in temperature thus increasing in cold tolerance between September and December (Goto et al. 2001). Contradictory from the expected lowering of the SCP temperature over the fall, the warmer SCP values in December might be due to a warm fall experienced at the population locations. Additionally, the difference exhibited in December could be due to the collection location within the kudzu patch of bugs screened for the SCP. In October and November, the kudzu bug had not yet begun to move to overwintering locations and all sampled bugs

came from the kudzu vines directly. By December, many bugs had begun to move to the leaf litter for overwintering. Sampled bugs in December included those from the leaf litter and vines though primarily from the vines. The insects in the leaf litter could have been at a different stage of cold accumulation than those in the vines.

For population location, I would have expected a latitudinal gradient in which the southern most location had the warmest SCP. For example, in *Liriomyza huidobrensis*, SCP was high in southern populations and lowered with increasing latitude (Chen and Kang 2004). Conversely, my results showed no latitudinal gradient between our population locations. Instead, I found the Suffolk population, the most southern location, had the lowest SCP while the other two locations were not significantly different from each other.

Given the recent arrival of the kudzu bug to North America and the constricted single mitochondrial haplotype lineage (Jenkins and Eaton 2011), a founder effect may be limiting local adaptation. The population from which the United States kudzu bug populations originate from is located on the subtropical island of Kyushu, Japan (Hosokawa et al. 2014). As such, United States populations are likely experiencing high selective pressure to adapt to the temperate climate of Maryland from the subtropical climate of Kyushu. However, a genetic bottleneck might cause a barrier to rapid adaptation even under such a potentially high pressure. Additionally, it may take many more generations before a latitudinal cline develops in the United States. According to Moran and Alexander (Moran and Alexander 2014), geographic clines for a trait often take between 50-150 generations in a newly invaded species.

Lacking a latitudinal gradient may also result from looking at three populations on too fine of a scale. The three climate zones studied represent a range of three hundred and twenty kilometers, which may not have been diverse enough to detect a difference based on location. However, the SCP does not always follow a latitudinal gradient even in established pests within an invaded range. Colorado potato beetle, *Leptinotarsa decemlineata*, was found to have no latitudinal pattern in SCP from Jalisco, Mexico to Vermont, United States (Izzo et al. 2013). Additionally, in the native harlequin bug, *Murgantia histrionica*, SCP was found to have no difference between Maryland and Virginia (DiMeglio et al. 2016).

LLT₅₀ values showed an effect of month but no consistent latitudinal trend between population locations. As expected, LLT₅₀ values decreased between October and November indicating a cold acclimation. Insects prepare for winter through the process of seasonal cold accumulation. During cold accumulation, insects may up regulate cryoprotectant proteins or evacuating the digestive tract, which ultimately lower responses such as LLT₅₀ (Slachta et al. 2002). Triggers for cold accumulation may include photoperiod or temperature responses (Clark and Worland 2008). Precise cues for cold accumulation in the kudzu bug remain unknown.

Similar to the SCP, there was no consistent latitudinal trend in the LLT₅₀ values between the three population locations. Different patterns emerged at each tested temperature in regards to population location. Location variation in LLT₅₀ values is likely due to phenotypic plasticity within the populations. Plasticity can allow for survival and establishment during the time needed for an invasive species to achieve local adaptation (Moran and Alexander 2014). Monitoring for local adaptation in the future will enhance

the predictive capabilities of overwintering survival on a small geographic scale. Future studies must also characterize the ice nucleating potential of the *B. bassiana* fungus in the kudzu bug, as this fungus is prevalent in southern populations. An infection of this fungus in the Suffolk population in November caused high rates of background mortality causing removal of those bugs from analysis. Fungi and bacteria have potential to lower cold tolerance thus decrease the predicted surviving spring population numbers (Lee et al. 1992, 1993).

Observations of caged and field movement show that the kudzu bug moved to overwintering locations in late November to early December in 2015. Maryland's average December temperature was 7°C warmer than the 100 year average and only 1°C cooler than the average November temperature (NOAA 2016). Therefore, temperature is unlikely the cue for movement. Though not tested, photoperiod is a probable cue for overwintering movement. Photoperiod regulation of overwintering induction is congruent with other insects such as the linden bug, *Pyrrhocoris apterus*. Linden bug overwintering induction is primarily regulated by photoperiod while temperature plays a secondary role in induction (Hodkova and Hodek 2004). In the brown marmorated stink bug *Halyomorpha halys*, short photoperiod increased nymph development and has been suggested as part of the overwintering induction cue (Niva and Takeda 2003, Cira et al. 2016). As the kudzu bug does not move to overwintering refuge until late fall, an early frost could be extremely hazardous to population survival. The kudzu bug overwinters in the leaf litter at the base of trees and dead kudzu vines as confirmed by both field and cage movement observations. This finding is consistent with a study published by Lahiri

et al. in which they determined the kudzu bug overwintering in the leaf litter as well (Lahiri et al. 2015).

Leaf litter provides thermal buffering for the kudzu bug against ambient temperatures. Leaf litter also moderates the diurnal temperature fluctuations which benefits the bugs by decreasing the metabolic rate thus reducing the use of stored energy reserves (Williams et al. 2012). Furthermore, the average nighttime temperature in the leaf litter was on average warmer than the vine or ambient temperatures. The thermal buffering of the leaf litter is clear when over 2014-2016 only 8% of the hours below the LLT_{50} in the ambient temperature were below the threshold in the leaf litter as well. Furthermore, though the ambient temperature reached the SCP, there were no occurrences of the SCP in the leaf litter. Kudzu bug cold temperature tolerance comprises a narrow range in which at 2°C nearly all bugs survive but by -10°C all bugs have died after a 12 hour exposure. Thus, a few degrees of thermal buffering could drastically affect the surviving proportion of the population. Thermal buffering of soil and even ice is well known (Bale and Hayward 2010) and can aide insects in surviving the winter. Adjustments between microhabitat and ambient temperatures are crucial in modeling winter survival. Best practice would be to have a temperature monitor in the overwintering location of concern rather than relying on a weather station, which may record vastly different temperatures.

Yearly overwintering population survival as well as future range expansion predictions based on cold tolerance are possible. In the IPCC RPC 4.5 model, winter temperatures will increase by 3°C (IPCC 5th Report 2013) and the kudzu bug will likely be able to expand northward through central Pennsylvania and up to costal

Massachusetts. Kudzu vines as well as soybean extend throughout the predicted range thus the range shift will not be limited by host plant availability. The northern most reaches of the possible range will most likely allow for establishment but will not allow populations to build to economically damaging levels. Maryland, the current northern limit, could see increased pest pressure of the kudzu bug in soybeans. Warmer winters will begin to allow population densities to build unlike under current conditions, which could cause higher pest pressure.

In summary, the kudzu bug is a freeze avoidant species as they cannot withstand ice nucleation and die before the SCP. Measured cold tolerance supports limited plasticity over local adaptation at this point in time. Cold tolerance can be slow to evolve due to a tight co-adaptation or the gene being pleiotropic in addition to bottlenecks (Kimura 2004). Plasticity is thus allowing the kudzu bug to become established but not expand or build to high densities at their northern distribution limit. The leaf litter, which the bugs move to in late fall, provides an overwintering refuge protected from ambient temperatures. The microclimate created may increase the surviving proportion of the population. Future climate shifts, particularly with warmer winters, will allow the kudzu bug to expand northward most likely into the 6B plant hardiness zone by the end of the century. Producers can utilize knowledge of cold tolerance within an integrated pest management plan to assess risk of an area. Future work should examine the lethal time to kill a proportion of the population. The temperature monitors indicated that not all exposures to lower temperatures are twelve hours. Integrating knowledge of time effects on temperature lethality could improve prediction of surviving population numbers. Studies should also address non-lethal affects of cold temperatures such as reduced spring

fecundity as the result of cold stress during winter. Reduction of fecundity could cause a lag in population growth the following year, which has direct applications to the pest pressure to early-planted soybeans. Finally, continued work on evaluating local adaptation would benefit predictions of range expansion. If kudzu bug populations are able to adapt and become more cold tolerant, it would be possible for the distribution to shift northward more rapidly than current predictions.

Chapter 3: Degree-Day Model and Validation of Kudzu Bug Phenology

Abstract

Megacopta cribraria, kudzu bug, is a recent invader to the United States and is a pest of soybeans. I sought to determine the phenology of life stages of the kudzu bug in Maryland as well as create a degree-day (DD) model for egg and nymph development. I also monitored microhabitat temperatures of both kudzu and soybeans over 2015-2016 to characterize the difference from ambient temperature, which DD models commonly use. Kudzu bug population numbers were low in 2014 during the phenology study and thus little resolution was gained from the field observations. I observed that the bug is persisting within Maryland but that its populations in 2013-2016 are low compared to the Southeast United States. Based on the degree-day model, kudzu bug eggs take 80 DD with a minimum temperature of 14°C to hatch. Nymphs require 545 DD with a minimum temperature of 16°C for development. Field validation of the DD model fit well when factoring in a biofix of May 1st and approximately 30 days for a preoviposition period. The model suggests two full generations per year and the beginning of a third, which is not completed. Microhabitat temperatures did not show a clear trend between the kudzu and soybeans differences from ambient temperatures. Standard air temperature monitors do not affect model predictions for pest management. Ultimately, producers can predict the timing of kudzu bug life stages with the DD model in soybean fields for use of timing management plans.

Introduction

The kudzu bug, *Megacopta cribraria*, invaded Georgia in 2009 from Kyushu, Japan (Eger Jr et al. 2010, Suiter et al. 2010, Hosokawa et al. 2014). Though the bug is widespread across Asia and Australia, in the United States it reached a northern limit in Maryland by 2013 (Leslie et al. 2014). Kudzu bug is an agricultural pest of soybeans in both its native and invasive range. Yield losses can reach up to 59% at high densities, 25+ adults per plant, if untreated (Seiter, Greene, et al. 2013). The United States produced 3.93 billion bushels of soybeans in 2015 making the risk of economic loss staggering (“Crop Production 2015 Summary” 2016).

Integrated pest management utilizes an understanding of the biology of the pest species. Phenology, timing of life stages, and life history have extensive implications on timing of pest management practices. Currently, the kudzu bug is believed to have two generations per year in the United States but can have up to three in its native range (Zhang et al. 2012). To date, the phenology of the kudzu bug in the United States has been primarily determined through observations in the Southeast. Current knowledge suggests that the kudzu bug emerges from overwintering to lay eggs in the late spring. Eggs hatch within one week and nymphs develop over four to six weeks (Ruberson et al. 2013). Peaks of adult activity based on flight intercept traps occurs in July-August and October in Georgia (Zhang et al. 2012).

Temperature determines the development of insects, as they are poikilothermic. Degree-days (DD) offer an approach to calculating phenology based on the relationship between temperature and development (Damos and Savopoulou-Soultani 2011). Degree-days are often used in timing pest management of agricultural crops (Pruess 1983, Damos

and Savopoulou-Soultani 2011). In a study by Shi, Cui, and Zhang (2014), degree-day development was determined for a kudzu bug population in Zhejiang, China. Using constant temperatures ranging from 17°C to 33°C they were able to determine it takes 849 DD at a minimum temperature of 14.25°C for eggs to develop into adults (Shi et al. 2014). This model, however, may not be applicable to the populations in the United States. The bugs in the degree-day model were field caught in the Zhejiang province of China; however, the US population comes from a single female haplotype originating in Kyushu, Japan (Jenkins et al. 2010, Hosokawa et al. 2014). Zhejiang, China has a temperate climate while Kyushu, Japan has a sub-tropical climate further distinguishing the possibility of differences of development in response to temperature.

In addition to development, female fecundity and adult longevity can influence pest management plans. For a Chinese population, kudzu bug female preoviposition period ranges from 14 to 54 days (Shi et al. 2014). Fecundity has been estimated to be 49-73 eggs in India (Srinivasaperumal et al. 1992), 102-157 eggs in another study from India (Thippeswamy and Rajagopal 2005) and 49-160 eggs per female in China (Shi et al. 2014). Such wide-ranging estimates can alter predictions of population growth. Longevity of adults has been equally disparate. Reports range from 2-5 days in India (Srinivasaperumal et al. 1992), 6-25 days in the United States (Zhang et al. 2012), 23-64 days in a different study from India (Thippeswamy and Rajagopal 2005), and 44-81 days in China (Shi et al. 2014).

To relate temperature to development and life history, microhabitat conditions are known to affect realized insect temperatures producing microclimates (Ferro et al. 1979). The kudzu bug aggregates together on the stems and undersides of leaves of both kudzu

and soybeans. These areas protected by the architecture of the plant create a microclimate. Differences in microclimate temperatures from macroclimate temperatures are well known within plant canopies (Rosenberg et al. 1983). Evaluation of microclimate differences from ambient temperature can enhance the predictive capability of degree-day and life history modeling.

I expect the kudzu bug to complete two generations in Maryland. Peaks in adult activity are expected in April, when the adults emerge from overwintering, in June-August, and in October (Leslie et al. 2014). To determine the Maryland phenology and microhabitat temperature, I first developed a degree-day model and determined fecundity and adult longevity. Second, I assessed the microhabitat temperature of both kudzu and soybean. Finally, I compared the degree-day model to field observations in 2014 and 2016, and determined the persistence of the kudzu bug at Maryland sites from 2013 through 2016. These key areas have either not been studied or need resolution to better aid producers in implementing integrated pest management plans within the Mid-Atlantic of the United States.

Methods

Determining Degree-Day Development

Kudzu bug adults were collected from kudzu, *Pueraria* sp., patches in central to southeast Maryland in 2015. Adults were maintained in a mesh cage (Bioquip, Rancho Dominguez, CA) in the lab under room temperature and fluorescent lighting. The cage was provisioned with organic edamame (*Glycine max* var. 'Midori Giant') plants. Plants were grown without insecticides in an environmental chamber at the University of Maryland set at 25°C, 60% humidity, a light level of 300 $\mu\text{m m}^{-2} \text{s}^{-1}$, and an 18:6 light dark cycle.

The effect of temperature on the development of the kudzu bug was determined using environmental chambers set at four constant temperatures with a 16:8 light cycle and 70% relative humidity. Initial chambers (Model I-35LLVL, Percival Scientific, Perry IA) were held at 17, 21, 25, and 29°C; however, due to a chamber abnormality, the 25 and 29°C were run a second time in new chambers (Model 5090, Conviron, Hendersonville, NC). Chambers had a temperature monitor (Model DS1921G iButton, Maxim Integrated, San Jose, CA) to record temperature and a pan of water to increase humidity. The lab colony was checked daily for egg masses and a minimum of six egg masses were selected for development in each chamber. Newly laid egg masses were placed in a petri dish (9 cm diameter, 1 cm tall) with a fine mesh vent (4 cm diameter) in the lid and provisioned with a wetted cotton ball. Upon hatching, individuals were allowed to uptake symbionts from the egg mass (Jenkins et al. 2010) before being moved to separate petri dishes. Individual dishes contained one edamame leaflet with cut end wrapped in a wetted cotton ball. Leaves were changed three times a week. Individuals were checked daily for exuvia or death, and to rewet the cotton ball. After eclosion, the individual was sexed. Adult bugs were then paired to determine pre-oviposition time, oviposition time, fecundity and adult longevity. Paired adults were monitored daily for egg laying or death and were otherwise reared the same as the nymphs. In the second run, only the 29°C chamber was monitored for fecundity of adults and no longevity measures were recorded.

Models were created for the degree-day development of both eggs and nymphs. Initially, first and second runs for the 25 and 29°C chambers were compared with a t-test to determine if the data could be pooled for further analysis. To create the degree-day

model, a regression was run between temperature and the inverse of development time, 1/days to develop (JMP Pro version 11, SAS Institute, Cary, NC). Outliers were assessed by Jakeknife distances and a Cook's D Influence and removed if the value exceeded 0.03. Parameters for degree-day calculations include developmental minimum (T_{\min}), developmental maximum (T_{\max}), and degree-days for development (DD). In the regression model, $y=b+mx$, DD equates to the inverse slope ($1/m$), and T_{\min} to the negative intercept divided by slope ($-b/m$). T_{\max} is unable to be calculated from this equation.

Microhabitat Temperature Measurements

In a kudzu patch in Calvert County Maryland (38.432125, -76.55215), five stakes were deployed with one temperature monitor (Model DS1921G iButton, Maxim Integrated, San Jose, CA) within the leaf canopy of the kudzu vine. Also, an additional monitor measuring ambient temperature was suspended on the north side of a tree within the kudzu patch area. Monitors record temperature once an hour throughout the year. After uploading the temperature data, the two hours before and after the download times were removed from analysis. Due to the potential for shifting foliage cover, extreme outliers were excluded from analysis.

In addition to the kudzu patch, five temperature monitors (Model DS1921G iButton, Maxim Integrated, San Jose, CA) were deployed in a soybean field at the University of Maryland research farm in Beltsville, Maryland (39.012959, -76.82631). One additional temperature monitor was placed in a nearby wooded edge approximately 0.5 meters from the ground to measure ambient temperature. Monitors recorded once an hour from June through September. Monitors in the soybean field were adjusted once a

month to be positioned at 50% the stand height of the plants. Due to the open canopy nature of field, extreme outliers were excluded from analysis.

Observations of Phenology and Persistence

Phenology of egg, nymph and adult stages was assessed from June 5th to November 5th 2014. Six sites were chosen for sampling, four kudzu patches (38.934299, -76.80395: Prince George's County (A); 39.003872, -76.85146: Prince George's County (B); 39.05584, -76.96204: Montgomery County; and 39.232861, -77.02729: Howard County) and two soybean fields (39.012959, -76.82631: Beltsville; and 38.861641, -76.77453: Upper Marlboro). All sites were checked once a week during the summer and every two weeks during the fall. Sampling at kudzu patches entailed collecting ten 0.5 meter stem samples, five from the tip of the vine and five interior sections. Cuttings were bagged and processed at the University of Maryland. Additionally, one minute of sweeping was performed with a canvas sweep net (40 cm diameter, 90 cm pole). At all sites, including the soybean fields, white PVC poles (11.5 cm diameter, 22 cm tall) were deployed wrapped in sticky tape (similar to Stable Fly Sticky Sleeve, Great Lakes IPM, Vestaburg, MI) that was checked and changed during each sampling date. In all sampling methods, number of egg masses, nymphs and adults were recorded.

Persistence of the kudzu bug in Maryland was checked at kudzu sites around the state during the summer and fall of 2014, 2015 and 2016. These sites represent those discovered in 2013 during the earliest work assessing the kudzu bug invasion in Maryland along with more newly discovered kudzu patches. Each site was swept for a minimum of ten minutes with a canvas sweep net and counts of nymph and adult stages were denoted.

To validate the degree-day model, daily temperature minimum and maximum in 2014-2016 were obtained from a weather station in Prince George's County through NOAA (Oxon Hill: 38.7853, -76.9948). Temperatures were adjusted for average kudzu microhabitats by subtracting 0.2°C from the summer month's maximum temperature and 0.5°C from the minimum temperature (see Microhabitat Temperature Measurements). Adjusted temperatures were then used to calculate degree-days for egg hatching through nymph development each year. May 1st was set to be the start of accumulating degree-days. Preoviposition time between generations was set at 30 days. Model predicted generational timing was observed from a kudzu site at Oxon Hill in 2016 (38.780685, -76.99818), which was 0.51 kilometers from the Oxon Hill NOAA weather station. Sampling at the Oxon Hill kudzu patch was conducted by sweep sampling for ten minutes with a canvas net. Resulting numbers of nymphs and adults were counted. Additionally, the estimated nymph instar was recorded.

Results

Determining Degree-Day Development

Egg development time between the two runs of the 29°C chamber were not significantly different ($t_{20}=0.913$, $p=0.37$) and therefore were pooled for analysis. Run significantly affected egg development in the 25°C chamber ($F_{2,97}=5.83$, $P=0.004$). The second run was different from both generations of the first run and was excluded from analysis because the first three egg masses of the second run were housed for a week in a different chamber while the development chambers were being acquired. Egg development was significantly affected by temperature ($F_{1,120}=740.45$, $P<0.001$). Egg hatch time was the longest and had the greatest egg mortality in the 17°C chamber. Mean

egg development time in the chambers ranged from 20.5 ± 1.1 days at 17°C to 5.1 ± 0.2 at 29°C (Table 6). The highest percentage of eggs hatching occurred in the 25°C chamber with an average of 98% hatch (Table 6). A linear regression of development rate (1/days) against temperature was modeled ($y = -0.177 + 0.012 * x$, $R^2 = 0.861$). Degree-days for development was calculated by the inverse of the slope and found to be 80.08 DD. The minimum threshold for development was determined to be 14.2°C .

Nymph development time between the two runs of the 29°C chamber were significantly different ($t_{17} = -2.29$, $p = 0.035$) and only the second run was used in the analysis. The second run was chosen as the representative run as it had a higher percent of nymphs (45%) reach the adult stage than in the first run (19%). Also, the rate of development was more consistent within egg mass cohorts of the second run. For example, in the first run, the development of 5th instar nymphs had greater variation (11-28 days) than the second run (11-16 days). In the 25°C chamber, the first and second run were not significantly different ($t_{14} = -1.02$, $p = 0.32$) and were pooled for the analysis. Nymph development time was significantly affected by temperature ($F_{1,40} = 281.02$, $P < 0.001$). Nymphs were only able to successfully develop in the 21°C , 25°C , and 29°C chambers. All nymphs in the 17°C chamber died within the first instar shortly after emerging from the egg mass. Nymph development took the longest and had the highest mortality, besides the 17°C , in the 21°C chamber (Table 4, Table 5). Mean nymph development time ranged from 87.2 ± 1.9 days at 21°C to 48.6 ± 1.6 days at 29°C (Table 5). A linear regression of the development rate was modeled ($y = -0.0294 + 0.0018 * x$, $R^2 = 0.878$). Degree-days for development were calculated by the inverse of the slope as 544.48 DD. The minimum threshold for development was 15.99°C .

The sex ratio of females to males was 60, 49, and 47 % in the 21, 25, and 29°C chambers, respectively, indicating an even sex ratio of adults in each chamber ($\chi^2_1=0.20$, $p=0.65$; $\chi^2_1=0.01$, $p=0.93$; $\chi^2_1=0.47$, $p=0.49$) (Table 4). Adult longevity in the 21°C chamber was on average \pm standard error 89.7 \pm 40.7 days with a maximum of 249 days. In the 25°C chamber, the average adult longevity was 97.8 \pm 10.0 days with a maximum of 334 days (Table 4). Preoviposition period ranged from 31 to 87 days with the longest time occurring in the 21°C chamber (Table 6). Though preoviposition period differed among temperature settings, oviposition period remained relatively constant with a range of 35 to 43 days. Total number of egg masses per female and total number of eggs increased with increasing temperature (Table 6). On average each egg mass had 19 \pm 1 eggs.

Microhabitat Temperature Measurements

Temperature in the kudzu vine was cooler than the ambient both during the day and night. Temperature differences in the day ranged from 0.09 \pm 0.08°C to 0.79 \pm 0.06°C cooler and in the night differences ranged from 0.76 \pm 0.03°C to 1.29 \pm 0.05°C cooler. Day difference declined from May to August but increased in September. Nighttime differences steadily decreased from May to September (Figure 13).

Temperature in the soybean did not show a consistent trend. In June, both day and night temperature differences indicate that the soybeans were warmer than the ambient temperature. In July, both day and night temperatures were cooler in soybeans than the ambient. August and September both show the day temperature warmer than ambient and the night temperature as cooler than ambient (Figure 14).

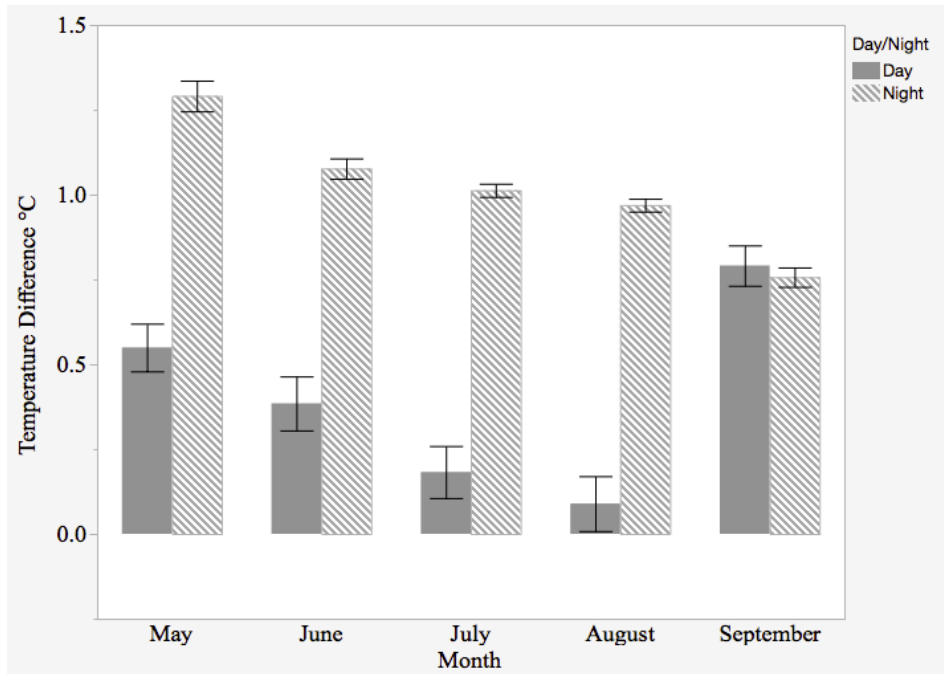


Figure 13: Average temperature difference (Ambient-Vine) for a kudzu patch in Calvert County, Maryland over 2015 and 2016.

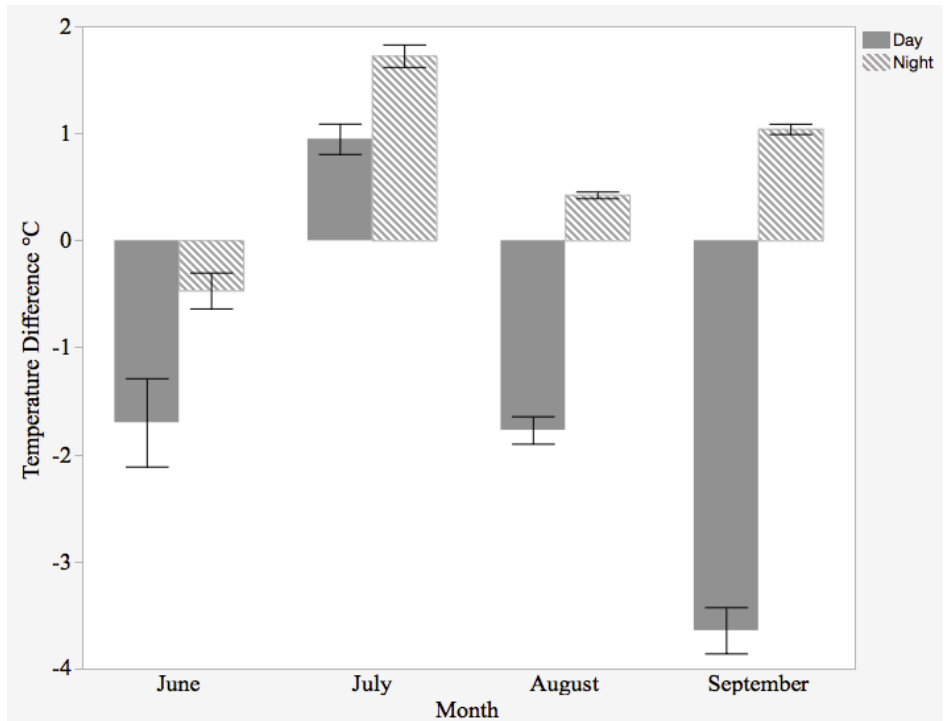


Figure 14: Average temperature difference (Ambient-Soybean canopy) of a soybean field in Prince George's County, Maryland over 2015 and 2016.

Table 4: Number of egg masses and individuals for each chamber. Average \pm standard error for percent of hatched nymphs to adulthood. Average \pm standard error (maximum) adult longevity except the 29°C chamber was omitted. The percent of reared adults that were females per chamber is shown. The 25°C chamber is pooled between the 1st and 2nd runs, 29°C chamber is only the 2nd run.

Chamber	Egg Masses	Number of Individuals	% To Adulthood	Adult Longevity	% Female
17	12	155	0	-	-
21	15	204	3.3 \pm 1.8	89.7 \pm 40.7 (249)	60.0
25	19	363	42.3 \pm 4.6	97.8 \pm 10.0 (334)	49.2
29	13	253	44.9 \pm 6.3	-	47.1

Table 5: Average \pm standard error of days between instars of egg mass cohorts per chamber temperature. The 25°C chamber is pooled between the 1st and 2nd runs, the 29°C Chamber is only the 2nd run.

Chamber	1-2	2-3	3-4	4-5	5-A	1-A
17	-	-	-	-	-	-
21	23.9 \pm 1.3	13.2 \pm 1.0	15.0 \pm 0.9	15.9 \pm 1.1	21.0 \pm 0.4	87.2 \pm 1.9
25	12.3 \pm 0.7	7.0 \pm 0.2	8.3 \pm 0.6	9.9 \pm 0.4	15.3 \pm 0.6	51.8 \pm 1.2
29	8.3 \pm 0.3	7.2 \pm 0.5	10.1 \pm 1.6	11.2 \pm 0.8	12.8 \pm 0.5	48.6 \pm 1.6

Table 6: Average \pm standard error of days between egg laying and hatch along with percent of total eggs laid that hatched per temperature. Average \pm standard error (maximum) number of egg masses per female and total number of egg per female. Average \pm standard error of preoviposition period, days between female eclosion and first egg mass laid, as well as oviposition period, the time between first and last egg mass laid in days. The 25°C chamber is pooled between the 1st and 2nd runs, the 29°C Chamber is only the 2nd run.

Chamber	Hatch Time	% Hatch	# Eggs Masses/ Female	# Eggs/ Female	Preoviposition Period	Oviposition Period
17	20.5 \pm 1.1	56.5 \pm 8.5	-	-	-	-
21	11.8 \pm 1.1	69.0 \pm 6.3	2.0 \pm 1.2 (4)	42.3 \pm 28.7 (97)	87.5 \pm 33.5	35.5 \pm 31.5
25	8.3 \pm 0.7	98.1 \pm 2.4	3.7 \pm 0.9 (13)	77.5 \pm 21.7 (302)	31.0 \pm 3.0	43.0 \pm 8.0
29	5.1 \pm 0.2	78.0 \pm 2.8	4.7 \pm 0.5 (9)	83.5 \pm 7.9 (201)	36.0 \pm 4.0	42.0 \pm 4.0

Observations of Phenology and Persistence

In 2014, kudzu bug population numbers were extremely low. No bugs were found during the phenology sampling at Howard County, Montgomery County, Beltsville Farm, or Upper Marlboro Farm sites (Table 7). At the Prince George's County (A), the first kudzu bug was captured was a single adult on June 5th. No additional adults or nymphs were found until October 1st. In October, two nymphs and ten adults were found. In early November eleven nymphs and fourteen adults were found (Table 7). Additionally, a single egg mass was found on August 7th. At Prince George's County (B) one adult was captured on August 21st.

Table 7: Each location was sampled once a week in the summer and once every two weeks in the fall. Counts for each location by month are broken into eggs, nymphs, and adults. Shaded boxes indicate a kudzu bug life stage was found.

	Howard County	Montgomery County	Prince George's County (A)	Prince George's County (B)	Beltsville Farm	Upper Marlboro Farm
June	0, 0, 0	0, 0, 0	0, 0, 1	0, 0, 0	0, 0, 0	0, 0, 0
July	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
August	0, 0, 0	0, 0, 0	1, 0, 0	0, 0, 1	0, 0, 0	0, 0, 0
September	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
October	0, 0, 0	0, 0, 0	0, 2, 10	0, 0, 0	0, 0, 0	0, 0, 0
November	0, 0, 0	0, 0, 0	0, 11, 14	0, 0, 0	0, 0, 0	0, 0, 0

Twenty-four sites were checked for persistence in 2014. Of those, eighteen were positive for kudzu bug and six were negative. Only one of the negative sites had been positive in 2013. In 2015, twenty sites were sampled with nineteen positive for kudzu bug and one negative, which had previously been positive from 2013-2014. In 2016, forty-eight sites were checked for persistence (Figure 15). Thirty-six of the sites were positive for kudzu bug including two new sites and fourteen sites that had been negative for kudzu bug in previous years. Twelve sites were found to be negative with six of those being

newly sampled sites and two having been sites previously positive for kudzu bug. In general, kudzu bug populations were low in 2014 and 2015 with nymphs and adults only reliably found in the fall. In 2016, population numbers were much greater. Moreover, nymphs and adults were easily found starting in June through the fall.

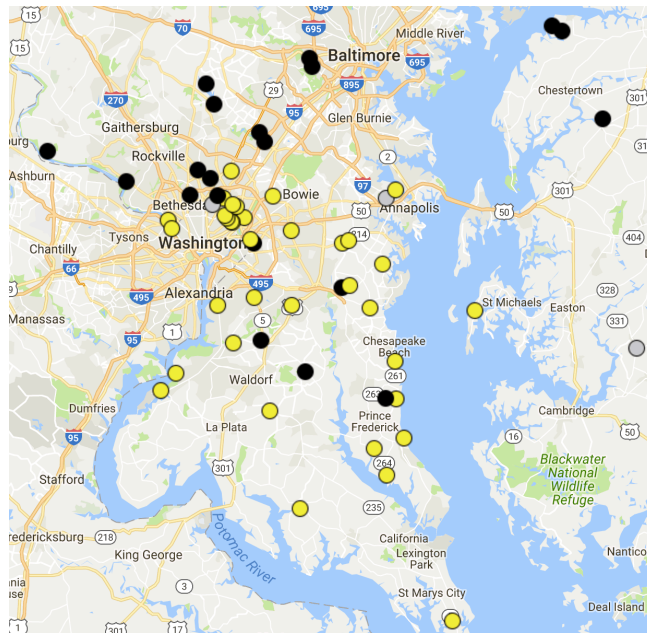


Figure 15: Sampled sites in 2016. Black were kudzu sites with no kudzu bug, grey are sites that were negative but have been positive in the past and yellow sites were positive for kudzu bug.

The degree day requirements calculated for eggs and nymphs were validated using temperature data from Prince George’s County: Oxon Hill. The accumulated degree-days over julian date show completion of two full generations and beginning a third (Figure 16). The first new generation of adults occurs in late June and the second generation occurs in late August to early September. Timing of the generations is consistent between years with variation due to temperature differences between the years. Examining field observations at the Oxon Hill kudzu site in 2016, the timing of the proportion of field caught nymphs shows a large proportion of late instar nymphs at julian date 180 (Figure 17). By julian date 209, the proportion has dropped below 10 percent indicating a large number of adults. This emergence of adults corresponds well with the predicted adult

emergence julian date of 187. Early instar nymphs spike in proportion at sampling date 229, which matches well to the expected date of 222. The last two sampling dates, 239 and 265, indicate a slow decrease in proportion of nymphs (Figure 17). Though late instar nymphs are found into late fall, a third complete generation is not possible based on temperature.

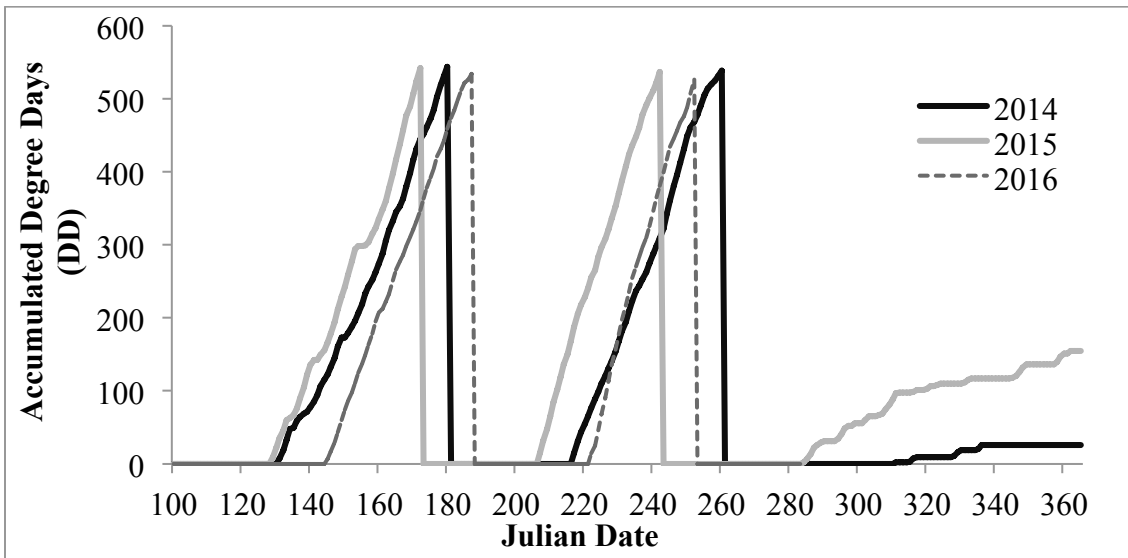


Figure 16: Accumulated degree-days for nymph development calculated from temperatures recorded in Oxon Hill, Maryland over 2014 to 2016. Nymphs reach adulthood at 545 accumulated DD and have a preoviposition period of thirty days.

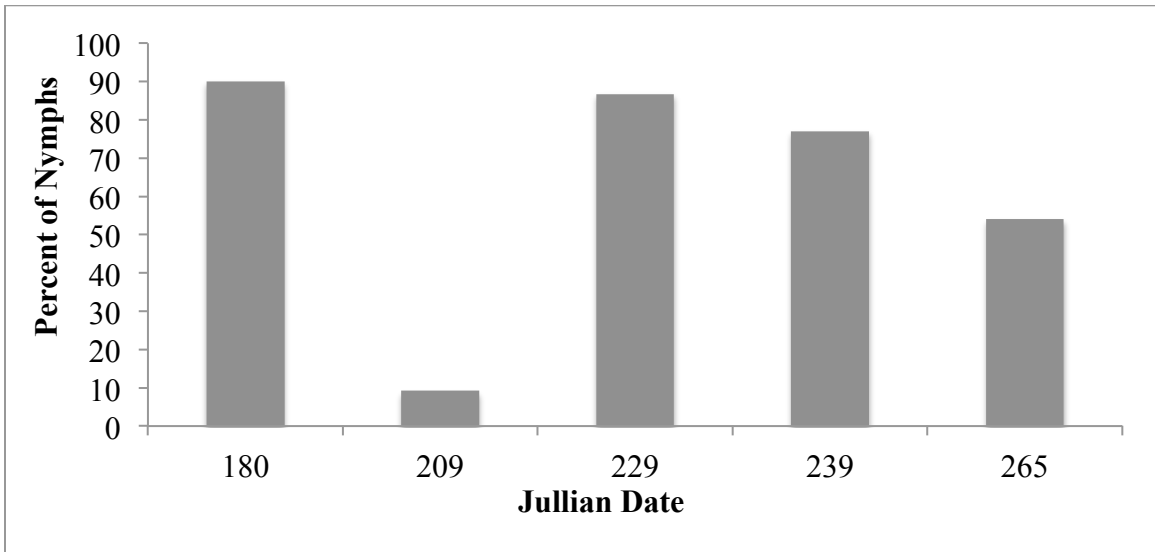


Figure 17: Percent of kudzu bug nymphs caught in 2016 during ten minutes of sweep net sampling at a kudzu patch in Oxon Hill, Maryland.

Discussion

The goals of this study were to (1) create a degree-day model for development and determine adult fecundity and longevity, (2) characterize the microhabitat differences between canopy temperature and ambient temperature, and (3) to validate the degree-day model through field observations of phenology and persistence. I found Maryland populations of kudzu bug eggs take 80 DD at a minimum of 14°C and nymphs require 545 DD at a minimum of 16°C. Females have a preoviposition period between 31-36 days in the 25 and 29°C chambers. Females can lay between 42 and 84 with a maximum of 302 eggs across all tested temperatures. Adult longevity ranged from 90 to 98 days with a maximum of 334 days. Microhabitat temperatures did not show a consistent trend within the soybean canopy but was on average cooler in the kudzu vine canopy by about 0.5°C during the day and 1°C at night. Field observations of the phenology in 2014 were inconclusive due to low population numbers at the study sites. However, the 2016 sampling at Oxon Hill matched the expected phenology based on temperatures from the Oxon Hill NOAA weather station. Persistence sampling within Maryland indicates that though population numbers were consistently low, the kudzu bug has been able to establish itself in Maryland.

Degree-days offer a direct relationship between development and temperature for Maryland kudzu bug populations. My Maryland degree-day model compared to the current degree-day model from China shows similarities and differences. In my model, egg development was found to take 80 DD with a minimum threshold of 14°C and the nymph development was found to take 545 DD with a minimum threshold of 16°C. A study by Shi et al. (Shi et al. 2014) in China found a similar minimum temperature

required for egg development but contrasting results for DD and for the nymph temperature minimum for development. The authors found eggs take 62 DD and eggs to adults require 850 DD with a minimum temperature of 14°C for development. Not only does the Chinese population take longer to develop, but the bugs can also develop at colder temperatures, perhaps reflecting local adaptation of the source population. The Chinese population was collected from a temperate zone and is thus likely to be more adapted to colder temperature than the US population, which is subtropical in origin. Though the US population was collected in Maryland, which is also temperate, it is likely local adaptation has not yet occurred. Therefore, populations in the US are currently constrained to their sub-tropical source population genetics. If US soybean producers use the DD model from China, it is probable that mismatches would occur from the model prediction and actual field timing.

One potential limitation of the new degree-day model is host plant impacts on development. The kudzu bug has several reproductive hosts including both legumes and non-legumes (Eger Jr et al. 2010, Medal et al. 2013, Blount et al. 2015). Non-legume host plants have shown greatly retarded development time compared to legume hosts (Eger Jr et al. 2010). The model in my study used soybeans, as they are the host plant of greatest concern. Systems other than soybeans particularly the non-legume plants should exercise caution in using this model.

Female fecundity and preoviposition times also indicated differences from the published literature. Shi et al. (Shi et al. 2014) found preoviposition ranged from 14 to 54 days and though my results lie within this range, unlike their study I did not find much variation between the upper two temperatures tested. Eggs per female have varied greatly

in the literature likely due to large variation in individual females consistent with my study. Number of eggs per female have been reported between 14 and 160 and my range is consistent with this array (Srinivasaperumal et al. 1992, Thippeswamy and Rajagopal 2005, Shi et al. 2014). Though previously not described in Maryland, I observed individual females producing anywhere from 0 to 302 eggs. Given the fecundity potential, in large populations the population growth of the kudzu bug can quickly intensify the pest pressure making early population suppression important to soybean producers.

My observed adult longevity is inconsistent with the literature which show a range from 2 to 81 days (Srinivasaperumal et al. 1992, Thippeswamy and Rajagopal 2005, Zhang et al. 2012, Shi et al. 2014). However, I show an average longevity of 90 ± 40 days in the 21°C chamber and 98 ± 10 days in the 25°C chamber exceeding any previously published measures. Furthermore, the maximum adult lifespan reached 334 days. Adult longevity may cloud the detection of distinct generations in the field. Additionally, adults may continue to feed and overlap with new generations to compound the pest pressure on soybean fields.

Accurate predictions based on temperature are dependent upon using temperatures that reflect the conditions of the kudzu bug. Microhabitat temperature within kudzu and soybeans shows a mixed trend. The kudzu canopy temperatures are about 0.5°C cooler during the day and 1°C cooler at night demonstrating the cooling effect of the canopy cover that is well known in plant systems (Rosenberg et al. 1983). Counter intuitively for the soybeans, most summer months have warmer soybean canopy temperatures during the day and cooler temperatures during the night. Due to the open

architecture of a soybean field, it is possible that the monitors were not accurately measuring the soybean microhabitat temperature. Producers collecting temperature for DD model predictions should use local temperature monitors if field monitors are not feasible.

Kudzu bug phenology in 2014 was inconclusive due to low population numbers. Adults during the study did not appear in number until the late fall. Though the timing of adults is congruent with the second generation, the first generation was likely missed due to low numbers making detection difficult. The Maryland phenology does support the phenology observed in the Southeast US. In Georgia, adults was also found in the fall persisting through December (Zhang et al. 2012). Comparisons earlier in the season are not possible and thus can neither support nor detract from observations in Maryland.

Persistence sampling shows that though population numbers were low in 2014 and 2015, most sites remained positive for the kudzu bug. Those sites, which did not, could be either a true negative or a false negative due to the power of detection. Many kudzu sites cover a large area where vines often twine up into the tree line. From my observation, kudzu bug adults frequently inhabit the upper canopy of a kudzu site where sampling is often not feasible. Thus it is possible that when finding a negative site, the bugs may still inhabit the area just in low numbers or in parts of the patch that are not reachable. In 2016, kudzu bug populations resurged in Maryland at most sites. Several sites that had been previously negative for the kudzu bug were positive. It is possible that migrating adults founded these sites; however, it is equally possible that the initial sampling occurred at a low population time thus missing a true detection.

Field sampling validation of the degree-day model shows a high degree of fidelity to the predicted model. The first new generation of adults and subsequent generation of nymphs match the model for accumulated degree-days at the Oxon Hill site. Field validation of degree-day models is necessary when a well-described phenology of the insect of interest is absent from the literature. The selected biofix date is the start of May, as I predict that to be the time when overwintering adults emerge and start to lay eggs based on temperature and field observations. The start of DD accumulation may need regional adjustment for the emergence of overwintering adults. Further developments might include studying the possibility of using flight intercept traps to begin the biofix. For example, apple producers concerned with codling moth management start counting degree-day accumulation when the first female is caught using a baited trap (Knight and Light 2005).

Based on the culmination of this study, I predict the following for the Maryland phenology of kudzu bug (Figure 18). Adults will emerge from overwintering in late April and begin laying eggs in May. Nymphs will begin to hatch and develop from May through the end of June. The first new generation of adults will be found in late June, will persist over the next three months, and will begin laying eggs in July. The second generation of nymphs will begin to develop in July through late September. Second generation adults will be found in September through the fall and will overwinter. Some of the second generation of adults will lay eggs in October giving rise to a third generation of nymphs from October through the end of the fall. These nymphs will not complete development and ultimately will die with the onset of winter temperatures.

Given the oviposition period of 36-43 days and the adult longevity, generations will overlap during the summer and fall.

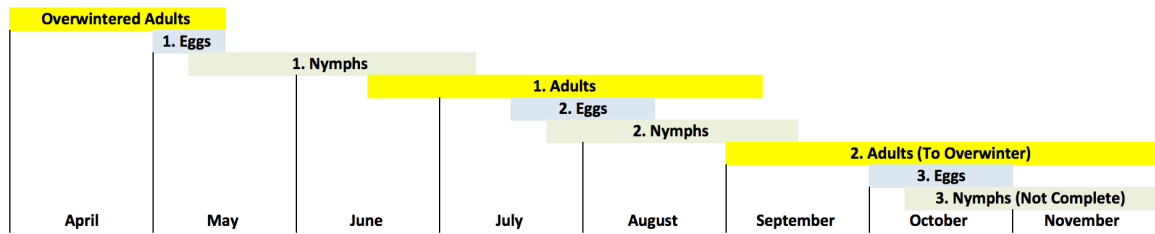


Figure 18: Predicted phenology of the kudzu bug in Maryland based on degree-days and field observations.

In summary, the objective of this study was to create a degree-day model for development, characterize the microhabitat temperature, and validate the degree-day model through field observations of phenology. The degree-day model for Maryland populations is eggs take 80 DD at a minimum of 14°C and nymphs require 545 DD at a minimum of 16°C. Field observations support this model well and the generalized phenology of Maryland, shown in Figure 18, should hold true unless the climate changes. Microhabitat temperature differences for soybean fields needs further study to resolve the conflicting trends. In general, producers should use local monitors over area wide monitors. Producers in the mid-Atlantic region concerned with pest management of the kudzu bug should use the Maryland degree-day model over the Chinese model.

Highlighted throughout my thesis, temperature influences the development and winter survival of the kudzu bug. An understating of the relationship between the kudzu bug and temperature can inform predictions of pest management timing, yearly surviving population numbers, and potential range shifts northward. Overall, I sought to determine the kudzu bug cold tolerance and degree-day model to predict the phenology of kudzu bug development in Maryland.

My cold tolerance research elucidates that the kudzu bug is a freeze avoidant species, which dies before ice nucleation in the body. Supercooling point, when the insect freezes, of the kudzu bug was on average -12.6°C . The temperature isoline, based on the USDA plant hardiness zones, corresponding to the supercooling point matched well with the current northern range limit of the kudzu bug indicating that the range is indeed bound by cold tolerance. The lower lethal temperature to kill 50% of the kudzu bug population was on average -5.1°C . Though this temperature occurs in Maryland, the leaf litter in which the kudzu bug overwinters provides thermal buffering that reduces the number of hours below this threshold. The kudzu bug moves to the leaf litter in late fall, placing them at risk of an early frost. Ultimately, under climate change, kudzu bug populations may begin to shift north to new soybean farmland. Knowing the cold tolerance will allow producers within the current range to predict how large spring population sizes are. With a greater spring population of overwintered adults, the potential damage to soybeans increases. Once adults emerge, producers can utilize the DD model to time management options and evaluate the potential for population growth given the fecundity and initial population size.

In effect, combining the work of cold tolerance and phenology allows for a comprehensive temperature based model to determine winter survival, range expansion, and phenology of the kudzu bug. Producers in the mid-Atlantic can use predictions to assess the threat of economic loss due to the kudzu bug in its current and future range as well as to determine timing of pest management options.

Literature Cited

- Addo-Bediako, A., S. L. Chown, and K. J. Gaston. 2000.** Thermal tolerance, climatic variability and latitude. *Proc. R. Soc. Lond. B Biol. Sci.* 267: 739–745.
- Altermatt, F. 2010.** Climatic warming increases voltinism in European butterflies and moths. *Proc. R. Soc. Lond. B Biol. Sci.* 277: 1281–1287.
- Andersen, J. L., T. Manenti, J. G. Sørensen, H. A. MacMillan, V. Loeschcke, and J. Overgaard. 2015.** How to assess *Drosophila* cold tolerance: chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits. *Funct. Ecol.* 29: 55–65.
- Archer, M. S. 2004.** The Effect of Time After Body Discovery on the Accuracy of Retrospective Weather Station Ambient Temperature Corrections in Forensic Entomology. *J. Forensic Sci.* 49: 1–7.
- Baker, R. H. A., C. E. Sansford, C. H. Jarvis, R. J. C. Cannon, A. MacLeod, and K. F. A. Walters. 2000.** The role of climatic mapping in predicting the potential geographical distribution of non-indigenous pests under current and future climates. *Agric. Ecosyst. Environ.* 82: 57–71.
- Bale, J. S. 1993.** Classes of Insect Cold Hardiness. *Funct. Ecol.* 7: 751–753.
- Bale, J. S., and S. A. L. Hayward. 2010.** Insect overwintering in a changing climate. *J. Exp. Biol.* 213: 980–994.
- Bale, J. S., G. J. Masters, I. D. Hodkinson, C. Awmack, T. M. Bezemer, V. K. Brown, J. Butterfield, A. Buse, J. C. Coulson, J. Farrar, J. E. G. Good, R. Harrington, S. Hartley, T. H. Jones, R. L. Lindroth, M. C. Press, I. Symrnioudis, A. D. Watt, and J. B. Whittaker. 2002.** Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Glob. Change Biol.* 8: 1–16.
- Bemani, M., H. Izadi, K. Mahdian, A. Khani, and M. Amin samih. 2012.** Study on the physiology of diapause, cold hardiness and supercooling point of overwintering pupae of the pistachio fruit hull borer, *Arimania comaroffi*. *J. Insect Physiol.* 58: 897–902.
- Blount, J. L., G. D. Buntin, and A. N. Sparks. 2015.** Host Preference of *Megacopta cribraria* (Hemiptera: Plataspidae) on Selected Edible Beans and Soybean. *J. Econ. Entomol.* 108: 1094–1105.
- Boggs, C. L. 2016.** The fingerprints of global climate change on insect populations. *Curr. Opin. Insect Sci.* 17: 69–73.
- Byrne, M. J., J. Coetzee, A. J. McConnachie, W. Parasram, and M. P. Hill. 2004.** Predicting climate compatibility of biological control agents in their region of introduction. *In Proc. XI Int. Symp. Biol. Control Weeds.* CSIRO Entomology.
- Cannon, R. J. C. 1998.** The implications of predicted climate change for insect pests in the UK, with emphasis on non-indigenous species. *Glob. Change Biol.* 4: 785–796.
- Cesaraccio, C., D. Spano, P. Duce, and R. L. Snyder. 2001.** An improved model for determining degree-day values from daily temperature data. *Int. J. Biometeorol.* 45: 161–169.

- Chen, B., and L. Kang. 2004.** Variation in Cold Hardiness of *Liriomyza huidobrensis* (Diptera: Agromyzidae) Along Latitudinal Gradients. *Environ. Entomol.* 33: 155–164.
- Chen, S., S. J. Fleischer, M. C. Saunders, and M. B. Thomas. 2015.** The Influence of Diurnal Temperature Variation on Degree-Day Accumulation and Insect Life History. *PLOS ONE.* 10: e0120772.
- Cira, T. M., R. C. Venette, J. Aigner, T. Kuhar, D. E. Mullins, S. E. Gabbert, and W. D. Hutchison. 2016.** Cold Tolerance of *Halyomorpha halys* (Hemiptera: Pentatomidae) Across Geographic and Temporal Scales. *Environ. Entomol.* 45: 484–491.
- Clark, M. S., and M. R. Worland. 2008.** How insects survive the cold: molecular mechanisms—a review. *J. Comp. Physiol. B.* 178: 917–933.
- Colautti, R. I., C. G. Eckert, and S. C. H. Barrett. 2010.** Evolutionary constraints on adaptive evolution during range expansion in an invasive plant. *Proc. R. Soc. Lond. B Biol. Sci.* 277: 1799–1806.
- Coleman, P. C., J. S. Bale, and S. A. L. Hayward. 2014.** Cross-generation plasticity in cold hardiness is associated with diapause, but not the non-diapause developmental pathway, in the blow fly *Calliphora vicina*. *J. Exp. Biol.* 217: 1454–1461.
- Couret, J., E. Dotson, and M. Q. Benedict. 2014.** Temperature, Larval Diet, and Density Effects on Development Rate and Survival of *Aedes aegypti* (Diptera: Culicidae). *PLOS ONE.* 9: e87468.
- (Crop Production 2015 Summary) . 2016.** Crop Production 2015 Summary.
- Crozier, L. 2003.** Winter warming facilitates range expansion: cold tolerance of the butterfly *Atalopedes campestris*. *Oecologia.* 135: 648–656.
- Damos, P., and M. Savopoulou-Soultani. 2011.** Temperature-Driven Models for Insect Development and Vital Thermal Requirements. *Psyche J. Entomol.* 2012: e123405.
- Danks, H. V. 2005.** Key themes in the study of seasonal adaptations in insects I. Patterns of cold hardiness. *Appl. Entomol. Zool.* 40: 199–211.
- David, J.-F., and G. Vannier. 1996.** Changes in supercooling with body size, sex, and season in the long-lived milliped *Polyzoniium germanicum* (Diplopoda, Polyzoniidae). *J. Zool.* 240: 599–608.
- Del Pozo-Valdivia, A. I., and D. D. Reisig. 2013.** First-Generation *Megacopta cribraria* (Hemiptera: Plataspidae) Can Develop on Soybeans. *J. Econ. Entomol.* 106: 533–535.
- Del Pozo-Valdivia, A. I., N. J. Seiter, D. D. Reisig, J. K. Greene, F. P. F. Reay-Jones, and J. S. Bachelier. 2016.** *Megacopta cribraria* (Hemiptera: Plataspidae) Population Dynamics in Soybeans as Influenced by Planting Date, Maturity Group, and Insecticide Use. *J. Econ. Entomol.* 109: 1141–1155.
- Denlinger, D. L., and R. E. Lee. 2010.** *Low Temperature Biology of Insects.* Cambridge University Press.
- DiMeglio, A. S., A. K. Wallingford, D. C. Weber, T. P. Kuhar, and D. Mullins. 2016.** Supercooling Points of *Murgantia histrionica* (Hemiptera: Pentatomidae) and Field Mortality in the Mid-Atlantic United States Following Lethal Low Temperatures. *Environ. Entomol.* nww091.

- (EDDMapS) Early Detection & Distribution Mapping System. 2016.** Kudzu Bug *Megacopta cribraria* (Fabricius). University of Georgia: Center for Invasive Species and Ecosystem Health, Tifton, GA.
- Eger Jr, J. E., L. M. Ames, D. R. Suiter, T. M. Jenkins, D. A. Rider, and S. E. Halbert. 2010.** Occurrence of the Old World bug *Megacopta cribraria* (Fabricius) (Heteroptera: Plataspidae) in Georgia: a serious home invader and potential legume pest. *Insecta Mundi*. 121: 1–11.
- Ellwood, E. R., J. M. Diez, I. Ibáñez, R. B. Primack, H. Kobori, H. Higuchi, and J. A. Silander. 2011.** Disentangling the paradox of insect phenology: are temporal trends reflecting the response to warming? *Oecologia*. 168: 1161–1171.
- Ferro, D. N., R. B. Chapman, and D. R. Penman. 1979.** Observations on Insect Microclimate and Insect Pest Management. *Environ. Entomol.* 8: 1000–1003.
- Forrest, J. R. 2016.** Complex responses of insect phenology to climate change. *Curr. Opin. Insect Sci.* 17: 49–54.
- Fritz, B. J., D. D. Reisig, C. E. Sorenson, A. I. Del Pozo-Valdivia, and T. E. Carter. 2016.** Host Plant Resistance to *Megacopta cribraria* (Hemiptera: Plataspidae) in Diverse Soybean Germplasm Maturity Groups V Through VIII. *J. Econ. Entomol.* 109: 1438–1449.
- Fukatsu, T., and T. Hosokawa. 2002.** Capsule-Transmitted Gut Symbiotic Bacterium of the Japanese Common Plataspid Stinkbug, *Megacopta punctatissima*. *Appl. Environ. Microbiol.* 68: 389–396.
- Gardner, W. A., J. L. Blount, J. R. Golec, W. A. Jones, X. P. Hu, E. J. Talamas, R. M. Evans, X. Dong, C. H. Ray, G. D. Buntin, N. M. Gerardo, and J. Couret. 2013.** Discovery of *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygasteridae), an Egg Parasitoid of *Megacopta cribraria* F. (Hemiptera: Plataspidae) in its Expanded North American Range. *J. Entomol. Sci.* 48: 355–359.
- Golec, J. R., and X. P. Hu. 2015.** Preoverwintering Copulation and Female Ratio Bias: Life History Characteristics Contributing to the Invasiveness and Rapid Spread of *Megacopta cribraria* (Heteroptera: Plataspidae). *Environ. Entomol.* 44: 411–417.
- Golec, J. R., X. P. Hu, C. Ray, and N. E. Woodley. 2013.** *Strongygaster triangulifera* (Diptera: Tachinidae) as a parasitoid of adults of the invasive *Megacopta cribraria* (Heteroptera: Plataspidae) in Alabama. *J. Entomol. Sci.* 48: 352–354.
- Goto, M., Y. Sekine, H. Outa, M. Hujikura, and K. Suzuki. 2001.** Relationships between cold hardiness and diapause, and between glycerol and free amino acid contents in overwintering larvae of the oriental corn borer, *Ostrinia furnacalis*. *J. Insect Physiol.* 47: 157–165.
- Grant, J., A. Leslie, and W. Lamp. 2014.** Kudzu Bug, a Potential Soybean Pest, Survives the Harsh Winter. *Agron. News Univ. Md. Ext.* 5: 2–3.
- Greenstone, M. H., P. G. Tillman, and J. S. Hu. 2014.** Predation of the Newly Invasive Pest *Megacopta cribraria* (Hemiptera: Plataspidae) in Soybean Habitats Adjacent to Cotton by a Complex of Predators. *J. Econ. Entomol.* 107: 947–954.
- Gunderson, A. R., and J. H. Stillman. 2015.** Plasticity in thermal tolerance has limited potential to buffer ectotherms from global warming. *Proc R Soc B.* 282: 20150401.

- Hahn, D. A., A. R. Martin, and S. D. Porter. 2008.** Body Size, but Not Cooling Rate, Affects Supercooling Points in the Red Imported Fire Ant, *Solenopsis invicta*. *Environ. Entomol.* 37: 1074–1080.
- Hallman, G. J., and D. L. Denlinger. 1998.** Temperature Sensitivity in Insects and Application in Integrated Pest Management. Westview Press.
- Hartley, S., P. D. Krushelnycky, and P. J. Lester. 2010.** Integrating physiology, population dynamics and climate to make multi-scale predictions for the spread of an invasive insect: the Argentine ant at Haleakala National Park, Hawaii. *Ecography.* 33: 83–94.
- Haynes, K. J., A. J. Allstadt, and D. Klimetzek. 2014.** Forest defoliator outbreaks under climate change: effects on the frequency and severity of outbreaks of five pine insect pests. *Glob. Change Biol.* 20: 2004–2018.
- Higley, L. G., and N. H. Haskell. 2001.** Insect Development and Forensic Entomology, pp. 287–295. *In Forensic Entomol. Util. Arthropods Leg. Investig.* CRC Press.
- Higley, L. G., L. P. Pedigo, and K. R. Ostlie. 1986.** DEGDAY: a program for calculating degree-days, and assumptions behind the degree-day approach. *Environ. Entomol.* 15: 999–1016.
- Hodgson, J. A., C. D. Thomas, T. H. Oliver, B. J. Anderson, T. M. Brereton, and E. E. Crone. 2011.** Predicting insect phenology across space and time. *Glob. Change Biol.* 17: 1289–1300.
- Hodkova, M., and I. Hodek. 2004.** Photoperiod, diapause and cold-hardiness. *Eur. J. Entomol.* 445–458.
- Holmes, L. A., S. L. Vanlaerhoven, and J. K. Tomberlin. 2012.** Relative Humidity Effects on the Life History of *Hermetia illucens* (Diptera: Stratiomyidae). *Environ. Entomol.* 41: 971–978.
- Hosokawa, T., Y. Kikuchi, M. Shimada, and T. Fukatsu. 2007.** Obligate symbiont involved in pest status of host insect. *Proc. R. Soc. B Biol. Sci.* 274: 1979–1984.
- Hosokawa, T., N. Nikoh, and T. Fukatsu. 2014.** Fine-Scale Geographical Origin of an Insect Pest Invading North America. *PLOS ONE.* 9: e89107.
- Inkley, D. B. 2012.** Characteristics of Home Invasion by the Brown Marmorated Stink Bug (Hemiptera: Pentatomidae). *J. Entomol. Sci.* 47: 125–130.
- (IPCC 5th Report) Intergovernmental Panel on Climate Change. 2013.** Climate Change 2013 The Physical Science Basis: Working Group I Contribution to the Fifth Assessment Report. Cambridge University Press.
- Izzo, V. M., D. J. Hawthorne, and Y. H. Chen. 2013.** Geographic variation in winter hardiness of a common agricultural pest, *Leptinotarsa decemlineata*, the Colorado potato beetle. *Evol. Ecol.* 28: 505–520.
- Jenkins, T. M., T. D. Eaton, D. R. Suiter, J. E. Eger Jr, L. M. Ames, and G. D. Buntin. 2010.** Preliminary genetic analysis of a recently-discovered invasive true bug (Hemiptera: Heteroptera: Plataspidae) and its bacterial endosymbiont in Georgia, USA. *J. Entomol. Sci.* 45: 62–63.
- Jenkins, T. M. J., and T. D. Eaton. 2011.** Population Genetic Baseline of the First Plataspid Stink Bug Symbiosis (Hemiptera: Heteroptera: Plataspidae) Reported in North America. *Insects.* 2: 264–272.

- Kelty, J. D., and J. Lee Richard E. 1999.** Induction of rapid cold hardening by cooling at ecologically relevant rates in *Drosophila melanogaster*. *J. Insect Physiol.* 45: 719–726.
- Khani, A., and S. Moharramipour. 2010.** Cold hardiness and supercooling capacity in the overwintering larvae of the codling moth, *Cydia pomonella*. *J. Insect Sci.* 10: 83.
- Kimura, M. T. 2004.** Cold and heat tolerance of drosophilid flies with reference to their latitudinal distributions. *Oecologia.* 140: 442–449.
- Knight, A. L., and D. M. Light. 2005.** Timing of egg hatch by early-season codling moth (Lepidoptera: Tortricidae) predicted by moth catch in pear ester- and codlemone-baited traps. *Can. Entomol.* 137: 728–738.
- Košt'ál, V., M. Šlachta, and P. Šimek. 2001.** Cryoprotective role of polyols independent of the increase in supercooling capacity in diapausing adults of *Pyrrhocoris apterus* (Heteroptera: Insecta). *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 130: 365–374.
- Labrie, G., D. Coderre, and É. Lucas. 2008.** Overwintering Strategy of Multicolored Asian Lady Beetle (Coleoptera: Coccinellidae): Cold-Free Space as a Factor of Invasive Success. *Ann. Entomol. Soc. Am.* 101: 860–866.
- Ladany, M., and L. Horvath. 2010.** A Review of the Potential Climate Change Impact on Insect Populations- General and Agricultural Aspects. *Appl. Ecol. Environmental Res.* 8: 143–152.
- Lahiri, S., D. Orr, C. Sorenson, and Y. Cardoza. 2015.** Overwintering Refuge Sites for *Megacopta cribraria* (Hemiptera: Plataspidae). *J. Entomol. Sci.* 50: 69–73.
- Lee, R. E., M. R. Lee, and J. M. Strong-Gunderson. 1993.** Insect cold-hardiness and ice nucleating active microorganisms including their potential use for biological control. *J. Insect Physiol.* 39: 1–12.
- Lee, R. E., J. M. Strong-Gunderson, M. R. Lee, and E. C. Davidson. 1992.** Ice-Nucleating Active Bacteria Decrease the Cold-Hardiness of Stored Grain Insects. *J. Econ. Entomol.* 85: 371–374.
- Lehmann, P., S. Kaunisto, V. Košťál, A. Margus, H. Zahradníčková, and L. Lindström. 2015.** Comparative Ecophysiology of Cold-Tolerance-Related Traits: Assessing Range Expansion Potential for an Invasive Insect at High Latitude. *Physiol. Biochem. Zool.* 88: 254–265.
- Leslie, A. W., C. Sargent, W. E. Steiner, W. O. Lamp, J. M. Swearingen, B. B. Pagac, G. L. Williams, D. C. Weber, and M. J. Raupp. 2014.** A New Invasive Species in Maryland: the Biology and Distribution of the Kudzu Bug, *Megacopta cribraria* (Fabricius) (Hemiptera: Plataspidae). *Md. Entomol.* 6.
- Lovejoy, R. T., and D. A. Johnson. 2014.** A Molecular Analysis of Herbivory in Adults of the Invasive Bean Plataspid, *Megacopta cribraria*. *Southeast. Nat.* 13: 663–672.
- Luedeling, E., K. P. Steinmann, M. Zhang, P. H. Brown, J. Grant, and E. H. Girvetz. 2011.** Climate change effects on walnut pests in California. *Glob. Change Biol.* 17: 228–238.
- Luna, E. K., R. A. Sitz, W. S. Cranshaw, and N. A. Tisserat. 2013.** The Effect of Temperature on Survival of *Pityophthorus juglandis* (Coleoptera: Curculionidae). *Environ. Entomol.* 42: 1085–1091.

- Marini, L., M. P. Ayres, A. Battisti, and M. Faccoli. 2012.** Climate affects severity and altitudinal distribution of outbreaks in an eruptive bark beetle. *Clim. Change.* 115: 327–341.
- Marshall, K. E., and B. J. Sinclair. 2012.** The impacts of repeated cold exposure on insects. *J. Exp. Biol.* 215: 1607–1613.
- Marshall, K. E., and B. J. Sinclair. 2015.** The relative importance of number, duration and intensity of cold stress events in determining survival and energetics of an overwintering insect. *Funct. Ecol.* 29: 357–366.
- McMaster, G. S., and W. W. Wilhelm. 1997.** Growing degree-days: one equation, two interpretations. *Agric. For. Meteorol.* 87: 291–300.
- Medal, J., S. Halbert, A. S. Cruz, T. Smith, and B. J. Davis. 2016.** Greenhouse Study to Determine the Host Range of the Kudzu Bug, *Megacopta cribraria* (Heteroptera: Plataspidae). *Fla. Entomol.* 99: 303–305.
- Medal, J., S. Halbert, T. Smith, and A. S. Cruz. 2013.** Suitability of Selected Plants to the Bean Plataspid, *Megacopta cribraria* (Hemiptera: Plataspidae) in No-Choice Tests. *Fla. Entomol.* 96: 631–633.
- M J Tauber, and C. A. Tauber. 1976.** Insect Seasonality: Diapause Maintenance, Termination, and Postdiapause Development. *Annu. Rev. Entomol.* 21: 81–107.
- Moerkens, R., B. Gobin, G. Peusens, H. Helsen, R. Hilton, H. Dib, D. M. Suckling, and H. Leirs. 2011.** Optimizing biocontrol using phenological day degree models: the European earwig in pipfruit orchards. *Agric. For. Entomol.* 13: 301–312.
- Moran, E. V., and J. M. Alexander. 2014.** Evolutionary responses to global change: lessons from invasive species. *Ecol. Lett.* 17: 637–649.
- Morey, A. C., W. D. Hutchison, R. C. Venette, and E. C. Burkness. 2012.** Cold Hardiness of *Helicoverpa zea* (Lepidoptera: Noctuidae) Pupae. *Environ. Entomol.* 41: 172–179.
- Musolin, D. L. 2007.** Insects in a warmer world: ecological, physiological and life-history responses of true bugs (Heteroptera) to climate change. *Glob. Change Biol.* 13: 1565–1585.
- Niva, C. C., and M. Takeda. 2003.** Effects of Photoperiod, Temperature and Melatonin on Nymphal Development, Polyphenism and Reproduction in *Halyomorpha halys* (Heteroptera: Pentatomidae). *Zoolog. Sci.* 20: 963–970.
- (NOAA) National Oceanic and Atmospheric Administration. 2016.** National Centers for Environmental information, Climate at a Glance: U.S. Time Series, Average Temperature. NOAA, Silver Spring, MD.
- Porter, J. H., M. L. Parry, and T. R. Carter. 1991.** The potential effects of climatic change on agricultural insect pests. *Agric. For. Meteorol.* 57: 221–240.
- Portilla, M., W. Jones, O. Perera, N. Seiter, J. Greene, and R. Luttrell. 2016.** Estimation of Median Lethal Concentration of Three Isolates of *Beauveria bassiana* for Control of *Megacopta cribraria* (Heteroptera: Plataspidae) Bioassayed on Solid Lygus spp. Diet. *Insects.* 7: 31.
- Pöyry, J., R. Leinonen, G. Söderman, M. Nieminen, R. K. Heikkinen, and T. R. Carter. 2011.** Climate-induced increase of moth multivoltinism in boreal regions. *Glob. Ecol. Biogeogr.* 20: 289–298.

- Pruess, K. P. 1983.** Day-Degree Methods for Pest Management. *Environ. Entomol.* 12: 613–619.
- Renault, D., C. Salin, G. Vannier, and P. Vernon. 2002.** Survival at low temperatures in insects: what is the ecological significance of the supercooling point? *Cryoletters.* 23: 217–228.
- Roltch, W. J., F. G. Zalom, A. J. Strawn, J. F. Strand, and M. J. Pitcairn. 1999.** Evaluation of several degree-day estimation methods in California climates. *Int. J. Biometeorol.* 42: 169–176.
- Rosenberg, N. J., B. L. Blad, and S. B. Verma. 1983.** *Microclimate: The Biological Environment.* John Wiley & Sons.
- Ruberson, J. R., K. Takasu, G. David Buntin, J. E. Eger, W. A. Gardner, J. K. Greene, T. M. Jenkins, W. A. Jones, D. M. Olson, P. M. Roberts, D. R. Suiter, and M. D. Toews. 2013.** From Asian curiosity to eruptive American pest: *Megacopta cribraria* (Hemiptera: Plataspidae) and prospects for its biological control. *Appl. Entomol. Zool.* 48: 3–13.
- Salin, C., D. Renault, G. Vannier, and P. Vernon. 2000.** A sexually dimorphic response in supercooling temperature, enhanced by starvation, in the lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). *J. Therm. Biol.* 25: 411–418.
- Salt, R. W. 1961.** Principles of insect cold-hardiness. *Annu. Rev. Entomol.* 6: 55–74.
- Seiter, N. J., A. I. Del Pozo-Valdivia, J. K. Greene, F. P. F. Reay-Jones, P. M. Roberts, and D. D. Reisig. 2016.** Management of *Megacopta cribraria* (Hemiptera: Plataspidae) at Different Stages of Soybean (Fabales: Fabaceae) Development. *J. Econ. Entomol.* 109: 1167–1176.
- Seiter, N. J., A. I. Del Pozo-Valdivia, J. K. Greene, F. P. F. Reay-Jones, P. M. Roberts, and D. R. Reisig. 2015.** Action Thresholds for Managing *Megacopta cribraria* (Hemiptera: Plataspidae) in Soybean Based on Sweep-Net Sampling. *J. Econ. Entomol.* 108: 1818–1829.
- Seiter, N. J., A. Grabke, J. K. Greene, J. L. Kerrigan, and F. P. F. Reay-Jones. 2014.** *Beauveria bassiana* is a Pathogen of *Megacopta cribraria* (Hemiptera: Plataspidae) in South Carolina. *J. Entomol. Sci.* 49: 326–330.
- Seiter, N. J., J. K. Greene, and F. P. F. Reay-Jones. 2013.** Reduction of Soybean Yield Components by *Megacopta cribraria* (Hemiptera: Plataspidae). *J. Econ. Entomol.* 106: 1676–1683.
- Seiter, N. J., F. P. F. Reay-Jones, and J. K. Greene. 2013.** Within-Field Spatial Distribution of *Megacopta cribraria* (Hemiptera: Plataspidae) in Soybean (Fabales: Fabaceae). *Environ. Entomol.* 42: 1363–1374.
- Shi, S.-S., J. Cui, and L.-S. Zang. 2014.** Development, Survival, and Reproduction of *Megacopta cribraria* (Hemiptera: Plataspidae) at Different Constant Temperatures. *J. Econ. Entomol.* 107: 2061–2066.
- Sinclair, B. J. 1999.** Insect cold tolerance: How many kinds of frozen? *Eur. J. Entomol.* 96: 157–164.
- Sinclair, B. J., L. E. Coello Alvarado, and L. V. Ferguson. 2015.** An invitation to measure insect cold tolerance: Methods, approaches, and workflow. *J. Therm. Biol.* 53: 180–197.

- Sinclair, B. J., P. Vernon, C. Jaco Klok, and S. L. Chown. 2003.** Insects at low temperatures: an ecological perspective. *Trends Ecol. Evol.* 18: 257–262.
- Slachta, M., P. Berkova, J. Vambera, and V. Kostal. 2002.** Physiology of cold-acclimation in non-diapausing adults of *Pyrrhocoris apterus* (Heteroptera). *Eur. J. Entomol.* 181–187.
- Soufbaf, M., Y. Fathipour, J. Karimzadeh, and M. P. Zalucki. 2010.** Bottom-Up Effect of Different Host Plants on *Plutella xylostella* (Lepidoptera: Plutellidae): A Life-Table Study on Canola. *J. Econ. Entomol.* 103: 2019–2027.
- Srinivasaperumal, S., P. Samuthiravelu, and J. Muthukrishnan. 1992.** Host Plant Preference and Life Table of *Megacopta cribraria* (Fab.) (Hemiptera: Plataspidae). *Proc. Indian Nat. Sci. Acad.* B58: 333–340.
- Suiter, D. R., J. E. Eger, W. A. Gardner, R. C. Kemerait, J. N. All, P. M. Roberts, J. K. Greene, L. M. Ames, G. D. Buntin, T. M. Jenkins, and G. K. Douce. 2010.** Discovery and Distribution of *Megacopta cribraria* (Hemiptera: Heteroptera: Plataspidae) in Northeast Georgia. *J. Integr. Pest Manag.* 1: 1–4.
- Sunday, J. M., A. E. Bates, and N. K. Dulvy. 2012.** Thermal tolerance and the global redistribution of animals. *Nat. Clim. Change.* 2: 686–690.
- Teets, N. M., and D. L. Denlinger. 2013.** Physiological mechanisms of seasonal and rapid cold-hardening in insects. *Physiol. Entomol.* 38: 105–116.
- Thippeswamy, C., and B. Rajagopal. 2005.** Life history of lablab bug, *Coptosoma cribraria* Fabricius (Heteroptera: Plataspidae) on field bean, *Lablab purpureus* var. *lignosus* Medikus. *Karnataka J. Agric. Sci.* 39–43.
- Tobin, P. C., S. Nagarkatti, G. Loeb, and M. C. Saunders. 2008.** Historical and projected interactions between climate change and insect voltinism in a multivoltine species. *Glob. Change Biol.* 14: 951–957.
- Tu, X., Z. Li, J. Wang, X. Huang, J. Yang, C. Fan, H. Wu, Q. Wang, and Z. Zhang. 2014.** Improving the Degree-Day Model for Forecasting *Locusta migratoria manilensis* (Meyen) (Orthoptera: Acridoidea). *PLOS ONE.* 9: e89523.
- Ungerer, M. J., M. P. Ayres, and M. J. Lombardero. 1999.** Climate and the northern distribution limits of *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae). *J. Biogeogr.* 26: 1133–1145.
- Van Dyck, H., D. Bonte, R. Puls, K. Gotthard, and D. Maes. 2015.** The lost generation hypothesis: could climate change drive ectotherms into a developmental trap? *Oikos.* 124: 54–61.
- Waagner, D., M. Holmstrup, M. Bayley, and J. G. Sorensen. 2013.** Induced cold-tolerance mechanisms depend on duration of acclimation in the chill-sensitive *Folsomia candida* (Collembola). *J. Exp. Biol.* 216: 1991–2000.
- Williams, C. M., K. E. Marshall, H. A. MacMillan, J. D. K. Dzurisin, J. J. Hellmann, and B. J. Sinclair. 2012.** Thermal Variability Increases the Impact of Autumnal Warming and Drives Metabolic Depression in an Overwintering Butterfly. *PLOS ONE.* 7: e34470.
- Zhang, Y., J. L. Hanula, and S. Horn. 2012.** The Biology and Preliminary Host Range of *Megacopta cribraria* (Heteroptera: Plataspidae) and Its Impact on Kudzu Growth. *Environ. Entomol.* 41: 40–50.