

**Abundance and Diversity of Urban Ant Species (Hymenoptera: Formicidae) and Water Relations of the Kudzu Bug (Hemiptera: Plataspidae)**

by

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## Abstract

Insect pests are heterogeneous group of organisms characterized by the fact that they damage or otherwise adversely affect people or their property. These insects cause pain, annoyance, disfigurement, emotional distress, disability, and damage because of bites, sting, feeding on humans. In fact, urban areas are extremely well suited for groups of insects that have associated with their lives with human and their activities. Ants (Hymenoptera: Formicidae) are among the most dominant group of insects and constitute significant biomass in a variety of ecosystems. Their economic effects on human health, property, and biodiversity make them a subject of interest. Temporal variation in temperature, rainfall and water availability are among the important factors governing the abundance and diversity of species within a locality. In the same vein, the kudzu bug, *Megacopta cribraria*, is also a nuisance urban pest species as well as an agricultural pest. Desiccation is a crucial stressor and plays an important role in insect survival, growth, development, behavior, and ultimately distribution. The management of these species is partially dependent on understanding these aspects of their physiology.

Thus, the primary objectives of the research with urban ants were to: (1) investigate the seasonal abundance and diversity of ant species around homes in Lee County, Auburn, Alabama; (2) determine the influence of seasonal conditions on the abundance and diversity of ant species. Between June 2018 and June 2019, field study was conducted to understand the population dynamics of ant species in Auburn, Alabama. The field study resulted in a total of 16 ant species belonging to 13 genera in four subfamilies being identified. Across seasons, Argentine ant,

*Linepithema humile*, big headed ant, *Pheidole dentigula*, and red important fire ant, *Solenopsis invicta* were most abundant. The greatest species diversity was in early September and lowest was in early March. The abundance and diversity of nuisance ant species is seasonal and species interaction dependent.

In laboratory experiment with *M. cribraria*, the primary research goals were to: (3) determine the total body water content (%TBW content), rate of mass, total body water loss (%TBW loss), and cuticular permeability (CP) of adult and nymphal *M. cribraria*, and to determine the desiccation sensitivity of all stages of kudzu bug. First instar nymphs from generation one had significantly greater %TBW than the other instars and across generations whereas generation two fifth instars had the lowest %TBW. The CP value of adult female generation one was the greatest across generations. In addition, when the mass losses of nymphs were compared, generation one first instars had the greatest mass loss compared with other instars across generations whereas overwintered generation two adult females had the lowest mass loss across generations. This study will aid in understanding how environmental stress may affect the survival and relative abundance of *M. cribraria* in fields and around homes.

Collectively, these studies give insight into physiology of the insects that can be leveraged to for control.

**Key words:** integrated pest management, physiology, urban pest, soybean pests, invasive pest.

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## Table of Contents

Abstract .....	ii
Acknowledgments.....	iv
List of Tables .....	vii
List of Figures.....	viii
Chapter 1: Introduction.....	1
Introduction.....	1
References.....	10
Chapter 2: Temporal Variation in Abundance and Diversity of Ant Species (Hymenoptera: Formicidae) Around Homes in Southeast of Alabama.....	18
Introduction.....	18
Material and method .....	21
Results.....	23
Discussion.....	28
References.....	34
Chapter 3: Water loss and Desiccation Tolerance of Various Stages of yearly generations of the Kudzu bugs, <i>Megacopta cribraria</i> (Hemiptera: Plataspidae).....	53
Introduction.....	53
Material and method .....	55
Results.....	58
Discussion.....	61

References..... 67

## List of Tables

### Chapter 2:

Table 1: Summary of ant species collected from around houses.....	46
---	----

### Chapter 3:

Table 1: Mean ( $\pm$ SE) initial fresh body mass, % total body water (%TBW), cuticular permeability (CP) ( $\mu\text{g cm}^{-2} \text{h}^{-1} \text{mmHg}^{-1}$ ), and mass loss (mg/g) of five nymphal instars and adults of <i>M. cribraria</i> .....	82
Table 2: Regression statistic of % mass loss of five nymphal instars and adults of <i>M. cribraria</i> at 30°C and 0-2% RH (means $\pm$ SE).....	83
Table 3: Regression statistics of %TBW loss of five nymphal instars and adults of <i>M. cribraria</i> at 30°C and 0-2% RH (means $\pm$ SE).....	84
Table 4: Mean ( $\pm$ SE) mortality of five nymphal instars and adults of <i>M. cribraria</i> after 10 and 24 h exposure to 30°C and 0-2% RH.....	85

## List of Figures

### Chapter 2:

Figures 1: Seasonal diversity of ant species during the 2018 - 2019 study period.....	47
Figures 2: Mean catch of <i>L. humile</i> , <i>S. invicta</i> , <i>P. dentigula</i> , and <i>S. molesta</i> at 30 homes 2018 – 2019.....	48
Figures 3: Mean catch of <i>B. patagonicus</i> , <i>M. minimum</i> , <i>C. pilosa</i> , and <i>T. pergandei</i> at 30 homes 2018 – 2019.....	49
Figures 4: Mean catch of <i>T. sessile</i> , <i>N. bourbonica</i> , <i>P. imparis</i> , and <i>C. pennsylvanicus</i> at 30 homes 2018 – 2019.....	50
Figures 5: Mean catch of <i>C. castaneus</i> , <i>D. bureni</i> , <i>Nylanderia</i> sp. and <i>P. pennsylvanica</i> at 30 homes 2018 – 2019.....	51
Figures 6: Temperature and precipitation in Lee County, Alabama during the 2018 –2019 sampling period.....	52

### Chapter 3:

Figures 1a: %Total body water lost over time for <i>M. cribraria</i> generation one nymphal instars.....	76
Figures 1b: %Total body water lost over time for <i>M. cribraria</i> generation two nymphal instars.....	77
Figures 1c: %Total body water lost over time for generation one and two <i>M. cribraria</i> adults.....	78



Figures 2a: %Mass loss over time for generation one *M. cribraria* nymphal instars ..... 79

Figures 2b: %Mass loss over time for generation two *M. cribraria* nymphal instars..... 80

Figures 2c: %Mass loss over time for generations one and two *M. cribraria* adults..... 81

## Chapter 1

### Insects

Insects are believed to have originated in the Late Silurian Period around 400 million years ago (Grimaldi and Engel 2005), making them one of the first terrestrial animals to appear on the planet. There are around 925,000 known species of insects, but estimates range from 2.5 to 10 million species with an average estimate of about 5 million total species (Grimaldi and Engel 2005). Approximately 80% of insect species comprises four major insect orders: Coleoptera, Diptera, Hymenoptera, and Lepidoptera (Grimaldi 2005). As insects were able to fly, some 90-170 million years before vertebrates (Grimaldi 2005), they began to reach their greatest diversity. The huge diversity (success) attributed to insects is due, in large part, to their ability to successfully exploit a large number of ecological niches. The implication is that insects have evolved to adapt to a wide range of conditions such as moisture, temperature, humidity, and atmospheric pressure. For a particular species, however, the range of moisture requirements is more restricted. Thus, species must conserve body water within permissible limits. Environmental water availability notwithstanding, insects obtain most, if not all, water from food, making feeding habit essential to equilibrate amount of water consumed. For example, sucking bugs are equipped with filter chambers to eliminate excess consumed water. Conversely, the bean weevil can survive on dry grains. Among terrestrial insects, there are a number of other adaptations help them overcome unfavorable conditions of aridity or excessive moisture, and such adaptations may be morphological, biological, and physiological. The need to find food and balance body water moderates where insects can be distributed. Ecologically speaking, this is the

concept of balance of life (Chapman 1971). Two sets of tendencies govern this balance: biotic potential tendencies to increase the population, and environmental resistance that tends to reduce the population. The interactions between these two forces must be maintained at a dynamic equilibrium. Disturbances in this balance precipitates the surge of insects as pests.

“Pest” is an interesting term that has been used to describe species of insects (and other animals/plants) that are home invaders, crop destroyers, and vectors of diseases. However, the actual percentage of insects that are considered pests is less than one percent (Hoffman and Frodsham 1993). Within this pest term are perimeter pests. Perimeter or peridomestic insect pests are insects that live predominantly and reproduce outdoors, but can move indoors to feed and drink. Adverse conditions such as extreme and unsuitable environmental conditions could drive indoor incursion (Eggleston and Arruda 2001). Primary perimeter pests in the southeastern United States include the American, *Periplaneta americana* (L.), Smokybrown, *Periplaneta fuliginosa* (Serville), and oriental, *Blatta orientalis* L., cockroaches; invasive Argentine ant, *Linepithema humile* (Mayr), red imported fire ant, *Solenopsis invicta* Buren, and little black ant, *Monomorium minimum* Buckley; and the Eastern subterranean, *Reticulitermes flavipes* (Kollar). Additional pests include, but not limited to, the Formosan, *Coptotermes formosanus* (Shiraki), red wasp, *Polistes carolina* (L.), yellow jackets, *Vespula maculifrons* (Buysson), fleas, *Ctenocephalides felis* (Bouché), bed bugs, *Cimex lecturalis* (Linnaeus), Asian tiger mosquito, *Aedes albopictus* (Skuse), and Kudzu bugs, *Megacopta cribraria*.

### **Peridomestic ants**

Of the common peridomestic insect groups, ants are economically important due to bites and stings on humans and animals, and their role as mechanical vectors of bacteria and allergens to sensitive people. The family Formicidae is one of the most invasive taxa worldwide. There are

dozens of invasive ant species worldwide (Suarez et al. 2009). Due to their high abundance and species diversity, regular occurrence throughout the year, and nest stability, ants are especially suitable for monitoring (Koch and Vohland 2008). More than 100 of the roughly 15,000 species of ants have developed populations beyond their native ranges (McGlynn 1999). These invasive species have negative impacts on natural and managed ecosystems, both direct and indirect (Holway et al. 2002, Lach and Hooper-Bui 2009). Invasive ants can be economically costly in both urban and agricultural areas. Their sudden incursion in homes qualifies them as annoying and nuisance pests, and can have economic impact on the public in the urban setting. For example, according to National Home and Garden Pesticide Use Survey, ants were ranked number one pest issue in homes, even exceeding cockroaches (Whitmore et al. 1993). This is not to say that all ant activities interfere with human activities. Some ant species can be beneficial, helping the decomposition of organic waste and nutrient recycling (Wagner and Jones 2004). Nevertheless, the notoriety of the ant species that occur in and around homes remain injurious to homeowners and residents. For example, the carpenter ant, *Camponotus pennsylvanicus* (De Geer), nests in wood or around homes producing considerable damage to beams and rafters (Klotz et al. 1998). Pharaoh ants, which are major pests in homes and hospitals, can act as disease vectors (Williams 1990). Some of the ant species such as odorous ants, *Tapinoma sessile* (Say), acrobat ants, *Crematogaster* spp., thief ants, *Solenopsis molesta* (Buren), and crazy ants, *Paratrechina longicornis* (Latreille), can become a household nuisance and troublesome when they enter human dwellings (MacGown and Forster 2005). For species that do not invade homes or cause damage to landscape, they may meticulously guard and watch over plant hoppers and aphids that cause damage to horticultural plants in return for the honeydew produced by these pests (Flatt and Weisser 2000, Schwartzberg et al. 2010). Fire ants, *Solenopsis* spp. and

harvester ants, *Pogonomyrmex* spp. may also occur in large numbers around homes. In particular, fire ants build mounds in sunny and open urban areas such as lawns, in yards, along paths and roads. In addition, the fire ants may nest indoor when the environmental conditions such as food shortages, hot and dry summer or heavy rainfall are not good for colony survival (Deshazo and Williams 1995). Fire ants are extremely aggressive, and, as its name indicates, can sting severely. The sting of the larger harvest ants is even more formidable, however. Similarly, Argentine ants, *L. humile*, nest in urban and suburban landscapes typically invading homes in search for sugar-diets and may prey on other insects (Suarez et al. 2001). In general, factors contributing to the proliferation of these peridomestic ant species include the availability of mulches, wood and stones (that provide harborage) and food sources emanating from trashcans and kitchens in/around homes. In addition, the biologies of these species such as polygony (multiple queens), polydomy (multiple colonies within a nest with little-to-no intraspecific aggression), monodomy (single colonies within a nest), and sociotomy (using reproductive budding) contribute to successful colonization of niches around homes (Vargo and Fletcher 1989, Passera 1994, Holway et al. 1998).

### **Peridomestic Kudzu bugs**

Another economically important urban pest is the invasive kudzu bugs, *Mecacopta cribraria* (Fabricius), that was first detected in the US in 2009 (Eger et al. 2010). After detection in 2009, it has spread throughout the southeastern United States including Alabama, Arkansas, Delaware, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, South Carolina, Tennessee, Virginia, and the District of Columbia (CISEH, 2018). Kudzu bug is a stinkbug that has piercing-sucking mouthparts and feeds on plant sap from stem, petioles, and nodes by extracting phloem sap that results in reduced plant growth and seed development.

Despite finding kudzu bugs on many different legumes and non-legume hosts, soybean and kudzu are the two primary reproductive hosts (Medal et al. 2013). They were also reported capable of reproducing on pigeon pea (*Cajanus cajan* L.), black eye pea (*Vigna sinensis* L.), lima bean (*Phaseolus lunatas* L.), and pinto bean (*Phaseolus vulgaris* L.). Kudzu bug is a pest of soybean and can cause soybean yield losses up to 60% if left uncontrolled (Seiter et al. 2013). Some people also consider adult kudzu bugs to have deleterious effects on urban settings. To overwinter, adult kudzu bugs migrate around buildings, particularly outside walls in the fall, and produce brown spots because of defecation and are general nuisance to residents (Suiter et al., 2010). *Megacopta cribraria* also invades homes and, when threatened, releases a defensive secretion that has a foul odor and can stain skin (Ruberson et al. 2013). Due to the abundance of kudzu plant in southeastern United States, high populations of *M. cribraria* can be seen around homes; kudzu plants have helped this insect spread extensively (Blaustein 2001). Kudzu and kudzu bug have created a serious pest problem involving a large number of insects flying around and landing in homes and on people. This species generally undergoes two generations per year in the U.S. (Zhang et al., 2012). It overwinters in the adult stage in reproductive dormancy (Golec and Hu, 2015) in protected locations, such as under loose tree bark, leaf litter or other debris (Lahiri et al., 2015). Following the activity of the overwintered adults in spring, the first generation is typically completed on kudzu plants or early soybeans (Del Pozo-Valdivia and Reisig 2013, Seiter et al. 2013), and second generation develops on soybeans (Zhang et al. 2012, Seiter et al. 2013). The two most important abiotic factors affecting the distribution and abundance of insects are water availability and temperature. Because of their small size and relatively large body surface area to volume ratios, kudzu bug, like most terrestrial arthropods, are prone to dehydration (Chown 2002, Edney 1977, Hadley 1994). The success of terrestrial

insects is largely due to their ability to conserve water, in addition to other physiological and behavioral adaptations (Thorat and Nath 2018). Insects show numerous physiological adaptations to counteract water loss, including a rigid, waterproof cuticle, frequently covered with a hydrophobic lipid layer, and a discontinuous ventilation pattern that eliminates the loss of respiratory water (Edney 1977, Hadley 1982, Kestler, 1984). Reducing water loss is accomplished by a relatively water impermeable cuticle covered with a thin layer of epicuticular lipids (particularly hydrocarbons) (Beament 1961, Hadley 1994, Chown and Nicolson, 2004). Water loss and desiccation stress play important roles in insect survival, growth, development, behavior, and distribution (Benoit et al., 2010).

### **Peridomestic insect management**

A number of pest control tactics have been employed to manage peridomestic insect pests. Baiting is a common practice for controlling ants around homes. Perimeter insecticide sprays are another imported method of pest control (Klotz et al. 2007). Despite a variety of chemicals and formulations that have been employed in the control of ants, no single chemical tactic has provided sustainable control of pest ants (Klotz et al. 2002, Rust et al. 2003). This has necessitated the continual use chemical formulations to control them. However, the secondary effects such as environmental contamination and effects on non-target organisms, associated with chemical use has made chemical approach to control unsustainable (EPA 2004). Thus, there is renewed interest in the development of tactics that can be used together to suppress peridomestic pests without incurring environmental and health costs.

Use of multiple tactics including physical, biological, ecological, as well as chemical methods is the concept of integrated pest management (IPM). IPM represents a long-term management strategy that employs a multiple, potentially lower cost control strategies with

minimal effects on health and environment to suppress pest population below economic injury levels (US EPA 2019). IPM integrates prevention, cultural practices, mechanical and/or physical pest control, biological pest control and chemical pest control to prevent and suppress pests. Similarly, IPM precepts can be adapted for the management of urban pests. Just as in IPM, the principles of urban pest management should range from the identification of pests and beneficial organisms before taking action, establishment of action thresholds for the pest, and evaluation of methods to determine success. The soundness of such a strategy is accessed by monitoring, evaluating and documenting results. The goal is to reduce the adverse impacts of pests and pest control method on human health, the environment and non-target organisms, while managing pests effectively. Applying IPM in urban environments, including home gardens, landscapes, and golf courses, presents special challenges. Urban IPM, or pest management programs that incorporate reduced use of pesticides in private and commercial landscapes, golf courses, and other urban landscape settings, is a rapidly expanding field that incorporates university and industry research. Unlike the overreliance on chemicals, IPM approaches involves improving sanitary and structural conditions to deny pests food, water, harborage, and movement, and includes the judicious use of pesticides after considering need and potential hazard to human occupants.

This strategy is not a single pest control method but, rather, a series of pest management evaluations, decisions, and controls. As a first line of pest control, the surrounding environment around homes should be monitored to prevent pests from becoming a threat in indoor space. Available data demonstrate that cockroach infestations and allergens in homes have been reduced by 50% using a combination of environment/landscape management strategies (Krieger et al. 2005, Kass et al. 2009), and in combination with low-toxicity pesticides (Brenner et al.



2003, Morgan et al. 2004, Condon et al. 2007). A notable trend discussed by these authors is the potentiality of integrated approaches such as education, commercial cleaning, and low-toxicity pesticides to reduce cockroach counts. Another study compared IPM that included repeated visits with the use of insect growth regulator devices to treatment with spray pesticide alone in public housing for German cockroach (Miller and Meek, 2004). The results suggested that integrated approaches might be superior to the traditional periodic insecticide application in reducing cockroach populations.

Nevertheless, a comprehensive integrated approach for perimeter ant pests does not currently exist. Ant management depends almost entirely on chemical control, with either a band of insecticide applied around the perimeter of the home or the application of granular or liquid baits around the perimeter of the home. Recommended sprays cover a 3 m wide band around the home and the lower 0.9 m of exterior of the home. However, typically, sprays usually provide control < 60 days control because the amount of insecticide is reduced by heavy irrigation, thick ground cover, exposure to direct sunlight, and elevated temperatures (Rust et al. 1996). This strategy allows high densities of perimeter pests to develop only a few m away from the home. Limited success has been obtained by combining an insecticide application with structural modifications such as caulking holes and cracks (Thoms and Robinson, 1987). Protection of the structure, however, does not control pests outdoors. High densities of perimeter pests can develop outdoors and serve as a reservoir for infestation of homes. Loss of insecticidal effectiveness occurs quickly in the Southeastern U.S. because high daily maximum temperatures in the summer rapidly degrade most insecticides (Harris 1972). Previous studies have shown that banded applications are ineffective against smokybrown cockroach < 10 days after treatment

(Smith et al. 1993, Smith et al. 1995). Consequently, infestation of homes occurs repeatedly throughout the summer.

Therefore, models that relate house and landscape characteristics with pest abundance are relevant to IPM. Such models would integrate, for a particular species, house characteristic and landscape items (age of home, brick exterior, etc.) by coefficients to determine relative importance of each factor. In other words, effective urban IPM is dependent upon some form of baseline data that estimate potential population before the IPM program is implemented. The purpose of evaluation is to improve efficiency, and collect high-quality data that inform sound decision-making and assess what factors are most critical to IPM program success. In this study, we investigated the seasonal abundance and diversity of ant species around homes. We hypothesized that ant species richness is related to temperature, precipitation, and age of the houses. Thus, the objective of this study was to determine the influence of seasonal conditions on the abundance and diversity of ant species around homes in Lee County, Auburn, Alabama.

We also investigated the water relation and desiccation tolerance of *M. cribraria*. Our objectives were to determine the total body water content (%TBW content), rate of total body water loss (%TBW loss), and cuticular permeability (CP) of adult and nymphal *M. cribraria*. We examined all developmental stages of both yearly generations, including pre-wintering and post-wintered adults of the second yearly generation. In addition, we determined desiccation sensitivity of all stages. This study will aid in understanding how environmental stress (temperature and humidity) may affect the survival and relative abundance of pests in fields and around homes.

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## Chapter 2

### Temporal variation in abundance and diversity of ant species (Hymenoptera: Formicidae) around homes in Southeast of Alabama

#### Introduction

Ants (Hymenoptera: Formicidae) are among the most dominant group of insects and constitute significant biomass in a variety of ecosystems (Wilson and Hölldobler 2005). Their economic effects on human health, property, and biodiversity make them subject of interest (Hölldobler and Wilson 1990, Andersen and Sparling 1997, Lobry de Bruyn 1999, Agosti et al. 2000). Ants also conduct different important activities such as nutrient recycling (Haines 1978), and seed collection and dispersal (Horvitz and Schemske 1986). Despite these benefits, some ant species are invasive pests and may inflict serious injuries on humans, livestock, or other beneficial arthropods with their bite and sting (Vinson and Greenberg 1986, Morel et al. 1990). Although only about 13% of the 12,000 known species of ants (Agosti and Johnson 2005) are considered to be pests in urban areas (Fisher and Cover 2007), five ant species (Argentine ants, *Linepithema humile*; Bigheaded ant, *Pheidole megacephala*; Red imported fire ants, *Solenopsis invicta*; Little fire ant, *Wasmannia auropunctata*; and Yellow crazy ant, *Anoplolepis gracilipes*) are listed among the world's worst invasive alien species (Global Invasive Species Database 2014, Lowes et al. 2000).

In the U.S., 40 ant species are considered as common urban pests (Hedges 1998). Of these, *Linepithema humile* (Mayr) and *Solenopsis invicta* (Buren) are ranked among the world's 100 worst invasive animals. Argentine ants have a widespread success and are an important

agriculture and urban pest (Knight and Rust 1990, Vega and Rust 2001). Argentine ants have strong invasive capabilities and form supercolonies that challenge control practices in managed habitats (Silverman and Brightwell 2008). Red imported fire ants are a major agricultural, urban, and wildlife pest, with an estimated \$6.7 billion annual losses in the U.S. (Lard et al. 2006). They inhabit much of the southeastern portion of the U.S., and infest row crops, nursery plants, and turf-grass (Banks et al. 1991, Morrison et al. 1997). They also damage irrigation system (Vinson 1997) and electrical equipment (Slowik et al. 1996). Red imported fire ants are medically and veterinary important because their sting and bite can cause life-threatening problems in wildlife species and birds (Allen et al. 2004, Morrow et al. 2015), poultry and domestic animals (Jemal and Hugh-Jones 1993), and humans (Richard et al. 2004). Additionally, they interfere with biological control (Eubanks 2001, Stahlschmidt et al. 2018) and their mounds can damage mowing and harvesting machinery (Klotz et al. 2003). Other ant species may cause severe property damage (Carpenter ants, *Camponotus* spp., Rust and Su 2012), carry pathogens that cause disease (Pharaoh ant, *Monomorium pharaonis* (L.), Beatson 1972), and are household nuisance pests troublesome when they get into human dwellings (MacGown and Forster, 2005), such as odorous ant, *Tapinoma sessile* (Say); acrobat ant, *Crematogaster* spp.; thief ants, *Solenopsis molesta* (Say); and crazy ants, *Paratrechina longicornis* (Latreille).

Ants have colonized almost all terrestrial habitats. If we understand changes in the spatial and temporal distribution patterns of an organism, we might be able to find differences in the structure of the local fauna (Gaston and Lawton 1988, Frith and Frith 1990) and exploit these for effective management. Ants respond quickly to environmental changes and are important elements in biological diversity studies along with other invertebrate groups (Agosti et al. 2000). Depending on the temporal and spatial scale, abiotic and biotic factors affect ant community

structure. Interspecific interactions result from dynamic changes in diversity within ecosystems (Levin 1992, Andersen 1995, Parr and Gibb 2010, Philpott et al. 2010). Among these factors, the main influences on species richness at local scale are considered as competitive interactions, microclimate conditions, availability of resources, and nesting site locations (Cornell and Lawton 1992, Godfray and Lawton 2001). In other words, ant activity changes depend on seasonal cycles, temperature, and food supply (Steinberger et al. 1992, Bollazzi and Roces 2002). Andersen (1995) stated that spatial and temporal foraging activity patterns in ant assemblages may be affected by vegetation, biotic interactions, and habitat disturbance regime. Dietrich and Wehner (2003) and Levings (1983) found that water availability affected ant species distribution in local fauna. In ground foraging ants, seasonal and spatial patterns were analyzed by Basu (1997), who reported noticeable seasonal fluctuations.

A way to manage perimeter and household pests such as ants effectively is the implementation of Integrated Pest Management (IPM) tactics. The key element in IPM programs and ecological research is sampling because of the need for accurate and precise estimates of species abundance (Binns and Nyrop 1992). Before deciding on the type of or need for treatment, correct sampling for diversity and abundance is critical. Early studies reported 47 ant species from urban areas (Murphree 1947) out of the 154 species in Alabama (MacGown and Forster, 2005). However, there has been no report on the peridomestic pest ant species in Alabama. Therefore, we investigated the seasonal abundance and diversity of ant species around homes. We hypothesized that ant species richness is related to temperature and precipitation. Thus, the objective of this study was to determine the influence of seasonal conditions on the abundance and diversity of ant species around homes in Lee County, Auburn, Alabama. We also correlated the abundance of species to test for interactions. In addition, we correlated the

abundance of species with the position of traps, age of the houses, and house and property areas to determine if those affected on ant species and populations.

## **Materials and methods**

### **Study site**

This study was carried out around homes in Auburn and Opelika, Lee County, Alabama, U. S. Fifty homes were surveyed and 30 were selected based on their age, type of exterior, and building design. These homes are primarily residential, with nearby neighborhood parks, gardens, and other open spaces. Weather information was obtained from a local weather station (Mesonet, AWIS). The climate in Auburn, Alabama is humid subtropical, with mild winters, early spring, during which almost all the annual precipitation occurs (with an average annual precipitation of 1369 mm), followed by long, hot, and humid summers, and warm autums (Weatherbase 2019). Summer is usually wetter than winter. Mean air annual temperature for the period of collection was between 1°C – 32 °C, and the average temperature was 18°C.

### **Ant sampling**

We sampled ant species around homes using baited traps from June 2018 through May 2019. The study period covered all four seasons: summer (June, July, and August), spring (March, April, and May), fall (September, October, and November), and winter (December, January, and February). Since ant species may have different feeding preferences (Blüthgen and Feldhaar 2010) and preferences of a colony may change during colony development (Dussutour and Simpson 2008), we developed a protein and sugar baited traps to meet nutritional preference of various ant species. Traps consisted of a 15 mL plastic centrifuge tube (12.7 cm long and 2.5 cm diameter; VWR, Radnor, PA). Uncapped plastic centrifuge tubes were baited with a drop of

honey and a piece of beef hot dog (about 2 cm) to attract ants into the tube. The hotdog was to lure protein-feeding ants while honey was for sweet-feeding ants. Traps were positioned horizontally on the ground next to vertical surfaces against the exterior of the house, fence, tree, or shrub to maximize trap catch. The traps were placed front and back of homes, against air conditioning compressor units, and against any nearby tree. Traps were retrieved 24 h after set-up, capped, and returned to the laboratory. Ants in capped tubes were stored in a freezer for preservation prior to identifying and counting. Five ant traps were placed at each house daily for three consecutive days, twice a month between spring and summer, and once a month in the fall and winter. In total, 500 traps were set for every collection cycle. Air temperature and rainfall data were obtained from a nearby weather station.

### **Ant identification and data analysis**

Ants were transferred from centrifuge tubes into 70% ethanol and examined using a dissection microscope. Ants were identified with the aid of the morphological key available at Mississippi Entomological Museum website: [https://mississippientomologicalmuseum.org.msstate.edu/Researchtaxapages/Formicidaepages/familialists/Common\\_Pest\\_Ants.html](https://mississippientomologicalmuseum.org.msstate.edu/Researchtaxapages/Formicidaepages/familialists/Common_Pest_Ants.html). Total number of individual for each ant species in each per trap was recorded for each house, sampling period, and date. Average abundance of each species was plotted over time to illustrate temporal abundance. Mean catch per trap of each ant species was calculated and relationships between means of ant species were estimated using SAS software (2013). Pearson correlation analysis was used to determine the relationship between ant species abundance around homes. All figures were developed using SigmaPlot 13 (SYSTAT software, San Jose, CA). Ant diversity and the abundance of each ant species were correlated

with mean temperature and rainfall. In addition, diversity and abundance of each ant species were correlated with position of traps and age of the houses.

## **RESULTS**

### **Species diversity**

A total of 16 ant species were captured from the 30 houses in Lee County in Alabama from June 2018 through May 2019 (Table 1). The 16 ant species belong to 13 genera and four subfamilies (Dolichoderinae, Formicinae, Myrmicinae, and Ponerinae). The most diverse subfamily was Myrmicinae (5 genera, 6 species), followed by Formicinae (4 genera, 6 species), Dolichoderinae (3 genera, 3 species), and Ponerinae (1 genus, 1 species). Myrmicinae and Formicinae comprised 37.5 % of the total numbers of ants collected, Dolichoderinae 18.75 %, and Ponerinae 6.25 %. Ant species diversity varied among the seasons. At the beginning of the study in early June 2018, 11 species were collected. The number declined to nine species in late June. A gradual increase in number of species was observed from early August and the number peaked in early September when 13 species were observed. Then the number of ant species declined with fluctuations of as low as seven in early March 2019 (Fig. 1). In general, most species exhibited a noticeable increase in activity from spring to summer. Ant diversity was greatest toward the end of summer and, not surprisingly, was lowest in winter months until March when the temperatures increased. Although *L. humile* was observed all year, *P. imparis* was just observed between November and February. *Nylanderia* sp., *Ponera pennsylvanica* (Buckley), and *Dorymyrmex bureni* (Trager) were observed just once time all year around and were present in summer.

### **Seasonal abundance**



A total of 260,050 individuals were collected during the one-year study period (June 2018 through May 2019). Ant species abundance was greater during the warmer season than in the dry and cold season. Most ant species were most abundant between June and October.

Among the 16 ant species, four were caught in numbers > 10,000, much more than the other ant species. Among these four species (Fig. 2), *L. humile* was the most abundant (189,922), followed by *S. invicta*, (20,214), *P. dentigula* (15,465), and *Solenopsis molesta* (Say) (10,250). Except for the absence of *S. molesta* in winter, the four most abundant species were present all year. The mean number of individual ants per trap of the 30 homes (per collecting week) *L. humile* was greatest ( $48.05 \pm 85.54$ , mean  $\pm$  S.D.) in late June 2018 and lowest in early March (0); there was a large fluctuation in catch of *L. humile* between months. The mean number of *L. humile* increased in February ( $30.38 \pm 53.26$ ), and then, dropped sharply in early March (0). The abundance trend for *S. invicta* was similar as *L. humile*, with the greatest catch in early June ( $9.49 \pm 34.79$ ) and the lowest in early March (0). The mean number of *P. dentigula* was the greatest ( $4.32 \pm 18.07$ ) in October and lowest ( $0.02 \pm 0.33$ ) in early March. Although mean trap catch of *P. dentigula* was not high throughout the study, it was present all year. Mean abundance of *L. humile*, *S. invicta*, and *P. dentigula* was high in February compared to December and January. The maximum trap catch of *S. molesta* was in early August ( $4.39 \pm 38.36$ ) and minimum catch from October to early April. Although the most abundant species were often seen in all study locations, *S. molesta* was observed in just seven study locations.

Ant species with total catch of 9896-2804 individuals were dark rover ant, *Branchymyrmex patagonicus* (Mayr) (9896), acrobat ant, *Crematogaster pilosa* (Emery) (4174), little black ant, *Monomorium minimum* (Buckley) (5901), and *Temnothorax pergandei* (Emery) (2804) (Fig. 3). The greatest mean number of *B. patagonicus* ( $4.61 \pm 24.40$ ) was in early

September and the lowest ( $0.52 \pm 3.11$ ) in early March (Fig. 3). A notable decrease in the mean number of *B. patagonicus* was observed between December and early March, with an increase in May. The mean number *C. pilosa*,  $1.51 \pm 11.66$ , and *M. minimum*,  $2.37 \pm 24.54$  were greatest in early June. While *T. pergandei* maintained low populations in cold season, the mean number ( $1.55 \pm 11.45$ ) was notably high in late September.

The abundances of the other eight ant species were low and some only occurred seasonally (Fig. 4 and 5). Odorous house ant, *Tapinoma sessile* (Say), was observed in summer (early July) and their populations peaked ( $0.68 \pm 9.40$ ) in late September. Robust crazy ant, *Nylanderia bourbonica* (Forel), was not observed from late May to late July, and was relatively abundant in cool seasons (Fall, Winter and Spring) compared to summer (Fig. 4). The presence of a few species like False Honey ant, *Prenolepis imparis* (Say), were distinctly seasonal, no catch during warm months and active in winter from November through January when few other species were foraging (Fig. 4). The black carpenter ant, *Camponotus pennsylvanicus* (De Geer), and reddish carpenter ant, *Camponotus castaneus* (Latreille) were observed between early June and October (Fig. 4 - 5). Mean number of three species, pyramid ant, *Dorymyrmex bureni* (Trager), *Nylanderia* sp., and *Ponera pennsylvanica* (Buckley) were observed only once throughout the entire study (Fig. 5).

### **Correlation between abundance and weather parameters**

The abundance of *L. humile* was positively correlated with high ( $r = 0.592$ ,  $P = 0.007$ ) and low temperature ( $r = 0.701$ ,  $P = 0.001$ ), but not with precipitation. There was a positive correlation between temperature and abundance in four ant species: *P. dentigula* (high temperature:  $r = 0.687$ ,  $P = 0.001$ ; low temperature:  $r = 0.718$ ,  $P = 0.001$ ), *S. molesta* (high:  $r = 0.678$ ,  $P = 0.001$ ; low:  $r = 0.710$ ,  $P = 0.001$ ), *C. pilosa* (high:  $r = 0.536$ ,  $P = 0.018$ ; low:  $r =$

0.581,  $P = 0.001$ ), and *C. pennsylvanicus* (high:  $r = 0.515$ ,  $P = 0.024$ ; low:  $r = 0.597$ ,  $P = 0.007$ ).

Two ant species displayed a negative correlation with temperature; *N. bourbonica* negatively correlated with high and low temperature (high:  $r = -0.501$ ,  $P = 0.028$ ; low:  $r = -0.517$ ,  $P = 0.023$ ) and *P. imparis* also negatively correlated with both high ( $r = -0.643$ ,  $P = 0.002$ ) and low temperatures ( $r = -0.557$ ,  $P = 0.013$ ). Interestingly, the abundance of *S. invicta*, *M. minimum* and *B. patagonicus* was not significantly correlated ( $P > 0.05$ ) to temperature or rainfall.

Interestingly, ant species was not correlated with precipitation.

### **Correlation between ant abundance, position of traps and age of houses**

The number total of ants collected ranged from 23,003 at traps located next to an air conditioning (AC) unit, 69,903 at traps next to tree, 77,614 at traps at the front of house, to 89,522 at traps at the back of house. On a per trap basis, the mean number of ants at AC traps was 22.42 ants, while back traps had 28.56 ants, front traps 26.19 ants, and 49.06 at traps located next to trees. Thus, traps at trees caught about twice as many total ants per trap compared with traps positioned in front, back or side of AC in a house. There was no significant difference in the number of ants trapped among the front, back, and AC traps. There was a positive correlation between position of traps and abundance of the ant species: *L. humile* with at traps AC ( $r = 0.935$ ,  $P < 0.001$ ), back ( $r = 0.905$ ,  $P < 0.001$ ), front ( $r = 0.828$ ,  $P < 0.001$ ), and tree ( $r = 0.871$ ,  $P < 0.001$ ) traps; *S. invicta* with at traps AC ( $r = 0.125$ ,  $P < 0.001$ ), back ( $r = 0.179$ ,  $P < 0.001$ ), front ( $r = 0.173$ ,  $P < 0.001$ ), and tree ( $r = 0.093$ ,  $P = 0.0004$ ) traps; *B. patagonicus* with at traps AC ( $r = 0.089$ ,  $P = 0.004$ ), back ( $r = 0.087$ ,  $P < 0.001$ ), front ( $r = 0.130$ ,  $P < 0.001$ ), and tree ( $r = 0.063$ ,  $P = 0.016$ ) traps; *P. dentigula* with at traps AC ( $r = 0.068$ ,  $P = 0.027$ ), back ( $r = 0.089$ ,  $P < 0.001$ ), front ( $r = 0.167$ ,  $P < 0.001$ ), and tree ( $r = 0.120$ ,  $P < 0.001$ ) traps. Also, *C. pilosa* was correlated significantly with at traps AC ( $r = 0.129$ ,  $P < 0.001$ ) and back ( $r = 0.049$ ,  $P = 0.005$ )

traps; *M. minimum* was correlated significantly with at back traps ( $r = 0.212$ ,  $P < 0.001$ ) and front ( $r = 0.125$ ,  $P < 0.001$ ) traps; *S. molesta* was correlated significantly with at front ( $r = 0.361$ ,  $P < 0.001$ ), and tree ( $r = 0.339$ ,  $P = 0.016$ ) traps. Interestingly, there was a negative correlation between at traps next to trees and *C. pennsylvanicus* ( $r = -0.058$ ,  $P = 0.026$ ). There was also no significant correlation ( $P > 0.05$ ) between the total number of ants and house area and property area. Although, there was no significant correlation between house age and the total number of ants caught ( $r = 0.233$ ,  $P = 0.215$ ), the numbers of *T. pergandei* ( $r = 0.398$ ,  $P = 0.0294$ ), *N. bourbonica* ( $r = 0.505$ ,  $P = 0.0044$ ), and *P. imparis* ( $r = 0.390$ ,  $P = 0.033$ ) were positively correlated with house age.

### Species interactions

Using correlation analysis for all possible pairs of ant species and all species combined, there were significant ( $P < 0.05$ ) positive correlations between the total number of ants collected and the numbers of *L. humile*, *S. invicta*, *C. pilosa*, *D. bureni*, *T. sessile*, *S. molesta*, *M. minimum*, *P. dentigula*, and *B. patagonicus*. These correlations are due to the large numbers of individuals of these species collected during the sampling period. There was a significant negative correlation between the abundance of *L. humile* and *S. invicta* ( $r = -0.063$ ,  $P = 0.001$ ). Also, the abundance of *L. humile* was significantly ( $P < 0.05$ ) negatively correlated with the abundance of *C. pilosa* ( $r = -0.034$ ,  $P = 0.001$ ), *B. patagonicus* ( $r = -0.046$ ,  $P = 0.001$ ), *T. pergandei* ( $r = -0.028$ ,  $P = 0.009$ ), *P. dentigula* ( $r = -0.056$ ,  $P = 0.001$ ), *M. minimum* ( $r = -0.024$ ,  $P = 0.023$ ), and *S. molesta* ( $r = -0.024$ ,  $P = 0.022$ ). Argentine ants clearly competitively excluded a number of different ant species. However, the correlation between the abundance of *L. humile* and *D. bureni* was positive ( $r = 0.025$ ,  $P = 0.017$ ). Also, there was a significant negative correlation between *P.*

*dentigula* and *S. invicta*. However, abundance of *S. invicta* only significantly correlated with a reduction (negative correlation) in abundance of big headed ants ( $r = - 0.025$ ,  $P = 0.021$ ).

## **DISCUSSION**

Information on the diversity and temporal variation of ant species around homes is a key element in control programs to ensure that informed decisions are made with respect to control tactics and chemicals. Here, using honey and sugar baited traps; we investigated the temporal diversity and abundance of ant species around homes in southeast Alabama. This type of sampling method is an inexpensive and harmless way to collect and monitor ant diversity and abundance in a given location. It also gives insights on where ants tend to forage on the property and what might be the reason that they congregate in these particular areas. In comparison to pitfall traps, bait traps confer some advantages. For example, baited traps provide much cleaner samples, which facilitates quick sorting and prompt identification. More so, these traps can be transported easily, and are less susceptible to surface rainwater runoff compared to pitfall traps. Further, the applicability of pitfall traps may be limited in urban areas where the excavation of a pristine garden and soil can be met with dissatisfaction among homeowners. Also, because the soil is disturbed by excavation during the setting of pitfall traps and baited traps are advantageous to prevent the "dig-in effect" in which some ant species interact with newly disturbed soil (Agosti et al. 2000). In these instances, use of pitfall traps become difficult to estimate the abundance and diversity of ants around homes – a solution bait traps easily provide.

In general, our results point to the strong effects of air temperature on ant species diversity and abundance around homes in Auburn, Alabama. Overall, we detected changes in diversity of ant species across seasons and, the greatest ant species diversity was calculated with

temperature in early September. The average high and low temperature in early September were 32°C and 22.5°C, respectively. In addition, precipitation in September was low when compared to other months. This might have resulted in environmental conditions (temperature and precipitation) suitable for most of ant species. Porter and Tschinkel (1987) reported that the primary abiotic factor affecting foraging activity of ants is temperature because ants tend to avoid surfaces during the period of day or year when the temperature is too cold or too hot. In general, foraging behavior of ants occurs when the environment temperature is between 10°C and 40°C (Hölldobler and Wilson 1990). Klotz (1984) reported that ant activity and movement may vary depending on the temperature. He observed a decreasing foraging activity in *Formica subsericea* (Say) at cooler temperature (below 12°C) and a reduction in movement at temperatures between 14 and 18°C. In this study, we observed more ants were trapped at 32°C and the number of ant species decreased seasonally as mean temperatures decreased, indicating a positive relationship between species diversity and average air temperatures. There was probably a reduction in movement of ants at low temperatures. The lowest ant species diversity was observed in early March. This is not surprising, because the average high temperature was 22°C in February and then it decreased to 16°C in early March, resulted in environmental conditions unsuitable for some ant species during this sampling period. Myrmicinae and Formicinae were the most diverse subfamily across seasons. This can be explained by the great adaptive radiation, range of foraging behaviors, nesting habits, and colony structure of these subfamilies (Hölldobler and Wilson, 1990). In addition, with more than 180 genera and 7,649 species worldwide, Myrmicinae is the most diverse and largest subfamily of Formicidae (Bolton, 2019). Ponerinae was the least diverse subfamily in this study. This subfamily often has small colonies with solitary foraging workers (Peeters and Crewe, 1987).

In this study, abundance of some species like *L. humile* and *S. invicta* was quite high. The increase in the abundance of *L. humile* in hot season (summer and fall) can be attributed to its thermal tolerance and capacity to adapt to a wide range of temperatures (Verble-Pearson et al. 2015), as observed in the *Solenopsis invicta* Buren (Helms and Vinson 2005). The abundance of *L. humile* can be ascribed to modifications in its social behavior and composition of colonies (Tsutsui et al. 2000, 2003, Giraud et al. 2002). Some studies have reported differences in the activity duration of native and non-native ants in the regions where they coexist (Holway et al. 2002). For example, in Japan, most native ant species are inactive in winter whereas *L. humile* is active all year unless the surface temperature falls below 10°C (Touyama 2004). Moreover, seasonal variation in *L. humile* abundance is probably related to its diet and reproductive cycle (Digirolamo 2006). *Linepithema humile* activity was at the lowest point in March probably because new worker production begins at that time (Markin 1970). The red imported fire ant, *S. invicta*, was also an abundant species during the summer, and its abundance varied over the course of the year. Our results agree with those of Stuble (2009), who found the density of *S. invicta* to be consistently greatest during the summer. The local climate is humid subtropical, with mild winters, early spring, during which almost all the annual precipitation occurs, followed by long, hot, and humid summers, and warm autums (Weatherbase 2019). It is possible that ant activity follows these environmental fluctuations which lead to changes in seasonal and daily rhythms. The highest abundance of *P. dentigula* was between June and October, a finding similar to Nunes et al. (2011), who reported higher abundance of *P. dentigula* between June and October. *Prenolepis imparis* differed from the other species because it was inactive during warm months and active in only cooler months. That is why, it is called “Winter Ant” and because it can forage at temperatures as low as 2°C (Wheeler 1930, Andersen 1997). According to

Tschinkel (1987), foraging and aboveground activity of *P. imparis* occur during the cold months (November through late March) however, activity of workers continued in underground nests through spring and summer in northern Florida.

Several studies suggest a strong correlation between ants and their habitats and nutrients (Bennett and Breed 1985, Holoway and Case 2000, Van Wilgenburg and Elgar 2007, Wagner and Nicklen 2010). These authors list a number of hypothesis, including a nutrition hypothesis to describe ant behavior. The nutrition hypothesis establishes a link between food rewards and ant behavior. This is particularly true as trees, compared to air conditioning compressor units (AC) and side of houses of host for food such as insects or honeydew may serve as a potential source of food for ants. The presence of debris, leaf litter, and other food may have been responsible for the higher abundance of ants near trees compared with those other trap position. Correlations found between *L. humile* and *S. invicta* ants, and position of traps is a reflection of the greatest abundance of these species over all collected species.

A number of studies have shown that interspecific competition between species contributes to distribution and abundance in ant communities (Lynch et al. 1980, Savolainen and Vepsäläinen, 1988, Andersen 1992, Andersen and Patel 1994). In the displacement of one ant species by another, interference competition in the form of aggressive conduct between worker ants is important (Savolainen and Vepsäläinen 1988, Andersen and Patel 1994). In this study, we found a significant negative correlation between *L. humile* and *S. invicta*, *B. patagonicus*, and *P. dentigula*. The negative correlation suggests that these species may compete for similar resources and can competitively exclude each other from a particular area. In addition, interspecific competition for resources through exploitative and interference processes is one of the variables regulating the group structure of ants (Wilson 1971, Fellers 1987, Hölldobler and Wilson 1990,



Davidson 1998, Holway 1999, Morrison 2000). A species discovers and exploits a limiting food source prior to the arrival of other species in exploitative competition; a species excludes competitors by territoriality using repellents or direct aggression in interference competition (Schoener 1983; Fellers 1987; Davidson 1998). Factors affecting the result of the interaction may vary based on body size, age, strength, experience, presence of sting or other forms of chemical defense, food size and shape (Cerdá et al. 1998; LeBrun 2005). The success of *L. humile* can be associated with colony size (Holway and Case 2001), foraging behavior (Gordon 1995, Herbers and Choiniere 1996), and effective fighting tactics (Holway 1999). On the other hand, we found positive correlation between *L. humile* and *D. bureni*. This is probably due to the small number (2) of *D. bureni* collected because it was only observed once in the study areas during the whole year. Also, the other significant negative correlation was observed between *S. invicta* and *P. dentigula*. To compete with other invasive species, numerical advantage is probably necessary, because in a *Pheidole* spp. nest only 8 to 20% of individuals are soldiers (Wilson 1971). These results seem to indicate that red imported fire ants may not directly compete or may not successfully compete with other ant species, except *L. humile*.

This work points to the variation and abundance of ant species around homes in Auburn, Alabama. In general, the greatest diversity of species was recorded in early September and the lowest in early March. Abundance of most species was greatest in the warmer seasons (summer and fall) and lowest in the cold season (winter). Also, we observed that there were interactions between species that affect abundance. These interactions lead to an increase in one species while causes a decrease in another species. Ants perform many ecological roles that can be beneficial to human beings, including the suppression of pest populations around homes. The information provided here can be used for the development of IPM programs against

peridomestic pest ants.

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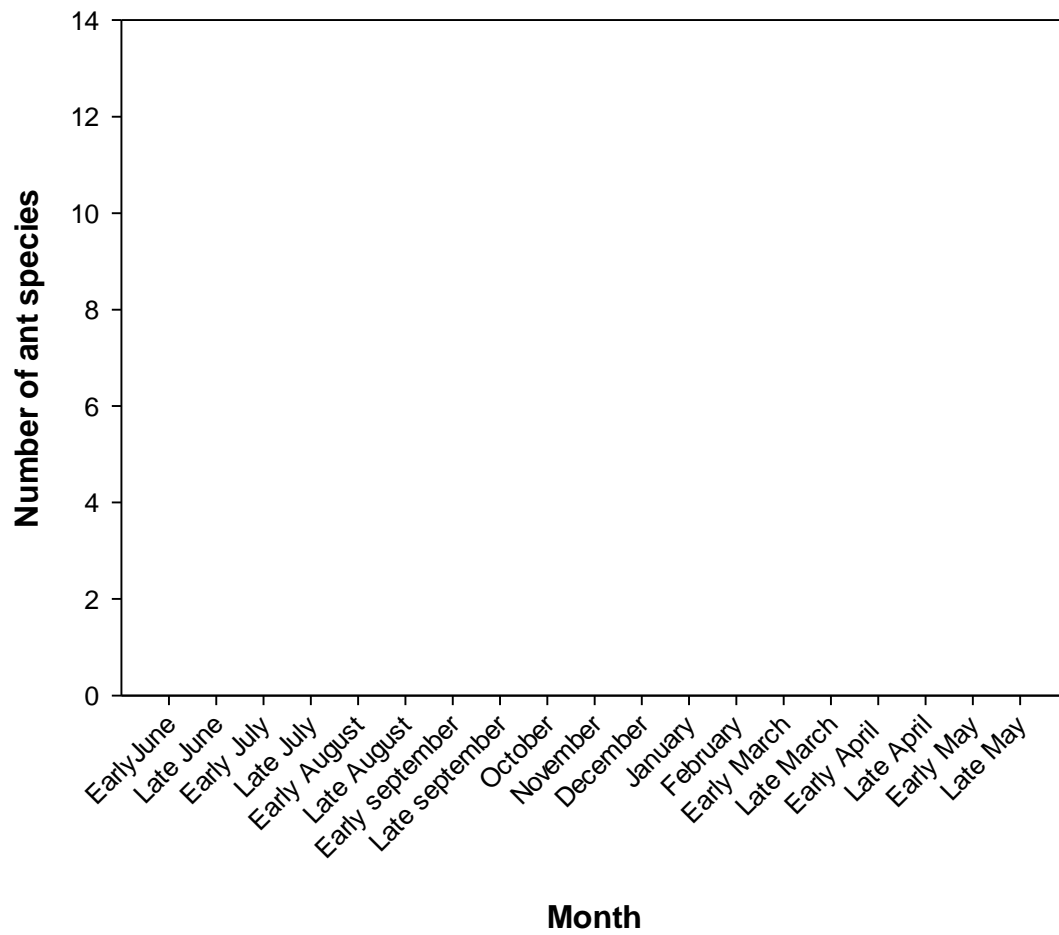
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**Table 1:** Summary of ant species collected from around houses

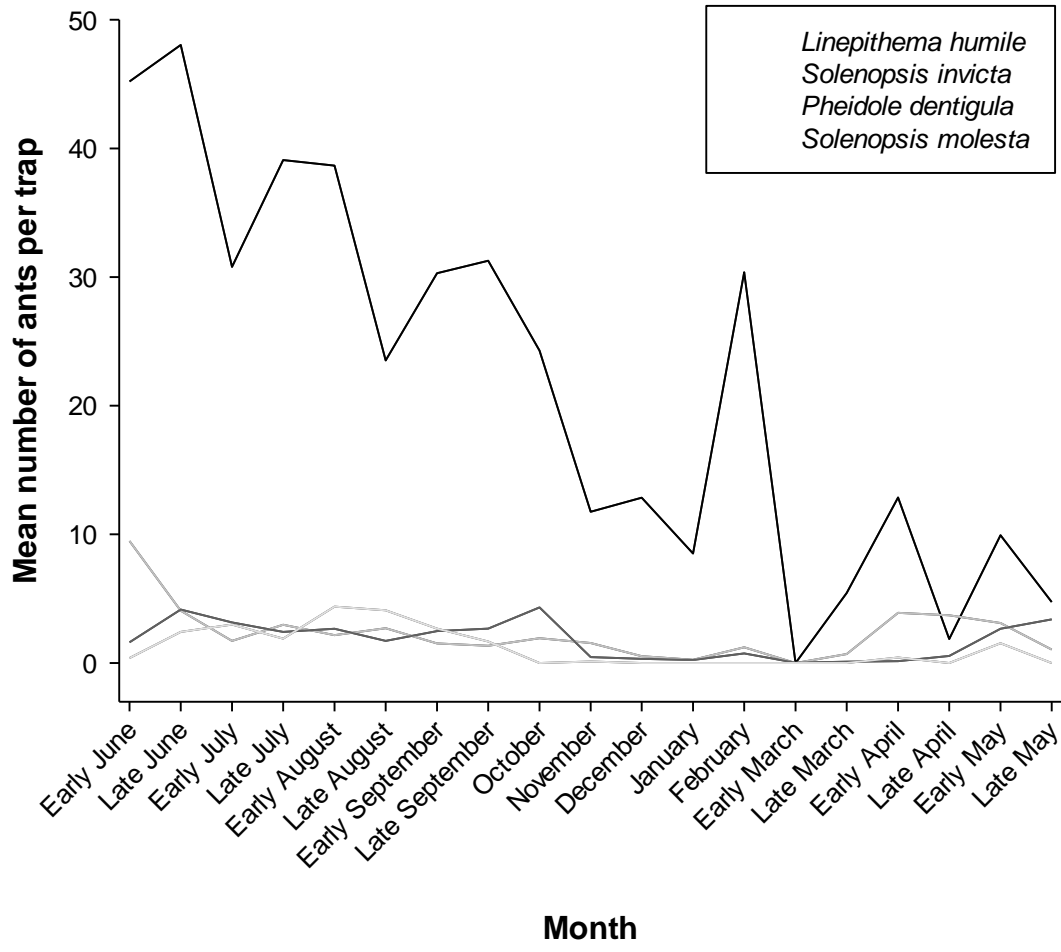
Subfamily	Species	Common name
Formicinae	<i>Camponotus pennsylvanicus</i> (De Geer)	Black carpenter ant
	<i>Nylanderia</i> sp. (Emery)	Crazy ant
	<i>Brachymyrmex patagonicus</i> (Mayr)*	Dark rover ant
	<i>Prenolepis imparis</i> (Say)	Winter ant
	<i>Camponotus castaneus</i> (Latreille)	Reddish carpenter ant
	<i>Nylanderia bourbonica</i> (Forel)	Robust crazy ant
Myrmicinae	<i>Crematogaster pilosa</i> (Emery)	Acrobat ant
	<i>Pheidole dentigula</i> (Smith)*	Big Headed ant
	<i>Monomorium minimum</i> (Buckley)	Little black ant
	<i>Solenopsis invicta</i> (Buren)*	Red imported fire ant
	<i>Solenopsis molesta</i> (Say)*	Thief ant
	<i>Temnothorax pergandei</i> (Emery)	No common name
Dolichoderinae	<i>Linepithema humile</i> (Mayr)*	Argentine ant
	<i>Tapinoma sessile</i> (Say)	Odorous house ant
	<i>Dorymyrmex bureni</i> (Trager)	Pyramid ant
Ponerinae	<i>Ponera pennsylvanica</i> (Buckley)	No common name

\*Most abundant species collected over season

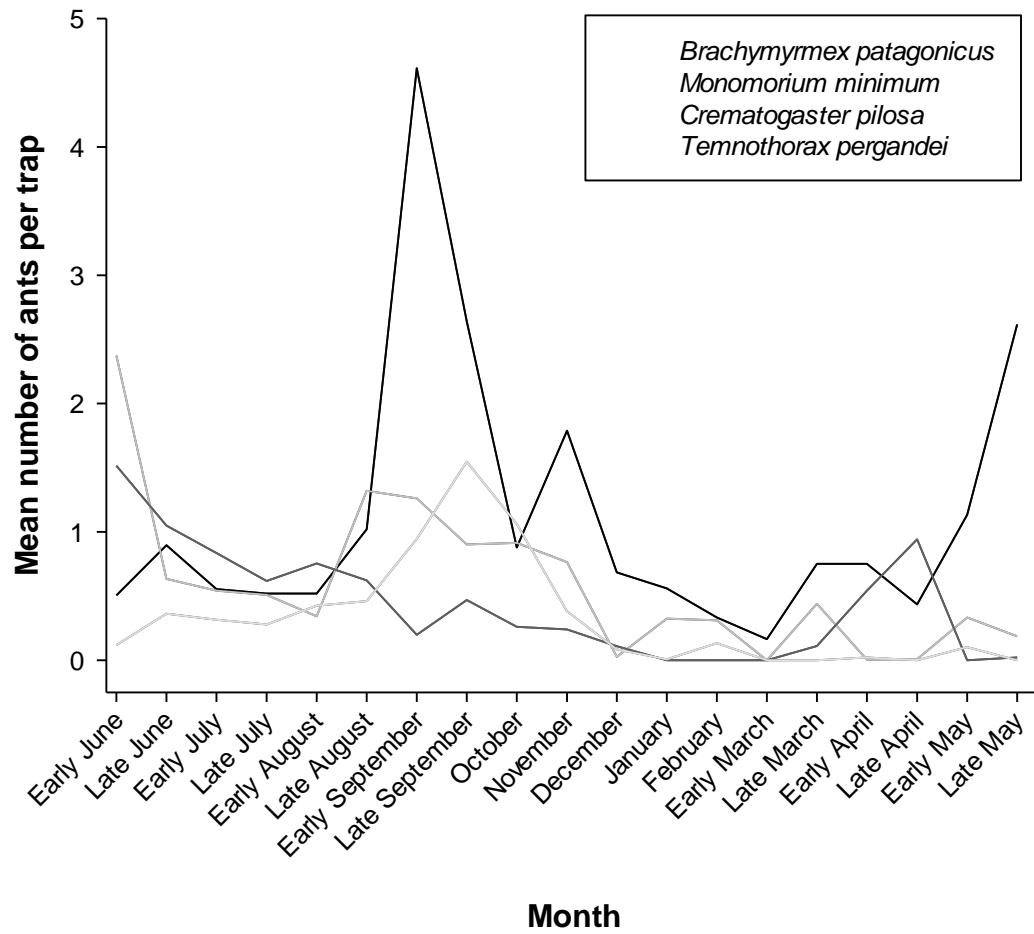


**Fig. 1.** Seasonal diversity of ant species during the 2018 - 2019 study period

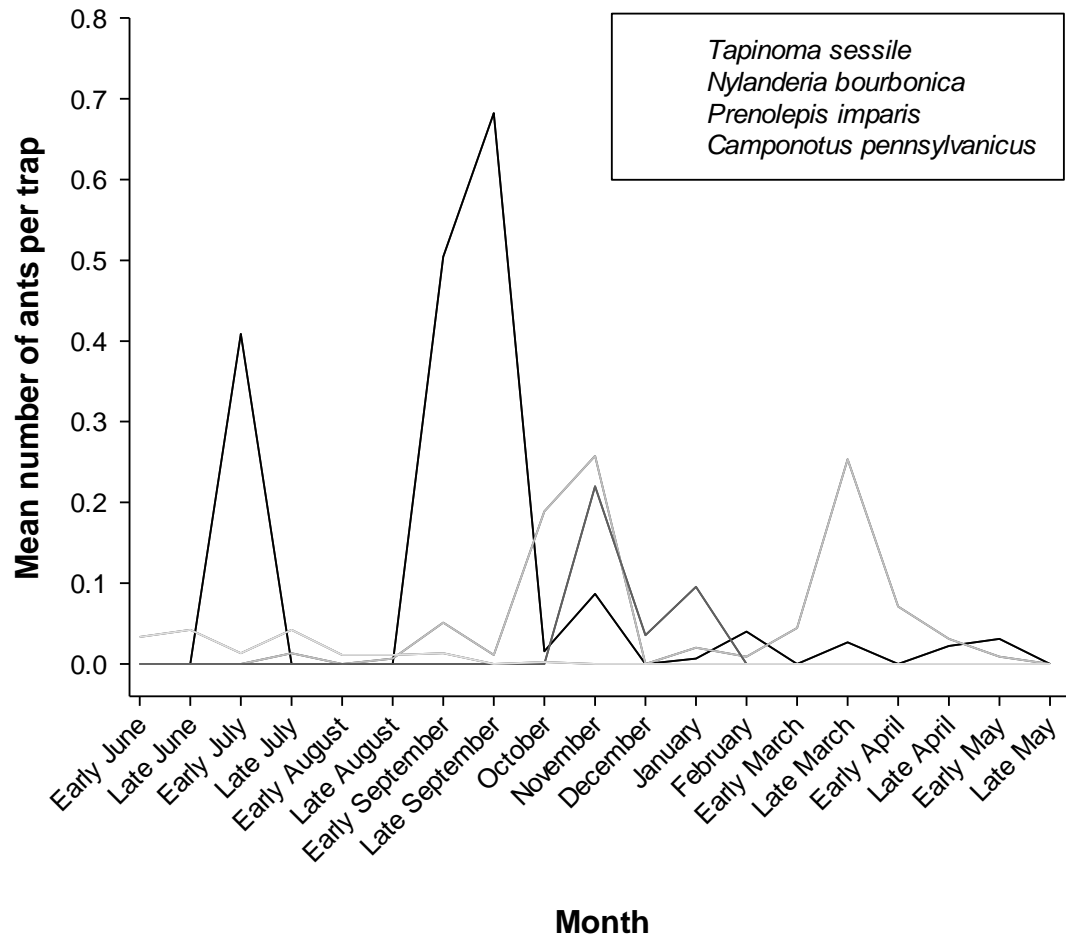




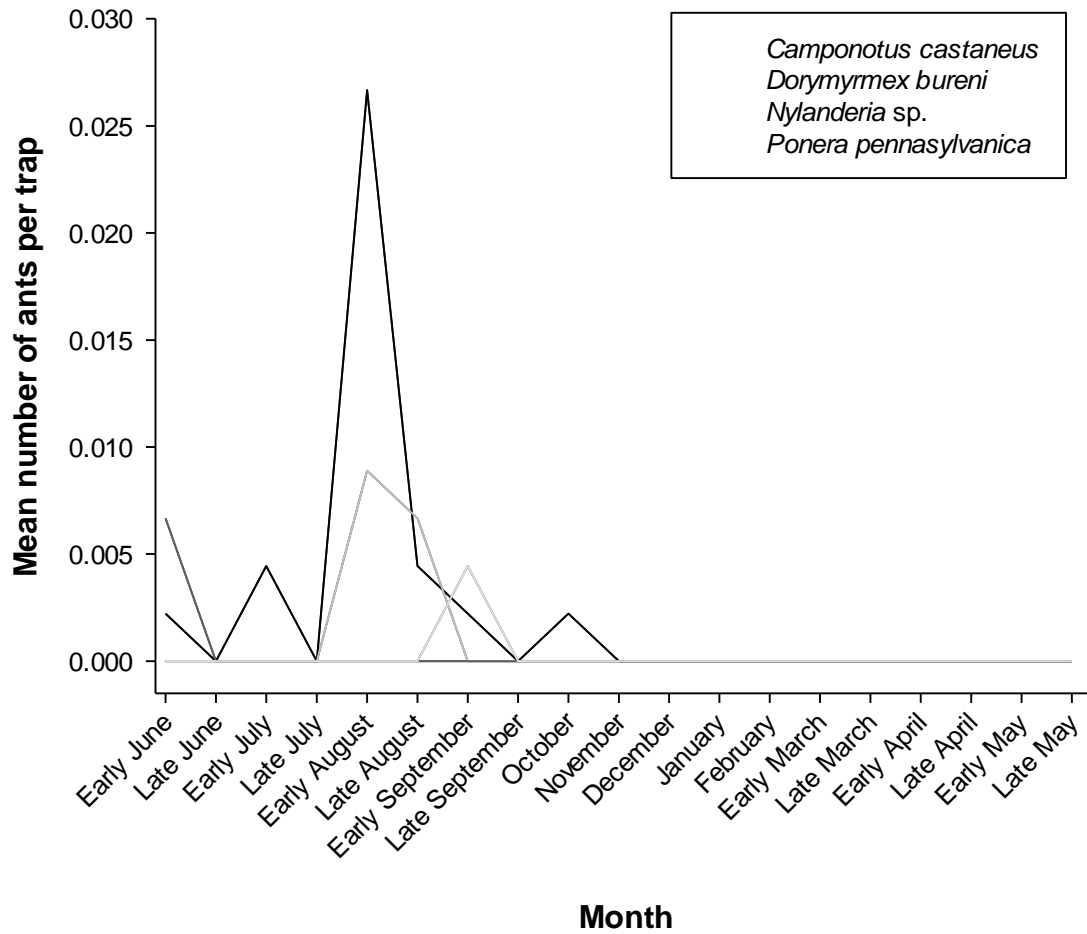
**Fig. 2.** Mean catch of *L. humile*, *S. invicta*, *P. dentigula*, and *S. molesta* at 30 homes 2018 – 2019.



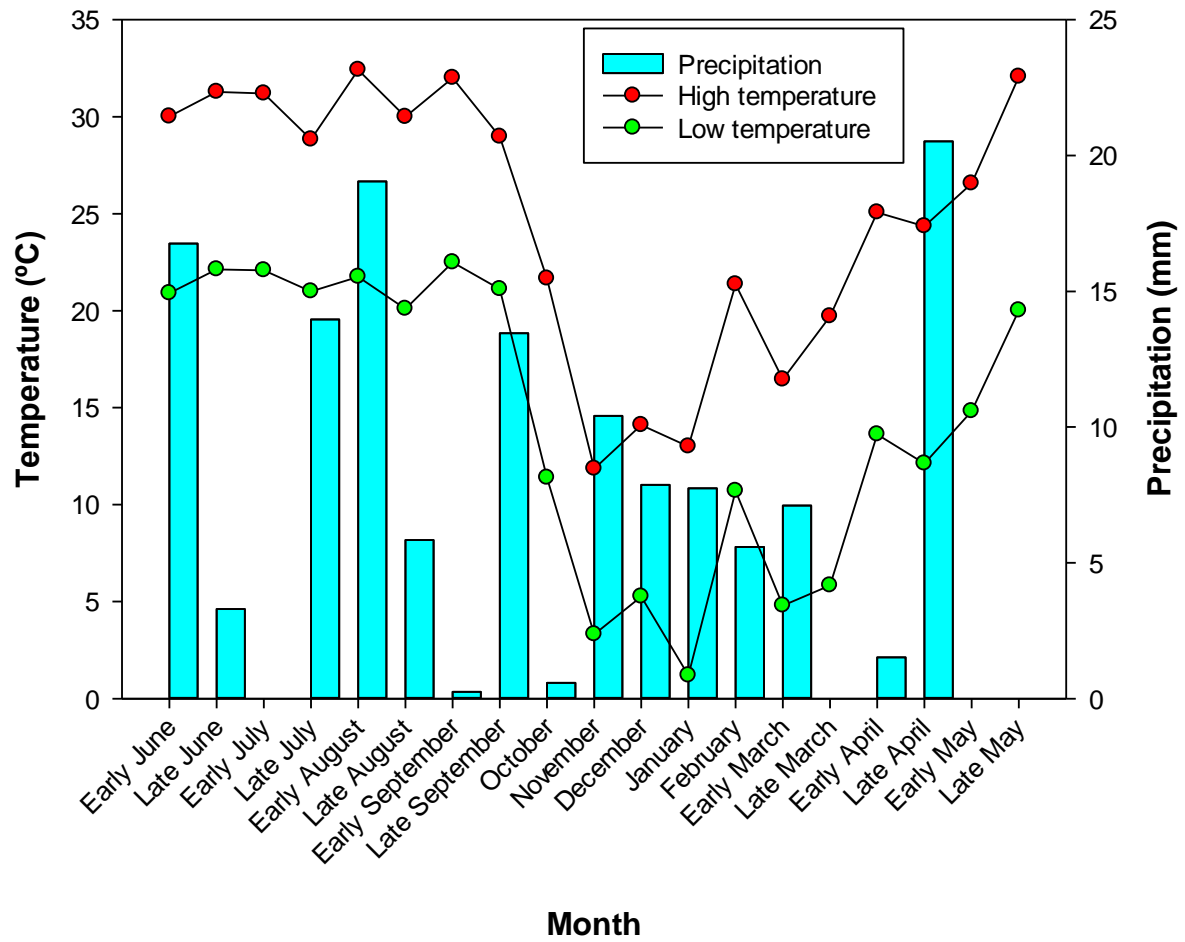
**Fig. 3.** Mean catch of *B. patagonicus*, *M. minimum*, *C. pilosa*, and *T. pergandei* at 30 homes 2018 – 2019.



**Fig. 4.** Mean catch of *T. sessile*, *N. bourbonica*, *P. imparis*, and *C. pennsylvanicus* at 30 homes 2018 – 2019.



**Fig. 5.** Mean catch of *C. castaneus*, *D. bureni*, *Nylanderia sp.* and *P. pennsylvanica* at 30 homes 2018 – 2019.



**Fig. 6.** Average air temperature and mean precipitation in Lee County, Alabama during the 2018–2019 sampling period.

## Chapter 3

### Water Loss and Desiccation Tolerance of Two Generations of Adult and Nymphal Kudzu Bugs, *Megacopta cribraria* (Hemiptera: Plataspidae)

#### Introduction

Terrestrial insects are particularly vulnerable to desiccation, given their small size and high surface area-to-body-volume ratio (Edney 1977, Hadley 1994). Their success is largely due to the ability to conserve water, in addition to other physiological and behavioral adaptations (Thorat and Nath 2018). This is accomplished by a relatively water impermeable cuticle covered with a thin layer of epicuticular lipids (particularly hydrocarbons) (Beament 1961, Hadley 1994, Chown and Nicolson 2004). Desiccation is a crucial stressor and plays an important role in insect survival, growth, development, behavior, and ultimately distribution. Benoit et al. (2010) demonstrated that desiccation stress reduced metabolic reserves and egg-laying capacity in the mosquito *Culex pipiens* (L.) (Diptera: Culicidae) and affected the seasonal population dynamics of this species. Water loss and cuticular permeability can vary among insect species (Edney 1977, Hadley 1994), castes within a species (Sponsler and Appel 1990, Bong et al. 2013), physiological status (Rolandi et al. 2014), and habitat conditions such as temperature, relative humidity, and airflow (Wang et al. 2016, Addo-Bediako et al. 2000, Appel et al. 1986, Appel et al. 1983).

The kudzu bug, *Megacopta cribraria* (Fab.) (Hemiptera: Plataspidae), is a relatively new invasive species to the U.S. Since the first report from northeast Georgia in 2009 (Eger et al.

2010), it has spread throughout the southeastern U.S. including Alabama, Arkansas, Delaware, Florida, Georgia, Kentucky, Louisiana, Maryland, Mississippi, North Carolina, South Carolina, Tennessee, Virginia, and the District of Columbia (Database at the Center for Invasive Species and Ecosystem Health, 2018). It has become a serious economic pest of commercial soybean crops (Zhang et al. 2012, Lahiri and Reisig 2016) and a nuisance invader of homes (Ruberson et al. 2013). Both immature and adult of *M. cribraria* feed on plants, using their piercing-sucking mouthparts to extract phloem sap (Stubbins et al. 2017) reducing plant growth and seed development. *Megacopta cribraria* can cause soybean yield losses of up to 60% if left uncontrolled (Seiter et al. 2013). *Megacopta cribraria* also invades homes and, when threatened, releases a defensive secretion that has a foul odor and can stain skin (Ruberson et al. 2013). This insect generally undergoes two generations per year in the U.S. (Zhang et al. 2012). It overwinters in the adult stage in reproductive dormancy (Golec and Hu, 2015) in protected locates, such as under loose tree bark, leaf litter or other debris (Lahiri et al. 2015). In soybean fields and kudzu patches, *M. cribraria* populations display nymphal aggregation, but no significant adult aggregation when populations are low (Knight et al. 2017). However, aggregations of overwintering populations that invade soybean fields are often observed at field edges (edge effect) adjacent to overwintering sites and feral kudzu patches (Seiter et al. 2013). Narrow row spaced soybean plants with a denser foliage canopy and a more humid microhabitat maybe more attractive to *M. cribraria* than plants of wide rows (Del Pozo-Valdivia 2013). Aggregation is reported to facilitate intake of atmospheric water and protect against desiccation in the Southern green stinkbug, *Nezara viridula* (L.) (Lockwood and Story 1986).

Although there have been a few studies on the water relations of Heteropterans, there are no studies on *M. cribraria*. We therefore investigated the water relation and desiccation tolerance

of this species. Our objectives were to determine the percentage of total body water content (%TBW content), rate of mass and percentage of total body water loss (%TBW loss), and cuticular permeability (CP) of adult and nymphal *M. cribraria*. We examined all developmental stages of both yearly generations, including pre-wintering and post-wintered adults of the second generation. In addition, we determined the desiccation sensitivity of all stages. This study will aid in understanding how environmental stress may affect the survival and relative abundance of *M. cribraria* in fields and around homes.

## **Materials and Methods**

### **Insects**

*Megacopta cribraria* were collected from patches of wild kudzu, *Pueraria montana* (Lour.) Merr. (Fabaceae), from Auburn, Lee County, Alabama, U.S.A. (32°35'52"N, 85°28'51"W). This location experiences a humid subtropical climate with mild winters, early springs, long, hot, muggy summers, and warm autumns. Average temperatures range from 1.2 to 12.8°C in January to 21.2 to 32.1°C in July. Rain occurs in every month and ranges from 77 mm in August to 165 mm in March; average annual rainfall is 1,337 mm.

First generation nymphs (instars 1-5) and adults were collected from May through July 2017. Second-generation nymphs and pre-wintering adult were collected from August through October 2017. Post-wintering adults were collected in March 2018. Adults were collected using a hand net and immatures were collected by cutting off and enclosing kudzu shoots on which they were feeding. Collections were placed in 3.8-L (1 gallon) wide mouth glass jars (15 cm diameter x 17 cm tall, Uline, Pleasant Prairie, WI), covered with mesh lids, and transported to laboratory immediately. Collections were maintained in the laboratory at  $24 \pm 2^\circ\text{C}$ ,  $70 \pm 5\%$  RH, and a photoperiod of 16:8 (L:D) h. Fresh kudzu shoots were provided daily. Experiments were



conducted within one week after the insects were captured. Adult genders and nymphal instars were identified using the characteristics described at <https://www.kudzubug.org/identification/>.

### **Measurement of water loss**

Initial body mass, percentage total body water (%TBW) content, %TBW loss over desiccation time, mass loss, and cuticular permeability (CP) were determined gravimetrically using methods similar to Appel (1993). Dead insects were used in these experiments to eliminate the effects of movement, fecal, oral, and respiratory water loss.

Live *M. cribraria* were separated by nymphal instar and adults by gender. Each group was confined in a small glass jar with screen-lid. These jars were placed in a 1 L glass chamber with  $\approx 10$  g NaCN and KCN and killed by exposure to HCN gas. The cyanide salts were activated with  $\approx 0.5$  ml hydrochloric acid to produce HCN gas. Exposures of approximately 5 min were necessary to kill adults and about 3 min to kill nymphs. This process was accomplished as quickly as possible under a laboratory hood because of the toxicity of cyanide. Dead insects were removed from the killing chamber, weighed to the nearest 0.01 mg individually (adults and nymphs above 3<sup>rd</sup> instar) or in groups (1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> instar nymphs were weighed in groups of 10) using an electronic digital analytical balance (A&D Company, LTD, Model GH-200, Japan). Weighed insects were placed into aluminum weighting pans (43 mm diameter x 12 mm tall, Fisher Scientific, Waltham, MA), and carefully placed into a 12 L glass desiccator containing 1 kg of fresh Drierite® (W.A. Hammond Drierite Co. LTD, Xenia, OH) that maintained the air in the desiccator at 0-2% RH. The desiccator was placed into an incubator at 30°C. Insects were weighed at 2 h intervals for 10 h and again at 24 h. After the 24 h weighing, the insects were transferred to an oven at 50°C for 48-72 h to determine dry weights. There were 10 to 21 replicates for each instar and adult gender of each generation.

Total body water content was calculated as the difference between initial mass and dry mass for each nymphal instar and adult gender. Percent total body water (%TBW) content and rate of percent total body water loss (%TBW loss) were calculated as follows:

$$\% \text{TBW content} = [(M_{\text{initial}} - M_{\text{dry}})/M_{\text{initial}}] * 100.$$

$$\% \text{TBW loss} = [(M_{\text{initial}} - M_{\text{dry}}/M_{\text{initial}})]/[(M_{\text{initial}} - M_{\text{dry}})] * 100$$

Mass loss was calculated using the mg of water loss between the initial and 2 h masses per initial mass (g) per unit time (h) and the formula is as follows: Mass loss = mg of H<sub>2</sub>O lost (T<sub>0</sub> - T<sub>2</sub>)/gram body weight (T<sub>0</sub>), where T<sub>0</sub> is initial mass and T<sub>2</sub> is mass after 2 h of desiccation.

Cuticular permeability (CP) was calculated using the mass loss between initial and 2 h masses because water loss at that time is greatest and represents maximal cuticular water loss (Sponsler and Appel 1990). The CP value was calculated using Edney's equation (Edney 1977):

[initial weight - weight at 2 h in µg] per unit surface area (cm<sup>2</sup>) per unit time (h) per unit saturation deficit (mmHg). Surface area of the insect was calculated using Meeh's formula

(Meeh 1897):  $S = 12 M^{2/3}$ , where  $S$  = body surface area (cm<sup>2</sup>) and  $M$  = initial mass (g) (Edney and McFarlane, 1974). Meeh's formula was used rather than the empirically derived model by Haagsma, et al. (1996) because it is more accurate for insects with sphere-like body shapes such as *M. cribraria*. The saturation deficit at 30°C and 0% RH is 31.824 mmHg (Harr et al. 1984).

### **Desiccation Tolerance**

All nymphal instars and adults were subjected to desiccation tolerance tests. Live insects were weighted initially and placed into the pre-weighted plastic tubes. (10 mm diameter x 39 mm height) with screen-lids to prevent escape. The tubes were placed into a desiccator and maintained as above at 30°C and 0% RH. Insects were examined for mortality every 2 h for 10 h

and again at 24 h and reweighed after death. There were 8 to 21 replicates for each instar and adult gender of each generation.

### **Statistical analysis**

Data were recorded directly into Microsoft Excel 2016 and analyzed using SAS software version 9.3 (SAS Institute 2013). Percentage of total body water (%TBW) was arcsine square root transformed prior to analysis. Body mass, CP value, and the transformed %TBW content values were checked for normality at  $\alpha = 0.05$  significance level using the univariate procedure in SAS (SAS Institute 2013). Analyses of variance (ANOVA) was used to determine if there were differences in initial body mass, percent total body water, water loss (mg/g) and CP values among instars (nymphs) and genders (adults), and generations. The Waller-Duncan K-ratio test was used to determine significant differences among instars and genders. Linear regression was used to estimate the rate of percentage of initial mass lost and %TBW lost over time.

## **Results**

### **Nymphs**

Mean initial body mass of first generation nymphs ranged from 0.045 mg for first instars to 20.95 mg for fifth instars and from 0.21 to 14.95 mg for generation two first and fifth instars, respectively (Table 1). Fifth instar nymphs had significantly greater mass ( $P < 0.05$ ) than the other nymphal stages for both generations. There were significant differences in initial body mass among most nymphal instars between generations; second generation first through third instar nymphs had significantly greater mass. Mean percentage total body water (%TBW) content of ranged from 60.54% for third instars to 88.89% for first instars in generation one and 62.41% for fifth instars to 68.43% third instars in generation two (Table 1). First instar nymphs had significantly greater %TBW ( $P < 0.001$ ) than the other instars in generation one and was not

different among instars in generation two (Table 1). The %TBW of both generations generally declined with increasing instar.

Mean mass loss ranged from 111.11 to 26.47 mg/g for first and third instar nymphs, respectively in generation one and 69.19 mg/g for first instars to 27.59 mg/g for second instars in generation two (Table 1). In general, mass loss decreased with increasing instar; first instar nymphs had highest mass loss. Cuticular permeability (CP) ranged from 2.87 to 11.06  $\mu\text{g cm}^{-2} \text{h}^{-1} \text{mmHg}^{-1}$  for third and fifth instar nymphs of generation one, respectively (Table 1). There was a similar range in CP values for the second generation however second instar nymphs had significantly ( $P < 0.05$ ) lower CP ( $3.10 \mu\text{g cm}^{-2} \text{h}^{-1} \text{mmHg}^{-1}$ ) than the other nymphal instars.

Percent initial mass loss and %TBW loss of all instars of both generations increased linearly with desiccation time (Fig. 1-2; Tables 2-3). First instars had the greatest rate (slope of regression) of initial mass loss 6.2 and 3.5 %/h for first and second generation, respectively (Table 2). First instars also had the greatest rate of %TBW loss 7.0%/h for first generation nymphs and 5.4%/h for second generation nymphs (Table 3.). Fifth instars had the lowest rates of initial mass loss (0.9 and 1.0%/h for first and second generations, respectively) (Table 2) and %TBW loss 1.4 for first generation and 1.5%/h for second generation (Table 3). After 10 h of desiccation, the greatest %TBW loss was of first instars, 62.5% for generation one (Fig. 1), and 57.5% for generation two (Fig. 2).

### **Adults**

Mean initial body mass of both generations increased as the nymphs developed into the adult stage resulting in significantly greater adult body mass than nymphs for both generation one and two ( $P < 0.005$ ) (Table 1). Adult females had significantly greater initial body mass than males in both generations. Total body water (%TBW) content for adults ranged from 51.36 to

60.09% for generation two prewinter adult females and overwintered females, respectively (Table 1).

Mass loss for generation one adults was 30.5 to 30.9 mg/g and did not differ significantly between the genders (Table 1). Generation two prewinter female mass loss was 22.94 mg/g which was significantly greater than mass loss of overwintered females (18.4 mg/g). There was no difference in mass loss between prewinter and overwintered males (Table 1). The cuticular permeability (CP) of adults was not significantly different between genders or generations (Table 1).

As with nymphs, both initial mass loss and %TBW increased linearly for adults of both generations during the 10-h desiccation period; prewinter-adults had the highest rates of initial mass loss and overwintered adults the lowest (Fig. 1-2, Table 2-3). Based on overlap of the SE of the slopes, there was no difference in rate of desiccation between males and females. Generation two prewinter adults had approximately twice the rate of %TBW loss compared with overwintered adults (Table 3).

## **Mortality**

No mortality was observed before 10 h of desiccation. At 10 h, there was 0 to 25% mortality; greatest mortality occurred with prewinter adults (Table 4). Later nymphal instars had greater desiccation tolerance at 24 h, indicated by lower mortality (10-45%), than early nymphal instars (first instar: 95-100%; second instar: 76.2-87.5%) and adults (75-100%) (Table 4). Of the later instars, the fourth and fifth instars had the greatest desiccation tolerance; mortality was 10-25%, lower than the 40-45% of the third instar. There was no significant difference in mortality between adult genders for both generations. However, second-generation prewinter adults tolerated desiccation better than did overwintered adults. The %TBW lost at death ranged from

25.6% for first generation adult females to 75% for first generation fifth instar nymphs (Table 4). First generation adults lost 25.6-33.3 %TBW at death compared with 54.2-67 %TBW for second generation prewinter adults. However, overwintered adults (second generation) lost similar %TBW at death as first generation adults.

## Discussion

Since the first report of kudzu bugs in the United States (Eger et al. 2010), this species has spread throughout the southeastern United States (CISEH, 2018) where it is an economically important pest of soybeans and a nuisance pest of homes. The climate where this species is active in the U.S. is predominantly warm and humid as it is in the kudzu bugs' native Southeast Asia (Liang et al. 2018). Desiccation is a major physiological challenge faced by terrestrial insects and can be measured in several ways. Change in mass in response to exposure to dry conditions (Chown and Nicolson 2004) can be calculated in terms of insect surface area (cuticular permeability), mass specific water loss (mg/g), and rates of initial and %TBW loss. The overall change in mass between measurements is attributed primarily to cuticular water loss, as respiratory water loss is generally considered negligible, especially in dead individuals such as those used in this study. Cuticular permeability and water loss of insects is generally correlated to the climate where they are distributed (Edney 1977, Hadley 1994). Body water content (%TBW) and desiccation sensitivity measured as the lethal amount of water lost also reflect adaptations to body water loss.

The %TBW content of insects ranges from about 40 to >90% (Edney 1977, Hadley 1994); immature stages generally have greater %TBW than adults. For Hemipterans, %TBW was about 69.6% in adult *Rhodnius prolixus* Stål (Gringorten and Friend 1982) and 78.3 to

81.3% in adult southern green stinkbugs, *Nezara viridula* (L.), (Piubelli et al. 2003). The %TBW of *M. cribraria* was similar to other Hemiptera and generally declined with increasing size (instar) in generation one; ranging from 88.9% for first instars to about 59% in adults (Table 1). Variation in %TBW among nymphal instars was  $\approx 28.4\%$ . In generation two, however, there was no significant difference in %TBW among most nymphal instars with only a  $\approx 6\%$  variation. Differences in %TBW between generations may reflect differences in the seasonal availability of plant sap, or insect growth rate. Fourth and fifth instar second generation nymphs were significantly heavier than first generation nymphs and contained slightly more body water.

The %TBW of *M. cribraria* for both generations one and two was higher compared with other insects that live in the same environment such as the imported red fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), *Geocoris punctipes* (Say) (Hemiptera: Lygaeidae), and *Reduviolus roseipennis* Reuter (Hemiptera: Nabidae) (Mack et al. 1988, Appel et al. 1991). This is not surprising because higher %TBW is expected for sap-sucking insects compared with chewing or predatory insects. Studies on insect energy budgets have shown that the standard metabolic rates are dependent on rates of energy (food) consumption. This is true for both sap-feeding and chewing insects. Sap-sucking insects have relatively low feeding efficiency (Llewellyn, 1982) and must therefore consume 100-1000-times their body weight per day of sap (Horsefield 1977, Mattson 1980), more than other herbivores. Plant sap is relatively nutrient poor so large amounts must be consumed and processed. It is therefore likely that consumption of water-rich plant sap may have contributed to the higher %TBW observed in *M. cribraria* compared with other insects.

The body water content of adult *M. cribraria*, was significantly lower in those preparing to overwinter than those collected in spring. This is not surprising, as many insects preparing to

overwinter typically reduce their water content through dehydration to avoid freezing and ice damage to cells (Danks, 2000, Holmstrup et al. 2002, Sinclair et al. 2013). Given the fat body and developing eggs in females, it is likely that overall body fat content reduced total body water content. Both prewinter and overwintered second-generation adult males had higher %TBW than females probably indicating they had lower fat reserves than females. Trade-offs between energy consumption and desiccation require that insects maintain balance between water-loss and thermal variability across all seasons (William et al. 2012, Sinclair et al. 2013). According to Tauber et al. (1986), insect diapause is accompanied by a corresponding decrease in metabolic activity, suppressed reproduction and more importantly, reduced water loss (Benoit and Denlinger, 2007). This suggests a scientific rationale for the lower water content recorded in the live adult *M. cribraria* preparing for overwintering compared to those collected in spring.

Insects lose water through various pathways such as transpiration through the cuticle, during respiration, excretion, and reproduction, but cuticular water loss generally accounts for the greatest proportion of total water loss (Hadley 1994, Gibbs and Johnson 2004). Loss of body water varies depending on the total body water and size of the insect, so smaller insects should lose water at a greater rate given their higher surface area to volume ratio than larger insects (Hadley, 1994). Our results clearly show that the rate of %TBW loss as well as initial body mass loss declined along with insect development; the greatest rate of %TBW loss occurred in the first instar and the lowest in the adult stages, particularly the prewinter adults (of both generations). Hull-Sanders et al. (2003) also reported that %TBW loss of first instar in juvenile stages of golden and mottled tortoise beetle was higher than other stages. The greater %TBW content we observed in younger instars may be a trade-off between their greater %TBW loss rate and desiccation tolerance, because insects with a higher initial body water content tend to have better



survival rates during desiccation than insects with a lower initial body water content (Gray and Bradley 2005). Additionally, the aggregation behavior of younger stinkbug instars facilitates intake of atmospheric water and protects against desiccation (Lockwood and Story 1986). Our finding that adults preparing to overwinter had lower %TBW loss rate compared with adults during the growing season and in spring which agrees with Ciancio's report (2018) on the brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), a species which was also introduced into the southeastern U.S. from Asia (Hoebeke and Carter 2003). Ciancio (2018) reported that overwintering adults *H. halys* had reduced water loss rates compared to non-overwintering individuals. The linear increase in %TBW and initial mass loss during 0 – 10 h desiccation for adults of both generations recorded has also been reported for many other insects including cockroaches (Blattodea: Blattidae, Ectobiidae, and Blaberidae) (Appel et al. 1983, Appel, 1991), termites (Isoptera: Rhinotermitidae) (Appel and Sponsler, 1990), other Hemiptera, and spiders (Mack et al. 1988).

Cuticular permeability of insects is related to the composition of epicuticular lipids, particularly hydrocarbons, with lower permeability correlated with greater amounts of longer chain saturated hydrocarbons (Hadley 1994, Blomquist and Bagnères 2010, Xu et al. 2018). Earlier studies reported a critical temperature or transition point of >30°C above which the water loss of many insects increased rapidly and abruptly (Ramsay 1935, Beament 1945). Xu et al. (2018) reported that the invasive red imported fire ant, *S. invicta* that is sympatric with *M. cribraria* in the U.S., has a higher water loss transition temperature and epicuticular lipid melting point than the black imported fire ant, *S. richteri*. Thus, *S. invicta* is able to retain more water under relatively higher temperature and occupy warmer and dryer environments than *S. richteri*. Cuticular permeability is also affected by other factors. The presence of a 'cuticular water pump'

controlled by epidermal cells removes water from the cuticle, which actively reduces water loss (Winston 1967, Winston and Beament 1969). Relative humidity affects saturation deficit, or pressure pulling water out of the insect. Temperature also affects saturation deficit and the phase of the lipid component of the epicuticular wax layer (Edney, 1977). Insects can acclimatize to microclimate changes by modifying the amount and composition of the cuticular wax layer (Gibbs et al., 1997, Marron et al., 2003). Hadley (1977) found that there were seasonal differences in the quantity of cuticular lipid in tenebrionid, *Eleodes armata* (Blaisdell), and showed beetles collected during the summer months had greater amounts of cuticular lipids than those collected during the winter. There can be differences in cuticular permeability among stages and between castes of the same species (Hadley 1994, Hu et al. 2012).

Xeric adapted insects classically have CP values ranging from 0.98 to 39.6  $\mu\text{g cm}^{-2} \text{h}^{-1} \text{mmHg}^{-1}$  whereas mesic adapted insects have CP values  $>50 \mu\text{g cm}^{-2} \text{h}^{-1} \text{mmHg}^{-1}$  (Edney, 1977). We found that the CPs of most stages of *M. cribraria*, were within the range of xeric insects even though they live in a humid subtropical climate. It is possible that the microclimates where *M. cribraria* lives are more xeric than the overall macroclimate. *Megacopta cribraria* is often observed on plants in direct sunlight, which might also increase the need for desiccation resistance and lower CP. Finally, the low CP may be related to desiccation sensitivity and allow the insects enough time to escape from otherwise lethal conditions.

There was relatively low mortality of first generation adult and nymphal *M. cribraria* at 10 h of desiccation (Table 4). Similarly, there was no more than 25% mortality of second generation *M. cribraria*; the greatest mortality was of prewinter adults. These prewinter adults had the lowest %TBW, probably due to increased fat content and reduction of water to prevent freezing, and likely lost lethal amounts of water more rapidly because less water was available.

Overwintered adults had low ( $\leq 10\%$ ) mortality at 10 h and had  $>36\%$  greater %TBW. This is probably due to reduction in body fat content by metabolism during the winter and egg laying by females immediately after overwintering in addition to feeding. Desiccation sensitivity, measured as lethal %TBW lost, was generally greater in younger instars. Fifth instars were the least sensitive in that they could tolerate ca. 75 %TBW loss in the first generation and 68 %TBW lost in the second generation. Final immature instars are often more resistant to desiccation than other stages (Edney 1977, Hadley 1994).

This study illustrates differences in body mass, %TBW, cuticular permeability and desiccation tolerance of first and second generation *M. cribraria*, an important new pest in the southeastern U.S. There are both seasonal (generation) and developmental stage differences in the water relations of this species. Parameters for first and second generation nymphs are similar, however first generation adults are most similar to overwintered second generation adults and not prewinter adults. Prewinter adults contain less body water than overwintered adults and are also less sensitive to desiccation. Overwintered adults have less fat and greater body water, but are more sensitive to body water loss. These results provide insights into developmental and seasonal changes in the water relations of *M. cribraria* and help to explain relative abundance in the fields and around homes.

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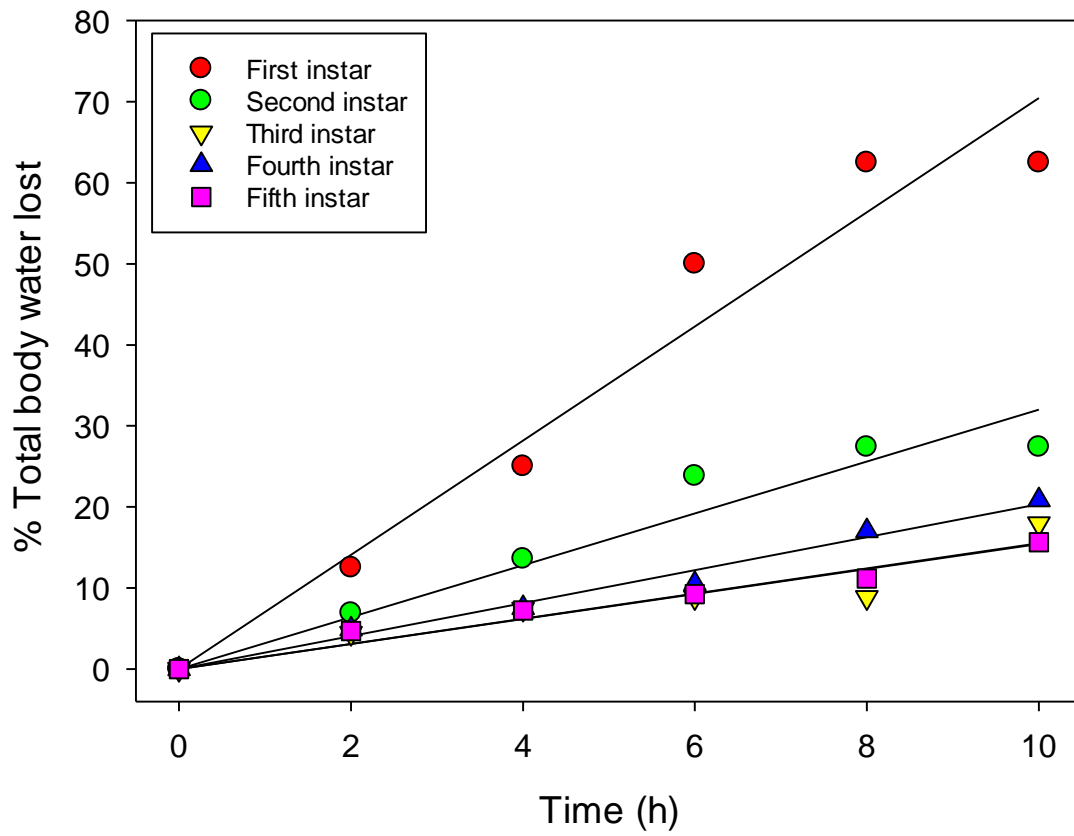
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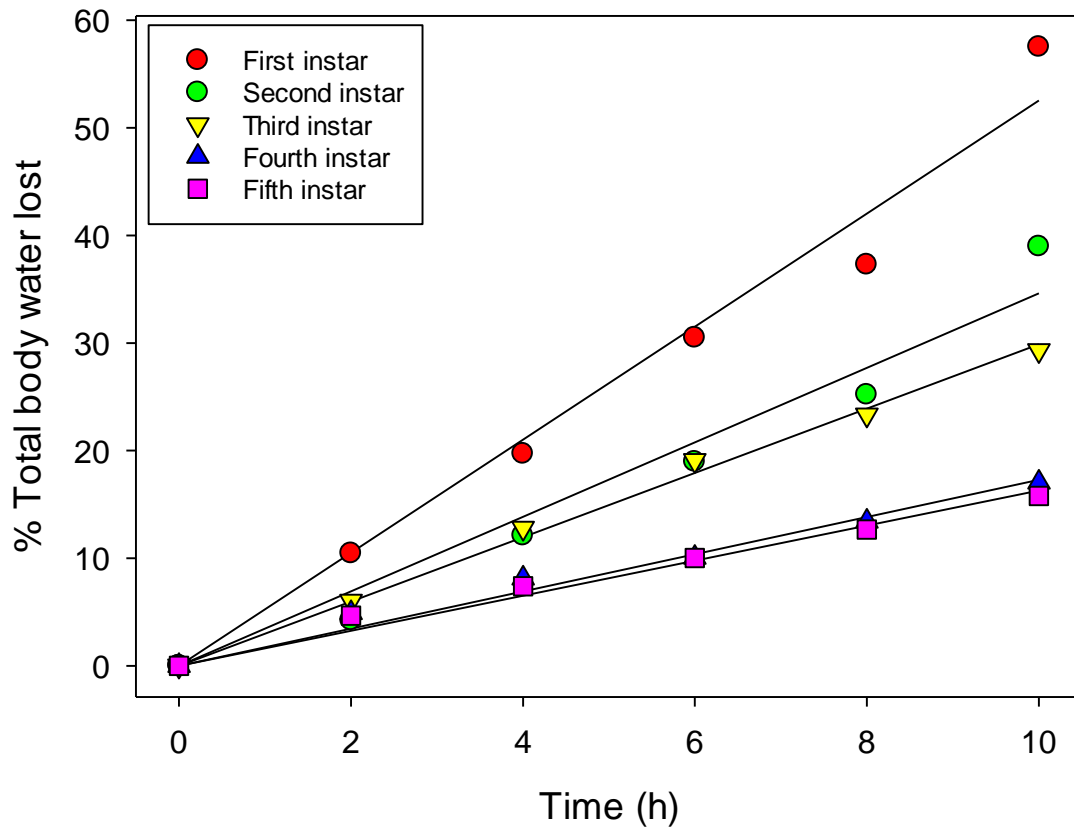
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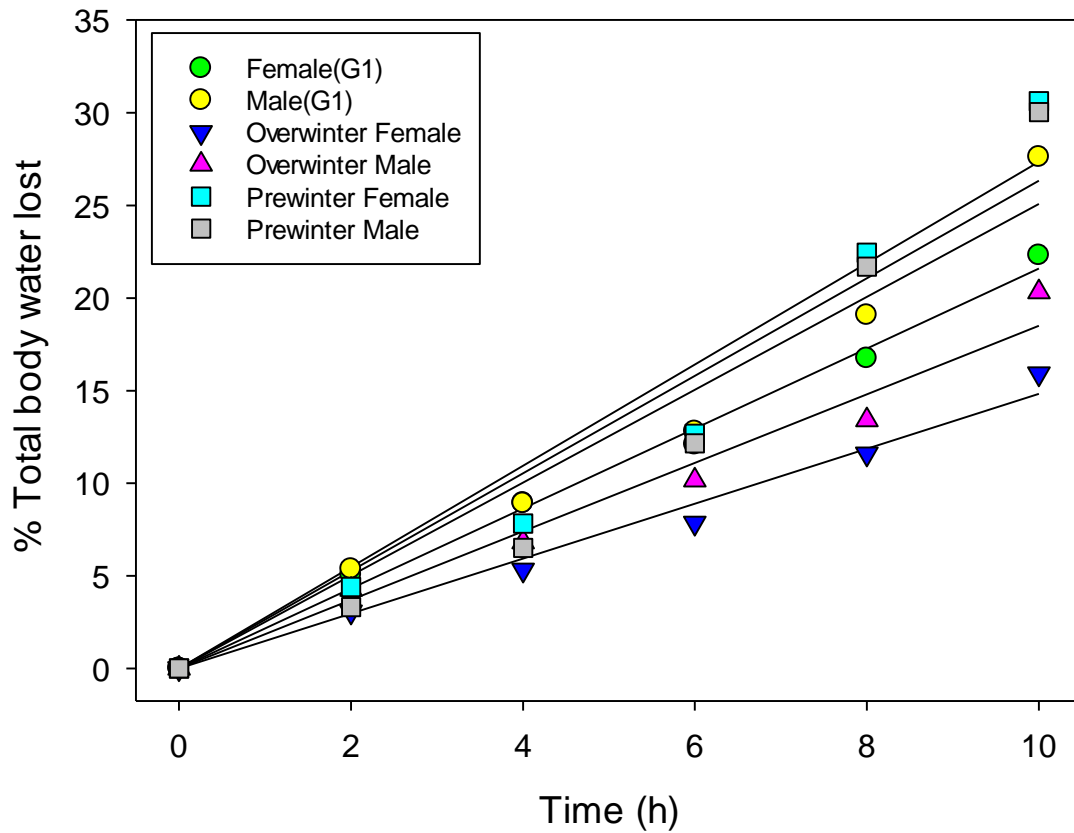
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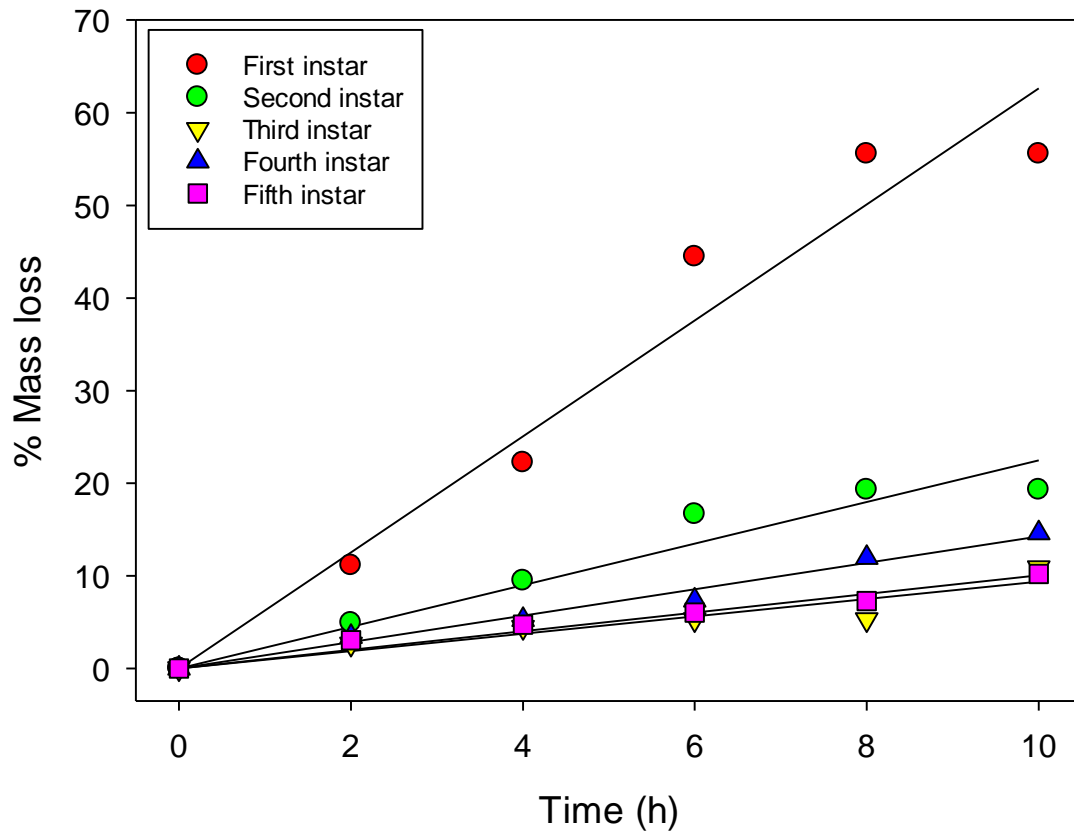
**Fig. 1a.** %Total body water lost over time for *M. cribraria* generation one nymphal instars



**Fig. 1b.** %Total body water lost over time for *M. cribraria* generation two nymphal instars

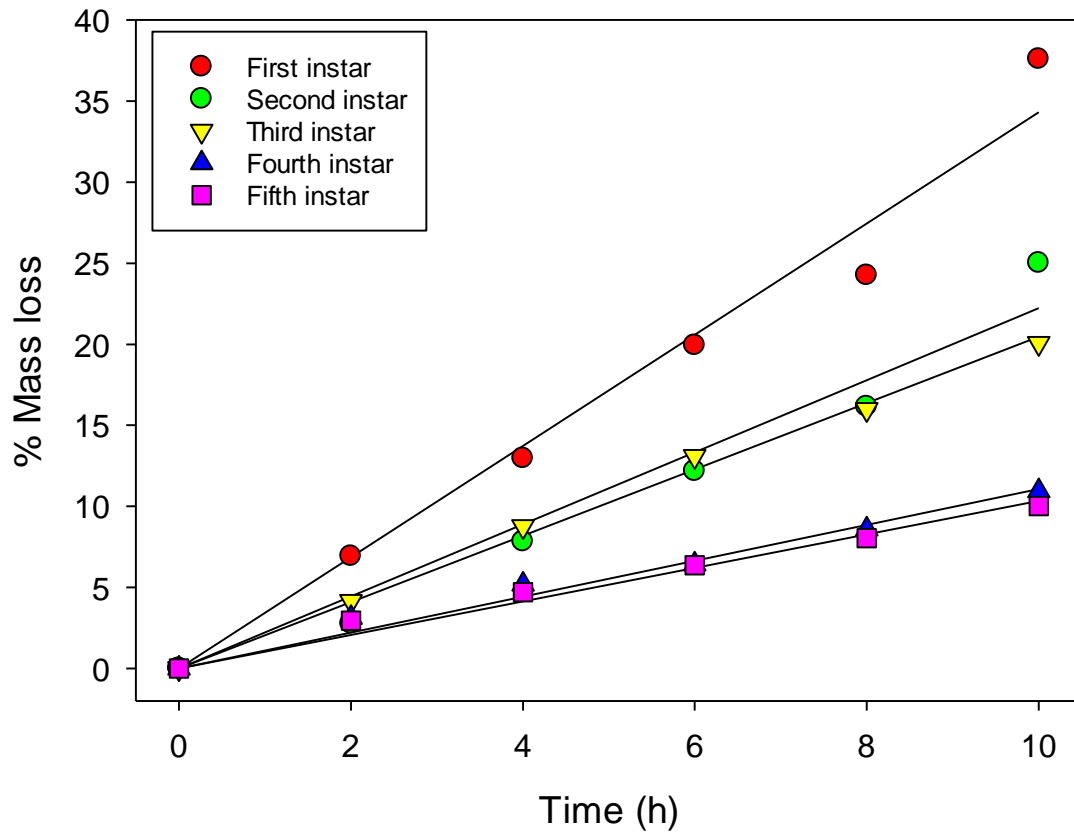


**Fig. 1c.** %Total body water lost over time for generation one and two *M. cribraria* adults

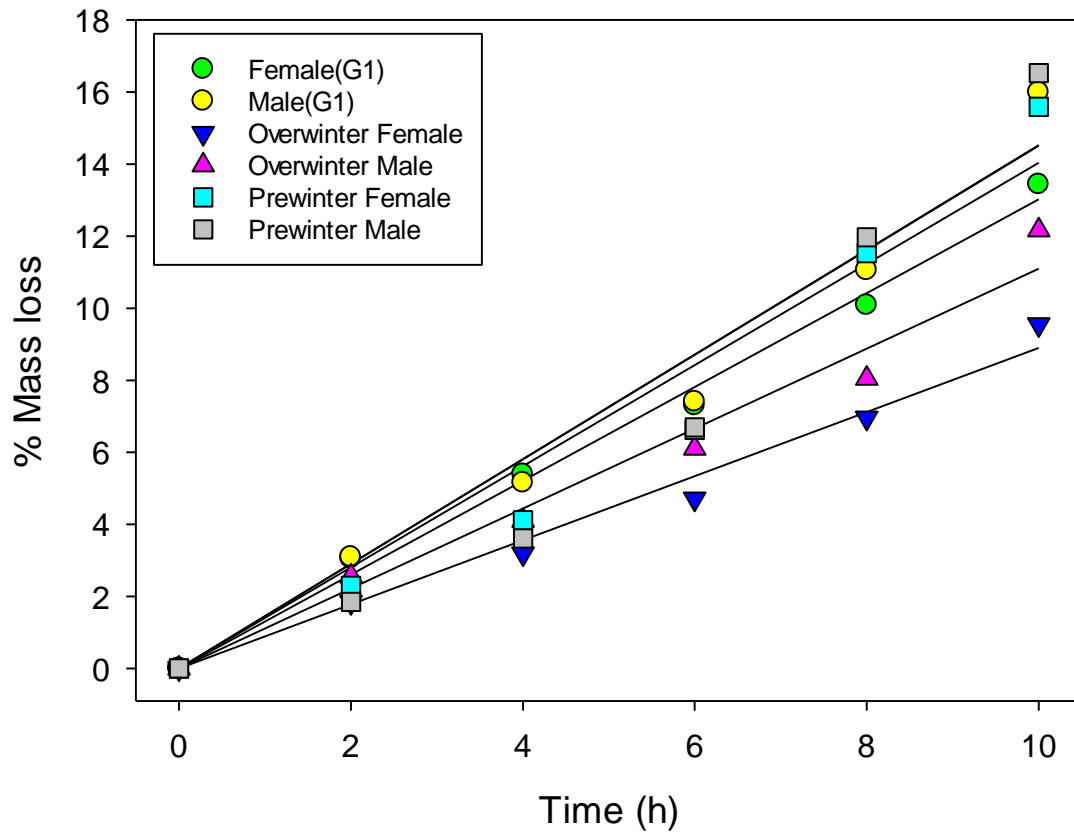


**Fig 2a.** %Mass loss over time for generation one *M. cribraria* nymphal instars





**Fig 2b.** %Mass loss over time for generation two *M. cribraria* nymphal instars



**Fig 2c.** %Mass loss over time for generations one and two *M. cribraria* adults

**Table 1.** Mean ( $\pm$ SE)\* initial fresh body mass, % total body water (%TBW), cuticular permeability (CP) ( $\mu\text{g cm}^{-2} \text{h}^{-1} \text{mmHg}^{-1}$ ), and mass loss (mg/g) of five nymphal instars and adults of *M. cribraria*.

Generation	Stages	No.	Initial mass			
		observations	(mg)	%TBW	CP value	Mass loss (mg/g)
1	1st Instar	20	0.045 ( $\pm$ 0.01)d	88.89 ( $\pm$ 0.00)a	5.17 ( $\pm$ 0.00)a	111.11 ( $\pm$ 0.00)a
	2nd Instar	20	0.205 ( $\pm$ 0.02)d	70.93 ( $\pm$ 2.75)b	3.78 ( $\pm$ 0.18)a	49.04 ( $\pm$ 3.59)b
	3rd Instar	20	0.56 ( $\pm$ 0.05)d	60.54 ( $\pm$ 2.21)de	2.87 ( $\pm$ 0.81)a	26.47 ( $\pm$ 6.86)b
	4th Instar	16	14.86 ( $\pm$ 1.34)c	68.37 ( $\pm$ 1.30)bc	10.35 ( $\pm$ 1.26)a	34.27 ( $\pm$ 5.49)b
	5th Instar	12	20.95 ( $\pm$ 1.02)b	64.94 ( $\pm$ 0.55)cd	11.06 ( $\pm$ 1.52)a	30.52 ( $\pm$ 4.01)b
	Female	10	30.28 ( $\pm$ 0.81)a	59.96 ( $\pm$ 0.76)de	12.33 ( $\pm$ 1.60)a	30.50 ( $\pm$ 4.17)b
	Male	10	20.95 ( $\pm$ 0.64)b	58.24 ( $\pm$ 1.13)e	11.11 ( $\pm$ 1.43)a	30.93 ( $\pm$ 4.03)b
2	1st Instar	150	0.21 ( $\pm$ 0.01)d	65.32 ( $\pm$ 1.55)ab	5.39 ( $\pm$ 1.11)bc	69.19 ( $\pm$ 14.28)a
	2nd Instar	90	0.64 ( $\pm$ 0.05)d	64.16 ( $\pm$ 1.52)b	3.1 ( $\pm$ 0.67)c	27.59 ( $\pm$ 5.97)b
	3rd Instar	40	1.88 ( $\pm$ 0.06)c	68.43 ( $\pm$ 0.53)a	6.68 ( $\pm$ 0.58)ab	41.43 ( $\pm$ 3.59)b
	4th Instar	10	12.44 ( $\pm$ 0.61)b	63.94 ( $\pm$ 1.25)b	9.48 ( $\pm$ 1.55)a	31.30 ( $\pm$ 5.08)b
	5th Instar	10	14.95 ( $\pm$ 0.83)a	62.41 ( $\pm$ 1.26)b	9.35 ( $\pm$ 1.56)a	29.54 ( $\pm$ 5.13)b
	Prewinter Female	21	26.09 ( $\pm$ 0.80)a	51.36 ( $\pm$ 1.77)a	8.71 ( $\pm$ 1.88)a	22.94 ( $\pm$ 5.09)a
	Prewinter Male	14	19.76 ( $\pm$ 0.66)b	54.79 ( $\pm$ 1.21)a	6.47 ( $\pm$ 1.15)a	18.46 ( $\pm$ 3.31)a
	Overwintered Female	20	28.44 ( $\pm$ 0.80)a	60.09 ( $\pm$ 0.38)a	7.32 ( $\pm$ 0.59)a	18.39 ( $\pm$ 1.49)b
	Overwintered Male	20	19.16 ( $\pm$ 0.39)b	59.96 ( $\pm$ 0.38)a	8.92 ( $\pm$ 1.03)a	25.55 ( $\pm$ 3.01)a

\*Means are the value of individual insects ( $\pm$  SE). Mean values within each column followed by the same letter are not significantly different according to the Waller Duncan k-ratio mean separation test ( $P < 0.05$ ).

**Table 2.** Regression statistic of % mass loss of five nymphal instars and adults of *M. cribraria* at 30°C and 0-2% RH (means  $\pm$  SE).

Generation	Stages	No. Observations	Slope	Intercept	R <sup>2</sup>	F	P
1	1st Instar	20	6.16 $\pm$ 0.69	0.53 $\pm$ 4.22	0.95	78.67	0.0009
	2nd Instar	21	2.09 $\pm$ 0.27	1.12 $\pm$ 1.61	0.94	61.81	0.0014
	3rd Instar	20	0.90 $\pm$ 0.17	0.24 $\pm$ 1.03	0.88	27.72	0.0062
	4th Instar	16	1.44 $\pm$ 0.09	-0.09 $\pm$ 0.56	0.98	237.98	0.0001
	5th Instar	12	0.93 $\pm$ 0.07	0.57 $\pm$ 0.45	0.97	155.32	0.0002
	Female	10	1.29 $\pm$ 0.05	0.09 $\pm$ 0.31	0.99	616.59	<0.0001
	Male	10	1.51 $\pm$ 0.12	-0.46 $\pm$ 0.74	0.97	151.09	0.0003
2	1st Instar	100	3.53 $\pm$ 0.27	-0.69 $\pm$ 1.66	0.97	165.48	0.0002
	2nd Instar	80	2.42 $\pm$ 0.20	-1.46 $\pm$ 1.23	0.97	140.64	0.0003
	3rd Instar	30	2.00 $\pm$ 0.06	0.32 $\pm$ 0.38	0.99	1026.17	<0.0001
	4th Instar	10	1.03 $\pm$ 0.05	0.54 $\pm$ 0.35	0.98	305.07	<0.0001
	5th Instar	10	0.96 $\pm$ 0.04	0.56 $\pm$ 0.30	0.99	369.51	<0.0001
	Prewinter Female	16	1.54 $\pm$ 0.14	-1.03 $\pm$ 0.90	0.96	107.68	0.0005
	Prewinter Male	8	1.65 $\pm$ 0.18	-1.51 $\pm$ 1.13	0.95	78.55	0.0009
	Overwintered Female	20	0.92 $\pm$ 0.05	-0.24 $\pm$ 0.33	0.98	271.12	<0.0001
	Overwintered Male	20	1.13 $\pm$ 0.09	-0.17 $\pm$ 0.55	0.97	155.42	0.0002

**Table 3.** Regression statistics of %TBW loss of five nymphal instars and adults of *M. cribraria* at 30°C and 0-2% RH (means  $\pm$  SE).

Generation	Stages	No.					
		Observations	Slope	Intercept	R <sup>2</sup>	F	P
1	1st Instar	20	6.96 $\pm$ 0.78	0.59 $\pm$ 4.75	0.95	78.67	0.0009
	2nd Instar	21	2.98 $\pm$ 0.38	1.61 $\pm$ 2.33	0.94	59.67	0.0015
	3rd Instar	20	1.49 $\pm$ 0.27	0.45 $\pm$ 1.68	0.88	28.72	0.0058
	4th Instar	16	2.04 $\pm$ 0.13	-0.11 $\pm$ 0.79	0.98	245.68	<0.0001
	5th Instar	12	1.42 $\pm$ 0.11	0.87 $\pm$ 0.69	0.97	155.6	0.0002
	Female	10	2.13 $\pm$ 0.09	0.15 $\pm$ 0.52	0.99	607.57	<0.0001
	Male	10	2.61 $\pm$ 0.21	-0.78 $\pm$ 1.29	0.97	150.48	0.0003
2	1st Instar	100	5.41 $\pm$ 0.41	-1.15 $\pm$ 2.49	0.97	172.34	0.0002
	2nd Instar	80	3.77 $\pm$ 0.31	-2.32 $\pm$ 1.93	0.97	139.97	0.0003
	3rd Instar	30	2.92 $\pm$ 0.09	0.47 $\pm$ 0.55	0.99	1027.78	<0.0001
	4th Instar	10	1.60 $\pm$ 0.09	0.88 $\pm$ 0.56	0.98	296.78	<0.0001
	5th Instar	10	1.50 $\pm$ 0.07	0.88 $\pm$ 0.47	0.99	371.51	<0.0001
	Prewinter Female	16	3.02 $\pm$ 0.31	-2.15 $\pm$ 1.88	0.96	95.07	0.0006
	Prewinter Male	8	3.01 $\pm$ 0.34	-2.78 $\pm$ 2.06	0.95	77.92	0.0009
	Overwintered Female	20	1.53 $\pm$ 0.09	-0.40 $\pm$ 0.56	0.98	270.91	<0.0001
	Overwintered Male	20	1.89 $\pm$ 0.15	-0.29 $\pm$ 0.93	0.97	151.32	0.0003

**Table 4.** Mean ( $\pm$ SE) mortality of five nymphal instars and adults of *M. cribraria* after 10 and 24 h exposure to 30°C and 0-2% RH.

Generation	Stage	No.	% Mortality		
		Observations	% Mortality (10 h)	(24 h)	%TBW lost at death
1	1st Instar	20	5 $\pm$ 0.02d	100 $\pm$ 0.37a	33.81 $\pm$ 5.33de
	2nd Instar	21	0 $\pm$ 0.00e	76.19 $\pm$ 0.29d	57.44 $\pm$ 4.56bc
	3rd Instar	20	20 $\pm$ 0.09b	45 $\pm$ 0.15e	50.13 $\pm$ 9.45c
	4th Instar	16	0 $\pm$ 0.00e	18.75 $\pm$ 0.07	63.84 $\pm$ 6.67b
	5th Instar	12	0 $\pm$ 0.00e	25 $\pm$ 0.09f	74.95 $\pm$ 2.11a
	Female	10	0 $\pm$ 0.00e	90 $\pm$ 0.34b	25.59 $\pm$ 6.52e
	Male	10	0 $\pm$ 0.00e	90 $\pm$ 0.34b	33.33 $\pm$ 3.01de
2	1st Instar	100	1 $\pm$ 0.08d	95 $\pm$ 0.36b	25.81 $\pm$ 8.92e
	2nd Instar	80	2.5 $\pm$ 0.01d	87.5 $\pm$ 0.33c	29.00 $\pm$ 4.44e
	3rd Instar	30	0 $\pm$ 0.00e	40 $\pm$ 0.15e	46.64 $\pm$ 7.20c
	4th Instar	10	10 $\pm$ 0.04c	20 $\pm$ 0.08g	58.94 $\pm$ 1.67bc
	5th Instar	10	10 $\pm$ 0.04c	10 $\pm$ 0.05h	67.79 $\pm$ 6.09b
	Prewinter Female	16	25 $\pm$ 0.11a	75 $\pm$ 0.25d	54.23 $\pm$ 4.99c
	Prewinter Male	8	25 $\pm$ 0.10a	87.5 $\pm$ 0.28c	67.02 $\pm$ 3.22b
	Overwintered Female	20	0 $\pm$ 0.00e	95 $\pm$ 0.36b	33.51 $\pm$ 1.21de
	Overwintered Male	20	10 $\pm$ 0.04c	100 $\pm$ 0.37a	32.95 $\pm$ 2.97de

Mean values within column followed by the same letter are not significantly different according to the Waller Duncan k-ratio mean separation test ( $P < 0.05$ ).