Biology and Management of *Megacopta cribraria* (Fabricius) (Heteroptera: Plataspidae): A Recent Invader to The United States

by

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Abstract

Identified as both an agricultural pest of soybean and a residential nuisance pest, *Megacopta cribraria* (F.) (Heteroptera: Plataspidae), is a recently invasive pest species to the southeastern United States. Isolated to a few northeastern Georgia counties upon initial detection in the fall of 2009, it has subsequently spread to 13 states where it can now be found as far north as Washington D.C. and as far west as Arkansas. Most of the research regarding this pest insect is focused on its impacts on agriculture and urban environments, however its biology and physiology seem currently overlooked. Information on these aspects could provide an important understanding of this insect in its new habitat. Therefore investigations were completed on some biological and physiological traits of *M. cribraria* that may provide an understanding of the rapid spread and success of this insect in its newly exploited geographical range. Through laboratory and field experiments, it was demonstrated that approximately 15% of females in three overwintering populations in Lee Co. (Auburn, AL) had mated before entering winter dormancy and sperm was stored in their spermatheca for up to seven months, oocytes in mated overwintering females proceeded to post-blastoderm stage before the onset of spring feeding/mating, all of the overwintering males had sperm in their testes, and the ratio of females gradually increased in populations during overwintering. This study indicates that both males and females are capable of reproductive quiescence. It also suggests that pre-overwinter mate females, containing both eggs and sperm, may be able to invade new territories and produce a subsequent generation in the absence of males; and the spread of this insect may be attributed to founders effect. The ability of *M. cribraria* to bypassing feeding on the presumed obligatory host, kudzu (*Pueraria montana* (Lour.) Merr. variety *lobata* (Willd.) Maesen and S. Almeida), was also addressed. Through no-choice greenhouse assays, overwintered generation *M. cribraria* could bypass feeding on kudzu to oviposition on mung bean (*Vigna radiata* (L.) R. Wilczek), soybean (*Glycine max* (L.) Merrill), butter bean (*Phaseolus lunatus* L.), and black-eyed pea (*Vigna unguiculata* (L.) Walp). No significant differences were observed in the number of egg masses, individual eggs, or in hatch rate among the five legume species. First-generation *M. cribraria* developed on all legume species except black-eyed pea, in approximately 55.6 d. Mortality from egg to adult was highest on black-eyed pea and butter bean followed by kudzu, mung bean and soybean. Results of this study suggest that kudzu is not an obligatory host for *M. cribraria*. Lastly, preliminary field surveys in the summer 2013 and 2014 discovered a native Dipteran (Tachinidae) species *Strongygaster triangulifera* utilizing adult *M. cribraria* for the development of its offspring, while the non-native, but natural occurring, *Paratelenomus saccharalis* (Hymenoptera: Platygastridae), was found in AL, GA, and MS.

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CHAPTER 1

GENERAL INTRODUCTION

Biology of *M. cribraria*

Taxonomy

The family Plataspidae contains a reported 560 species and 59 genera (Davidova-Vilimova and Stys 1980, Henry 2009). It is nested within superorder Pentatomoidea and resides within the order Hemiptera. *Megacopta cribraria* was described by Fabricius in 1798 as *Cimex cribrarius*, and since several species have been placed in several genera, *Tetyra*, *Thyreocoris* and *Coptosoma*, before being placed in the genus *Megacopta Coptosoma xanthochlora* (Walker 1867) is a synonym.

Megacopta cribraria, commonly kudzu bug, was originally identified in the United States by general and genital morphological studies (Eger et al. 2010, Ruberson et al. 2010), followed by mitochondrial and nuclear DNA sequencing of the primary and secondary endosymbionts (Jenkins and Eaton 2011) and genetic studies of the cytochrome oxidase I sequence (Jenkins et al. 2011). Results of genetic testing matched with *M. cribraria* and the closely related, *M. punctatissima* (Montandon), however, the two species are likely conspecific (Eger et al. 2010, Hosokawa et al. 2014) and have been considered synonymous (Yang 1934, Davidová-Vilímová 2006).

External Morphology

Morphological characteristics can be used to distinguish the Plataspidae from other members of the Pentatomoidea of North America, and dimorphic characteristics aide in the differentiation between male and female *M. cribraria*. One of the most distinctive traits of this family is the complex folding of the hind-wing under the scutellum that is evident in adult insects (Schuh and Slater 1995). However, to aide in the identification to species level, Eger et al. (2010) provided a key to families of Pentatomoidea in America north of Mexico that includes *M. cribraria*. The main diagnostic characteristics used in the separation of Plataspidae, and thus *M. cribraria* (as it is the only Plataspidae in the U.S.), from other Pentatomoidea are 1) two segmented tarsi and 2) a greatly enlarged scutellum covering the majority of the abdomen. Overall, the appearance of adult insects can be defined as such: 3.5- 6.0 mm in length, globular in shape, and brown to olive green in color with dark punctuations along the dorsal side (Figure 1.1) (Eger et al. 2010).

Dimorphic characteristics differ with respect to the coloration of the sternites, curvature of the terminus, and shape of the genitalia capsule. The sternites of females are lighter in color, but with an area of dark coloration restricted medially, while in males the sternites are usually entirely dark in coloration (Figure 1.2). Females have a rounded terminus, whereas males are truncated or blunt terminally. Lastly, the genital segments of the male form a circular cup, unlike those of the female, which appear triangular in shape (Figure 1.3).

As is common with other stinkbugs and most Heteroptera (Aldrich 1988), *M. cribraria* contain scent glands used in the secretion of both defensive substances and attractant pheromones (Kitamura et al. 1984). Nymphal *M. cribraria* contain dorsal abdominal scent glands that function primarily for defensive purposes (Vilímová and Kutalova 2011) and open through ostia located between tergal segments 3-4, 4-5, and 5-6 (Ahmad and Moizuddin 1975b, Schuh and Slater 1995). Adult *M. cribraria* will additionally develop ventral metathoracic scent glands.

Secretions from the ventral metathoracic glands of adult Plataspidae are similar to other pentatomid stinkbugs in that they are composed of alkanes and unsaturated hydrocarbons. More specifically the secretion is a blend of undecane, dodecane, tridecane, pentadecance, decenyl acetate, octenal, decenal, and oxo-hexenal (Baggini 1966, Kitamura 1984).

Figure 1.1. Overall appearance of *M. cribraria* (Photo by J. E. Eger, Jr. 2010).

Figure 1.2. Ventral view of female (left) and male (right) *M. cribraria* showing sternite coloration and terminus shape.

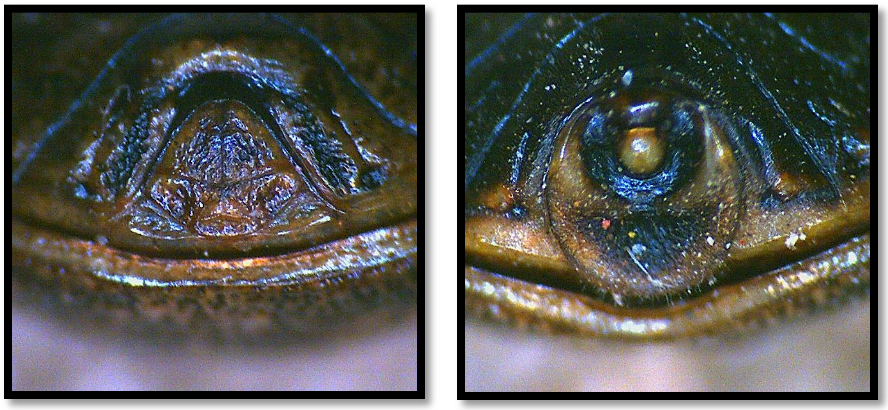

Figure 1.3. Posterior view of the genital capsule of female (left) and male (right).

Reproductive Morphology

Relatively few studies have addressed the internal morphology of the kudzu bug (Ahmad and Moizuddin 1975a). Moreover, only three studies have focused on the internal reproductive structures of the family Plataspidae. Pendergrast (1957) conducted a study of the reproductive organs of a single member of this family to better place Plataspidae amongst other Heteroptera. Using only two species from the family

Plataspidae, Miyamoto (1957) determined the ovariole number of females. Ahmad and Moizzuddin (1975a) provided the most extensive examination of the reproductive morphology of both male and female *M. cribraria*. Descriptions of the reproductive tracts of adults are contained within the report by Ahmad and Moizzuddin (1975a) and will not be repeated here in detail. Briefly, however, the authors reported seven ovarioles in females (Figure 1.4) and seven testicular follicles in males (Figure 1.5) in each of the paired sexual organs. These findings are considered generalized features of lower Pentatomoidea, including the Plataspidae and primitive pentatomids (Ahmad and Abbasi 1971). Also included in the generalized reproductive features of this family is the female spermathecae morphology; e.g., having a highly sclerotized spermatheca, containing a small pump region, lacking medial dilation in the spermathecal duct, and lacking ectodermal sacs (Ahmad and Abbasi, 1971).

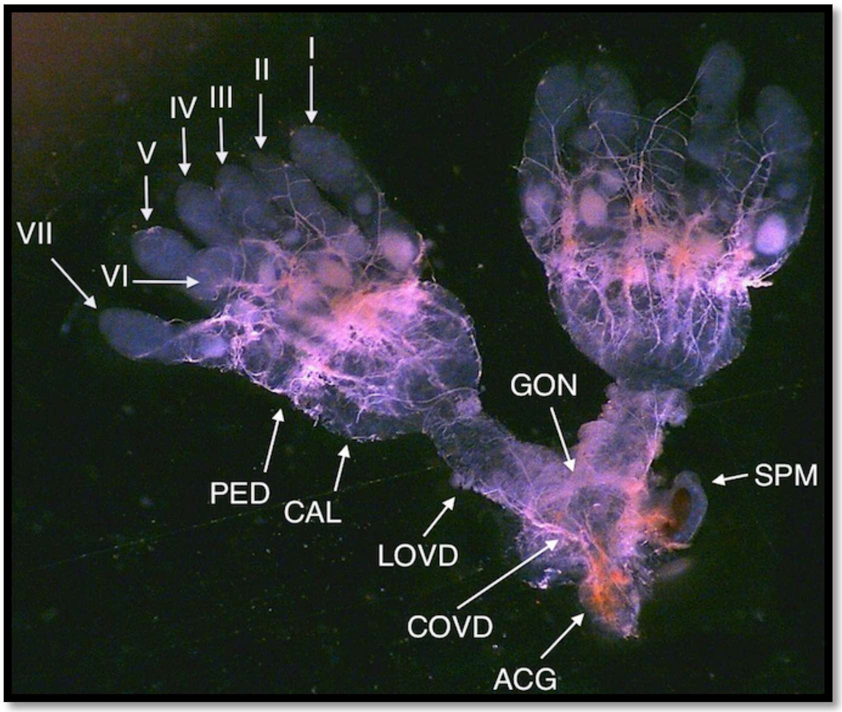

Figure 1.4. Female reproductive tract. Abbreviation definitions; I-VII Ovarioles; PED: pedicel; CAL: calyx; LOVD: lateral oviduct; COVD: common oviduct; GON: gonopore; ACG: accessory gland; SPM: spermatheca.

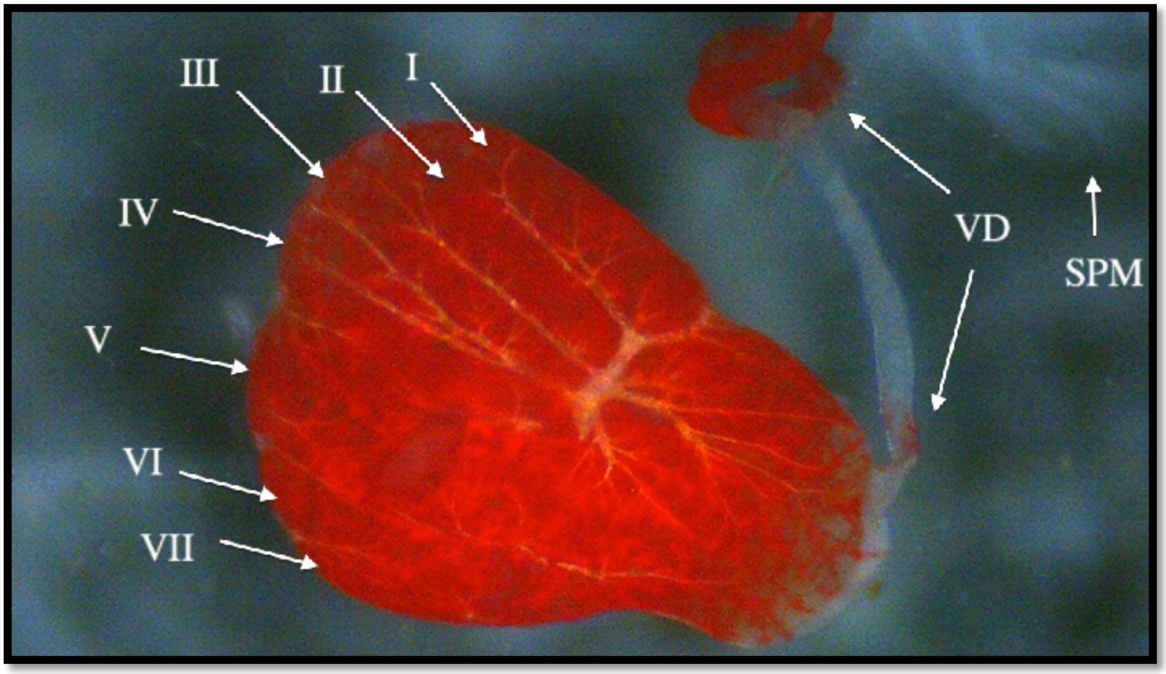

Figure 1.5. Male testis. Testicular follicles represented by roman numerals I-VII; VD: vas deferens; SPM: spermatozoa.

Geographical Distribution

Megacopta spp. are native to the Old World and Oceania where they have been reported from India, China, Australia, Indonesia, Japan, Korea, Macao, Myanmar, New Caledonia, Pakistan, Sri Lanka, Thailand, Vietnam, Taiwan, and Malaysia (Montandon 1986, 1987, Distant 1902, Kirkaldy 1910, Matsumura 1910, Shroff 1920, Esaki 1926, Hoffman 1931, 1935, Yang 1934, Ishihara 1937, Esaki and Ishihara 1950, Ahmad and

Moizuddin 1975, Hsiao and Ren 1977, Lal 1980, Ren 1984, Hirashima 1989, Easton and Pun 1997).

Megacopta cribraria was first detected in the United States in Hoschton, Jackson County, GA in October 2009, and represents the first report of a Plataspidae on the North American continent (Eger et al. 2010), although the closely related *Coptosoma xanthogramma* (White) (Hemiptera: Plataspidae) has been reported from the Hawaiian Islands (Beardsley and Fluker 1967). Since the initial detection in 2009, it has subsequently been identified in 13 U.S. states: Alabama, Arkansas, North Carolina, South Carolina, Mississippi, Louisiana, Kentucky, Florida, Tennessee, Virginia, Delaware, Maryland, and Washington D.C. (www.kudzubug.org).

Life cycle

Like all other Heteroptera, *M. cribraria* undergoes hemimetabolous development. Beginning from an egg, it completes 5 nymphal instars before eclosing to an adult (Figure 1.6) over a period of 25-56 days in its native regions (Eger et al. 2010). General features of each instar from first-to-fifth can be seen in the coloration of the body, scalloping of the lateral margin, increased number of setae, and development of the wing pads (Moizuddin and Ahmad 1975). However a more reliable determination of the instar is the width between the eyes and body size (Zhang et al. 2012). Adult longevity differs between subsequent generations. In a bivoltine population of *M. cribraria* from China, Zhang and Yu (2005) reported that first generation adults live 1.5-3 months, whereas the second generation lives 9-10 months. These differences may be due to the overwintering dormancy of the second-generation adults.

Males and females engage in large mating aggregations where females select their mates before copulation (Hibino and Itô 1983, Hibino 1986, Hosokawa and Suzuki 1993, 1999, 2001). Pairs engage in copula by aligning in an end-to-end configuration and may remain so in excess of 10 hours, even though sperm transfer is regularly completed within 2-4 hours (Hoskawa and Suzuki 2001). This act of prolonged copulation is thought to act as mate guarding by preventing re-mating of females by other males (Parker 1970, Simmons and Siva-Jothy 1998).

Following copulation there is an incubation period of approximately 4-7 days (Ahmad and Moizuddin 1977, Srinivasaperumal et al. 1992, Hosokawa and Suzuki 2001), after which females will deposit egg masses in parallel rows. Females prefer to oviposit on actively growing leaf sheaths of the kudzu vine (*Pueraria montana* (Lour.) Merr. variety *lobata* (Willd.) Maesen and S. M. Almeida) (Zhang et al. 2012). During egg deposition, the female simultaneously places small, brown-colored endosymbiont capsules underneath the egg mass. These contain two essential obligate bacterial endosymbionts: the primary gamma-proteobacterial, *Candidatus Ishikawaella capulata*, and the secondary alpha-proteobacterium, *Wolbachia* (Figure 1.7) (Fukatsu and Hosokawa 2002, Hosokawa et al. 2007, Zhang et al. 2012). After a 3-5 day incubation period, first instars hatch (Ahmad and Moizuddin 1977, Sprinivasaperumal et al. 1992) and ingest the bacteria contained in the fecal capsules before dispersing to obtain plant food. The acquisition of these bacteria is essential for proper development and reproduction in the Plataspidae (Fukatsu and Hosokawa 2008, Hosokawa et al. 2010). Without the mother-mediated transfer and ingestions of these symbiotic bacteria, development of the insect is severely retarded and mortality increases dramatically (Müller 1956, Hosokawa et al. 2007).

Figure 1.6. Instar development from first (left) to fifth (right) (Photo by Zhang et al. 2012).

Figure 1.7. Endosymbiont capsules indicated by arrows (Photo by J.E. Eger, Jr. 2010).

Seasonal Activity and Voltinism

In the United States, adult *M. cribraria* have three peaks of activity. The first peak is seen after overwintering adults become active in late March through early May. The second peak occurs after the first generation has developed, approximately late June to August. The last peak is seen as adults migrate from feeding sites to overwintering sites beginning in early October (Zhang et al. 2012). Nymphal development is gradual and nymphal instars overlap with one another. First, second, third, and fourth instars can be seen in development from late April through late June. Fifth instars are often seen late June through July (Zhang et al. 2012).

Activity peaks of adults in the southeastern U.S. approximately correspond with the activity of this insect in its Asian distribution (Tayutivutikul and Yano 1990, Wang et al. 1996). While the seasonal activity of this insect occurs almost equally throughout its range, its voltinism differs between newly invaded areas as compared to native regions. One to three generations a year have been reported from eastern China (Wang et al. 1996), one generation a year has been observed in Southern Japan (Hibino and Itô 1983), and two generations a year have been reported from south-central Japan and Thailand (Tayutivutikul and Yano 1990). In warmer climates it has been indicated that *M. cribraria* may be active throughout the entire year (Tippeswamy and Rajagopal 1998a). In the U.S. it is reported as a bivoltine species (Zhang et al. 2012).

Plant Damage and Pest Status

The Plataspidae is one of few Hemipteran taxa that preferentially feed on legume species (Schaefer 1988), and has thus been problematic to the production of legume crops throughout the Old World (Yang 1934, Hasegawa 1965, Lal 1980, Ren 1984). The kudzu bug prefers to feed on new plant growth, where it extracts photosynthate from the phloem (Zhang et al. 2012). It can commonly be found feeding along plant stems, petioles, leaves, pods, and possibly flowers (Zhang et al. 2012, Seiter et al. 2013b) of various plants. Damage resulting from the feeding activity is seen as purple spots that later coalesce to form large black necrotic regions (Figure. 1.8) (Thippeswamy and Rajagopal 2005), and extensive feeding may result in defoliation (Chaterjee 1934). As it is a phloem feeder, *M. cribraria* produces copious amounts of honeydew that results in secondary plant issues such as black sooty mold leading to reductions in photosynthetic ability of the plant (Zhang et al. 2012).

The preferred host plants of *M. cribraria* in its native regions are the wild legume kudzu, *Pueraria montana* (Lour.) Merr. variety *lobata* (Willd.) Maesen and S. M. Almeida, and the cultivated soybean, *Glycine max* var. Merill (Ishihara 1950, Zhang et al. 2012), where it is most frequently reported as a pest of this bean in Asia (Hoffman 1932, Ishihara 1950, Kobayashi 1981, Kono 1990, Wang et al. 1996, Wu and Xu 2002, Hosokawa et al. 2007, Xing et al. 2008). In India it is commonly reported to attack *Dolichos lablab* (L.) (Ahmad and Moizuddin 1975b, 1977; Thippeswamy and Rajagopal 1998, Thejaswi et al. 2008). However the bean plataspid is not limited to these species, as it appears to have a broad host range of nearly 20 leguminous (Fabaceae) and 14 non-legume species spanning 14 plant families in its native range (review by Eger et al. 2010).

Reports from the United States are limited on the host range of this insect *in agro*, however it likewise feeds on kudzu and soybean. As *M. cribraria* is a recently invasive species to the U.S., researchers are currently trying to determine whether or not it similarly displays a broad host range. To date, Zhang et al. (2012) present the only field choice experiments regarding this issue. They observed *M. cribraria* to feed on 10 forest legume species and non-selectively oviposit on 8 species, although adults developed only on soybean. While the development of the insect was apparently not possible on other plants during the study, it indicates that *M. cribraria* is capable of utilizing additional plant species for a nutrient source and can survive for an extend period of time on their respective feeding plant.

Other studies such as Medal et al. (2013) conducted no-choice greenhouse experiments and similarly found the plataspid to non-selectively oviposit on some of the tested legume species. The authors found the plataspid to be capable of successful development on the pigeon pea (*Cajanus cajan* L.), black-eyed pea (*Vigna unguiculata* (L.) R. Wilczek), lima bean (*Phaseolus lunatus* L.), and pinto bean (*Phaseolus vulgaris* L.) in addition to kudzu and soybean.

Figure 1.8. Feeding damage caused by *M. cribraria* on soybean (Photo by Seiter et al. 2013b).

Economic Importance

The kudzu bug is a well document pest of soybean throughout its native distribution, effecting the production of this crop in India (Thippeswamy and Rajagopal 2005), China (Wang et al. 1996, Wu and Xu 2002, Xing et al. 2008), and Japan (Ishihara 1950, Kobayashi 1981, Takasu and Hirose 1985, Kono 1990, Hosokawa et al. 2007, Kikuchi and Kobayashi 2010). Its impact on this bean can be extremely high. For instance in China, researchers have reported yield reductions up to 50% (Wang et al. 1996).

In the cultivation of the lablab bean, *Lablab purpureus* (L.), a key edible bean crop in India and the Middle East, *M. cribraria* has repeatedly been reported to occur at high levels in fields throughout the cropping season (Ahmad and Moizuddin 1975, Thippeswamy and Rajagopal 1998, 2005a; Rekha and Mallapur 2007, Thejaswi et al.

2008, Sujithra et al. 2008). This feeding activity has resulted between 9-44% reductions in seed yield and size of this crop in India (Thippeswamy and Rajagopal 1998).

Similarly in the United States, the kudzu bug has begun to disrupt the cultivation of the economically important soybean (Ruberson et al. 2012). In 2010, *M. cribraria* was observed in soybean fields during early vegetative growth stages (V1-V3) in Georgia and South Carolina (Seiter et al. 2013b). However no quantitative information on plant impacts existed at that time. Limited studies have been conducted since then on the effects of *M. cribraria* feeding, and have demonstrated that this insect is capable of severely impacting soybeans. Preliminary studies by Greene et al. (2012) showed that *M. cribraria* reduced crop yields in untreated fields upwards of 47%, however an average yield loss was observed at 18%. Another study by Seiter et al. (2013b) demonstrated that naturally occurring peak densities were capable of not only reducing yield by nearly 60%, but feeding stress reduced seed weight, and effected pod size and seed-set per pod of soybean.

In economic terms, 2011 marked the first year yield losses were reported from the kudzu bug. According to a report by the Midsouth Entomologist in 2011 (Musser et al. 2012), a total of 31,349 bushels were lost per acre at cost of pesticide treatment plus loss of yield at US$988,034.00 throughout the Southern U.S. Comparing reports from 2011 to 2012 (Musser et al. 2013), 2012 showed an additional 366,600 acres contaminated by this insect, and a further 61,000 acres treated with insecticides. Overall, representing a 24-fold increase in areas contaminated with the kudzu bug, and a 6.4-fold increase in acres sprayed from 2011 to 2012. In Alabama, this represents an average loss plus cost of control around US$28.50 per hectare (Musser et al. 2013).

As this insect appears to be expanding its range, these numbers will likely increase over time. This is extremely alarming as soybeans are the second most abundant crop grown in the U.S., with the first being corn (www.epa.gov). In total, the U.S. produced over 3 billion bushels of soybeans in 2012, and exported nearly 45%, or 1.345 billion bushels internationally that same year. The total value of the crop in 2012 was US$43 billion dollars (According to the American Soybean Association via www.soystats.com).

Natural Enemies and Predators

Natural Enemies and Predators Abroad

In Asia and regions once endemic to the kudzu bug, there exists a large egg parasitoid complex, minor predatory insects and arthropods, and a single fungus that all may offer some control of populations of *M. cribraria*. This includes 7 reported egg parasitic Hymenopteran species, 2 predatory Heteroptera, and 2 separate reports of an arachnid and fungus.

The Hymenoptera species include, *Ensarsiella boswelli* (Hayat), *Ablerus* sp. (Howard), *Dirphys boswelli* (Girault) (Aphelinidae), *Paratelenomus saccharlis* (Dodd) (Platygastridae), *Trissolcus* sp. (Scelionidae), and *Ooencyrtus nezarae* (Ishii) (Encyrtidae). These all appear widespread throughout the Old World (Ahmad and Moizuddin 1976, Srinivasaperumal et al. 1992, Polaszek and Hayat 1996, Rajmohan and Narendran 2001, Zhang et al. 2003).

The occurrence of additional natural enemies throughout areas native to the kudzu bug include the Heteropteran predators *Reduviius* sp. (Reduviidae) and *Antilochus coqueberti* (Fabricius) (Pyrrhocoridae) (Ahmad and Moizuddin 1976), the spider *Oxyopes shweta* (Tikader) (Araneae: Oxyopidae) (Borah and Sarma 2009), and the entomopathogenic fungus *Beauveria bassiana* (Borah and Dutta 2002) found infecting 31% in nymphs and 19% in adults sampled in Assam, India.

Rates of Parasitism Abroad

The least dominate of the egg parasitoids are the *Trissolcus* sp. (Hymenoptera: Scelionidae) that co-exists with *O. nezarae* in China. Collection of these wasps in a soybean field in China accounted for less than 2% of the total parasitoids *in agro* (Zhang et al. 2003). *Alberus* sp. (Hymenoptera: Aphelinidae), a wasp from southern India, appears more dominate, being discovered developing within nearly 60% of *M. cribraria* eggs collected from a lablab bean field (Rajmohan and Narendran 2001).

The most successful and frequently reported parasitoids of *M. cribraria* in Asia are *Paratelenomus saccharalis* (Hymenoptera: Platygastridae), a specialist of the family Plataspidae, and *Ooencyrtus nezarae* (Hymenoptera: Encyrtidae), a generalist egg parasitoid of numerous phytophagous Hemipterans. Parasitism by these wasp species can be very high, for example Wall (1928, 1931) found that parasitism by *P. saacharalis* occurred in 51% of eggs that were field-collected from June-August in Guangdong Providence, China. In Japan, Takasu and Hirose (1986) found parasitism of *M. punctatissima* (=*cribraria*) eggs between 43-100% in a kudzu patch. While parasitism by *P. saccharlis* in southern India ranged from 4.3-20.6% depending on both the crop and the month (Srinivasaperum et al. 1992).

Natural Enemies and Predators in the U.S.

Being previously restricted to the Eastern Hemisphere many natural enemies and insect predators that may keep populations of the kudzu bug below economic thresholds in Asia and elsewhere, have not been found in the newly invaded region of the southeast United States. Nonetheless, there have been various observations and discoveries of native natural enemies attacking the kudzu bug in the United States.

Through field observations and molecular gut-content analysis Ruberson et al. (2013) and Greenstone et al. (2014) reported several existing generalist predators including *Euthyrhynchus floridanus* (L.) and *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), *Geocoris uliginosus* (Say) and *G. punctipes* (Say) (Hemiptera: Geocoridae), *Zelus renardii* (Kolenati) (Hemiptera: Reduviidae), *Hippodamia convergens* (Guérin-Méneville) (Coleoptera: Coccinellidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae), the spiders *Oxyopes salticus* (Hentz) and *Peucetia viridans* (Hentz) (Araneae: Oxyopidae), the entomopathic fungus *Beauveria bassiana* (Balsamo) Vuillemin, and *Phasia robertsonii* (Townsend) (Diptera: Tachinidae) found parasitizing a single adult. Additionally surveys by Golec et al. (2013) detected the presence of the adult parasitoid, *Strongygaster triangulifera* (Loew) (Diptera: Tachinidae), and collaborative efforts of Auburn University (Auburn, AL), University of Georgia (Griffin, GA), and Emory University (Atlanta, GA) detected the presence of the egg parasitoid, *Paratelenomus saccharalis* (Gardner et al. 2013).

Control Strategies: Urban & Agricultural

Urban Control Strategies

The seasons that urban residents will most frequently encounter kudzu bugs are during fall, when the insect seeks overwintering sites, or in spring when it emerges from overwintering sites in search for food (Suiter et al. 2010a, 2010b). Due to its attraction to light colors, especially white (Horn and Hanula 2011), *M. cribraria* frequently ends up on or in homes (Figure. 1.9) and when populations are high, will often come in contact with vehicles and people (Eger et al. 2010). When homeowners first reported seeing large aggregations of this lesser known urban pest to professional pest management companies, their response was the application of pyrethroid-based sprays to the outside of effected homes (Suiter et al. 2010), even though efficacies of pyrethroids had not been tested in residential settings at this time. Seiter et al. (2013a) addressed this fundamental question, and are so far, the only researchers to have tested the efficacy of insecticides on residential surface materials (including vinyl, metal, bare and painted wood, and brick). It showed that multiple pyrethroid and pyrethroid-neonicotinoid compounds (Thiamethoxam + λ-cyhalothrin, Bifenthrin, Acetamiprid + Bifenthrin, and Imdicacloprid + β-cyfluthrin) caused 100% mortality within twenty-four hours, yet efficacies of the treatments were reduced when applied to more porous building materials such as wood and brick. However as this was a controlled laboratory study, not much is understood on the extent to which environmental factors like UV-light and rain, have on the degradation of these chemicals (Konstantinou et al. 2001). It has since been recommended that broad-spectrum insecticides not be applied to residential structures due to their potential toxicity to residents and unintended environmental consequences (Ruberson et al. 2010, U.S.

Environmental Protection Agency [EPA] 2013). Nonetheless, these results have broadened our understanding of the use of insecticides against this invasive pest, and may increase the efficacy of which spot treatments are performed on public structures.

To date, the most safe and effective methods and primary means for preventing *M. cribraria* in urban settings are a combination of integrated pest management approaches (IPM), which include physical, cultural, chemical, and biological practices. The most effective means of preventing entry of nuisance pests into homes is to seal cracks and crevices (Appel 2003); this can be done by caulking window seals, replacing screening, and/or placing steel wool inside ventilation chimneys or pipes (Suiter et al. 2010, Golec et al. 2014a). Aggregating adults may be power-washed off residential structures (Golec et al. 2014a). It is advised not to crush adults that are inside of homes; they will release a foul odor and they may stain fabrics and skin (Figure 1.10) (Suiter et al. 2010), and cause irritation of the skin and eyes (Wong and Mak 2012). They should be removed by using either a household vacuum with pantyhose secured around the vacuum tube with a rubber band, or they can be vacuumed with a shop-vac where the canister is filled with soap water (Golec et al. 2014a).

Adult *M. cribraria* may enter home gardens and ornamental plantings around residential structures, infestations of this manner may be mitigated by washing of the infested plant(s) with water, knockdown techniques (placing a bucket containing soapy water under effect plant(s)), or deterring insects from gardens/homes by the use of white cross-vane bucket traps as described by Horn and Hanula (2011).

Another approach to lessen problems associated with the kudzu bug is to treat kudzu patches adjacent to residential areas. This can be done by one or two ways: directly exterminating the insects via application of insecticides, or by removing kudzu patches in nearby residential areas. The best time to treat kudzu patches with insecticides is in early spring when insects form large feeding aggregations in kudzu patches. Many products are available in common local retail garden centers, and the most efficient insecticides for homeowner use are those containing pyrethroids. Popular amongst these retail stores are the deltamethrin-based D-Fense^{TM} (Control Solutions Incorporated [CSI], Pasadena, TX, USA), and Delta Dust^{TM} (Bayer Environmental Science, Research Triangle Park, NC, USA). The second method, removal of the kudzu patch(es), can be accomplished by both summer and fall mowing of vines, or by applying foliar herbicides to the leaves. Removing kudzu by mowing is an intensive process that must be repeated nearly weekly during the growing season, for multiple years. When pruning the vines and overall biomass, it must be completely defoliated and brought flush with the ground. Although this is a long-term method of kudzu control, it is the most environmentally friendly means of eradication (Enole and Loewstein 2014).

When adopting to treat with herbicides, it is recommended to first treat with a foliar applied herbicide when leaves are fully expanded in late spring or early summer. This should be followed up with a second application in late summer or early fall to disrupt the growth of any secondary leaves or shoots following the first application. Again, treatment with herbicides, as with mowing, should be carried out for multiple years. Herbicides containing triclopyr amine or glyphosate, active ingredients seen in common products such as Bayer Advanced Brush Killer Plus Concentrate^{TM} and Roundup Pro^{TM}, are very effect against kudzu (Enole and Loewstein 2014).

Figure 1.9. *M. cribraria* aggregations on residential structures (Photo by D. R. Suiter 2009).

Figure 1.10. Staining of human skin (ankle) (Photo by D. R. Suiter 2012).

Agricultural Control Strategies

In China, *M. cribraria* can be successfully controlled by the application of broad-spectrum insecticides such as methamidophos, chlorpyrifos, beta-cypermethrin, deltamethrin, beta-cypermethrin, or sumicidin. Susceptibility to insecticidal treatments differs between nymphs and adults, yet when targeted appropriately these chemicals offer extremely high rates of control in soybeans (Wang et al. 1996, Zhang and Yu 2005).

During the 2010 and 2011 soybean growing seasons in Georgia, field insecticidal trails were completed on a wide range of chemicals, this reveled those containing bifenthrin, cyhalothrin, zeta-cypermethrin, carbaryl, or acephate offered more than 80% control 2 to 5 days after their application. Moreover the trade name insecticides Hero, Brigade, Karate+Orthene, Endigo, Brigadier, Discipline, and Sevin had greater or equal to 90% control 2 to 5 days after treatment (Roberts and Whitaker 2012). The results of these trials were used to create guidelines on managing this insect in soybean fields throughout the southeast, and have been incorporated in the Alabama (2014) and University of Georgia (2013) Pest Management Handbooks, which recommend the use of nine different insecticides spanning three classes; pyrethroids, neonicotinoids, and organophosphates.

Insecticide treatments should always be applied in response to adult and nymphal infestations, and only when field populations of the kudzu bug exceed the economic threshold of 1 nymph (Reisig and Bacheler 2012) or 5 adults per sweep (Roberts 2013) across the whole field. Adult Infestations typically begin at field edges and insects will move inward as the season progresses (Seiter et al. 2013b). As field edges are the first areas contacted by migrating adults, boarder rows should be prioritized for insecticide applications early in the season. Subsequent applications should focus on where the insects have since penetrated. Chemicals offering longer residual times should be considered as adults actively migrate from overwintering to feeding sites, which lasts approximately 3 months (Zhang et al. 2012).

While the results from Roberts and Whitaker (2012) come from limited field trials (Green et al. 2012) the selected chemistries appear to offer good control of the kudzu bug. Chemical controls, however, often represent a quick solution to the problem and in the case of the kudzu bug there is often a need for multiple insecticide applications during the adult migratory season (Roberts and Whitaker 2012). This makes a strictly chemical based approach uneconomical and unsustainable. Furthermore many of the recommended trade name insecticides are broad spectrum in nature and may be toxic to beneficial arthropods, thus inhibiting their role in regulating pest insect populations. For instance the newly discovered *P. saccharlis* and *S. triangulifera* may be sensitive to current insecticidal treatments as research completed on other members of the Platygastridae (Hymenoptera) show sensitivity to delamethrin (Rauno et al. 2010), chlorpyrifos, and the entomopathogenic fungus, *Bacillus thuringeiensis* var. *kurstaki* (DiPel^{TM}) (Amaro 2013), while fenoxycarb (Grenier and Plantevin 1990, 1991) appears to be toxic to Tachinidae (Diptera). Therefore it is imperative that the use of insecticides be timed appropriately with adult migration, which should be monitored carefully, and insecticides should be used cautiously towards the end of May to allow for the successful establishment of parasitoids *in agro.*

Objectives

The kudzu bug, *Megacopta cribraria* (F.) (Heteroptera: Plataspidae), native to Asia, was first detected in the U.S. in Georgia during October of 2009. Since then it has invaded 13 southeastern states and is spreading northward and westward at a rapid rate. When first observed it was determined only as an urban nuisance, however this status has been upgraded to that of a serious agricultural pest. Currently, scientific literature concerning this bug in the U.S. does not address biological and physiological traits that have enabled the rapid invasion and exponential population growth of this insect in its North American distribution. Therefore, the immediate project has set out to examine such traits. The long-term goal of this project is to gain a comprehensive understanding of some biological and physiological aspects of this insect that will aide in the development of IPM programs against the kudzu bug. This project consists of three supporting objectives.

1. Investigate I) development of the reproductive system, II) sperm storage, III) oocyte development, and IV) sex ratio of overwintering adults
2. Determine if kudzu is a necessary host plant for successful development, or if feeding on this plant can be bypassed
3. Preliminary survey of natural enemies, their distribution, and potential as biological control agents.

For the three objectives, there are two separate hypotheses.

1. Overwintering second-generation adults are fertile at these times; females will contain stored sperm.
2. Kudzu bugs will be able to develop on multiple legume species without the need to have a preliminary feeding period on kudzu

CHAPTER 2

PRE-OVERWINTERING COPULATION AND FEMALE RATIO BIAS: LIFE HISTORY CHARACTERISTICS CONTRIBUTING TO THE INVASIVENESS AND RAPID SPREAD OF *MEGACOPTA CRIBRARIA* (HETEROPTERA: PLATASPIDAE)

Abstract

Pre-winter copulation and sperm storage in overwintering adult *Megacopta cribraria* (F.) was examined. Microscopic examinations of the spermathecae and ovaries were made in females and of the testes in males that were collected weekly before, during, and after dormancy. The results indicated that approximately 15% of females mated before entering winter dormancy and sperm was stored in their spermatheca for up to seven months, oocytes in mated overwintering females proceeded to post-blastoderm stage before the onset of spring feeding/mating, all of the overwintering males had sperm in their testes, and the ratio of females gradually increased in populations during overwintering. This study indicates that both males and females are capable of reproductive quiescence. The biological significance of these life cycle aspects is discussed from the viewpoints of invasiveness and adaptation.

Introduction

The kudzu bug, *Megacopta cribraria* (F.) (Heteroptera: Plataspidae), is native to Asia and India (review provided by Eger et al. 2010). Many authors indicate that this insect is an occasional to serious pest of many leguminous crops and vegetables in its native distribution (Takasu and Hirose 1985, Chen et al. 2009). It is commonly reported as a key pest of the soybean (*Glycine max* L.) in China and Japan (Takasu and Hirose 1985, Wu and Xu 2002, Xing et al. 2006) as feeding by this insect significantly reduces crop yield (Chen et al. 1996).

The kudzu bug was first discovered in the United States in Georgia in October 2009, when adults were found aggregating on residential homes in abundance apparently seeking overwintering sites (Eger et al. 2010, Suiter et al. 2010). At that time it was defined only as a nuisance pest, yet the following year kudzu bugs were subsequently discovered infesting vegetative soybean fields in Georgia and South Carolina (Seiter et al. 2013b). This quickly elevated its status from an invasive urban nuisance to that of a serious economic pest of the soybean in the U.S. (Greene et al. 2012), and a concern for international trade (Ruberson et al. 2013). The distribution of the kudzu bug has rapidly expanded since its initial detection, and can now be found in 13 southeastern states as well as Washington D.C. (http://www.kudzubug.org/distribution_map.cfm).

In its temperate Oriental distribution the kudzu bug completes one to three generations a year (Hibino and Itô 1983, Tayutivuikul and Kusigemati 1992, Wu et al. 2006), whereas in more tropical areas it may be active year round (Thippeswamy and Rajagopal 1998). In its U.S. range, the kudzu bug undergoes two generations per year (Zhang et al. 2012). It has a hemimetabolous life cycle that consists of eggs, five nymphal instars, and adults (Ramakrishna Ayyar 1913, Ahmad and Moizuddin 1977, Tayutivutikul and Yano 1990). When temperatures increase in spring, overwintering adults become active and colonize kudzu (*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.)), where they aggregate, feed, mate, and lay eggs. The first generation nymphs develop on kudzu, and adults may switch to additional host plants such as soybean, or remain on kudzu to develop the second generation (Zhang et al. 2012). The first instars ingests bacterial symbionts from capsules deposited beneath the egg mass by their mother, and will spend a quiescent period near the egg mass before dispersing to obtain plant food (Fukatsu and Hosokawa 2008). Kudzu bug nymphs and adults feed on plant phloem by inserting their stylet into stems, leaves, petioles, pods, and possibly flowers of host plants (Zhang et al. 2012, Seiter et al. 2013b). In the fall, adults prepare for overwintering by migrating to areas where they aggregate and shelter from adverse environmental conditions. Reported overwintering shelters include under tree bark, on or in structures, and in or under ground litter adjacent to kudzu patches, or near soybean fields. At this time they often become a nuisance pest as they commonly invade residential structures and home gardens (Eger et al. 2010, Waldvogel and Alder 2012). Although many legume and non-legume species are reported as host plants to this insect, only kudzu, soybean, pigeon pea (*Cajanus cajan* L.), black-eyed pea (*Vigna sinensis* L.), lima bean (*Phaseolus lunatas* L.), and pinto bean (*Phaseolus vulgaris* L.) are confirmed as being the primary reproductive hosts in the southeastern United States (Zhang et al. 2012, Del Pozo-Valdivia and Reisig 2013, Ruberson et al. 2013, Medal et al. 2013)

Despite the studies and reports on various aspects of its biology and control methods in the U.S., kudzu bug overwintering biology has not been studied in detail. To date, there is limited knowledge of ecological and behavioral information during this life history period. This includes adult insect overwintering locations as well as habitat use and aggregation behaviors of overwintering adults (Eger et al. 2010, Zhang et al. 2012). Zhang et al. (2012) reported a small peak of egg laying that occurred in October in Georgia, yet this generation did not complete development. We also observed oviposition on 21 November 2013 by females collected on 05 November 2013 from a residential area when kept in screen-capped glass jars and held at room temperature. However a separate collection of females restricted to identical screen-capped jars, but kept outside did not lay eggs. These observations suggested that adults may be capable of pre-winter mating, and that adults do not enter a strong cold-season diapause, but rather a reproductive quiescence or dormancy that can be broken with a period of warmer cold-season weather. Thus environmental stimuli that induce overwintering may prevent fertilization and subsequent embryogenesis in females, allowing the insect to stay reproductively dormant at these times.

To address the limited understanding of kudzu bug overwintering biology, this study investigated whether or not pre-winter and early spring copulation commonly occur, if sperm is stored in the female spermatheca and male testes throughout dormancy, if oocyte development occurs in overwintering females, and to determine the ratio and variation in overwintering survival between males and females. Previous literature has been limited to descriptions of the morphology of adult reproductive systems (Pendergrast 1957, Ahmad and Moizuddin 1975, Kim and Lee 1993), and has not reported on sperm storage in *M. cribraria*, or the family Plataspidae, although this has been documented in other Hemipterans (Koshiyama et al. 1996, 1997a, 1997b; Kott et al. 2000, Kobayashi and Osakabe 2008). While sex ratios of this species have been observed previously, they have only been determined in post-winter populations (Hibinio and Itô 1983, Zhang et al. 2012, Seiter et al. 2013b). Finally, we discuss how our findings may offer an explanation of the invasiveness and rapid range expansion of kudzu bug in the southeastern United States.

Materials & Methods

Collection of Insects. Adult *M. cribraria* were collected at approximately weekly intervals before, during, and after dormancy in Auburn, Alabama (Lee, Co.) from September 2013 through March 2014 when adult activity resumed in full. Populations were sampled at three locations, including a large patch of kudzu, two mature pecan trees (*Carya* sp.), and an area of ground litter adjacent to an additional kudzu patch. Pre-and-post dormant individuals were collected with a sweep net, while dormant insects were collected by hand from cracks, crevices or beneath bark on the main trunk of pecan trees, as well as beneath ground litter, and from fallen logs adjacent to the kudzu patches. Bugs were placed in a glass jar (10.16 x 7.62 cm) and transported to the laboratory. Individuals were counted and separated, and the sex ratio was determined.

Microscopic examinations of male and female reproductive anatomy. Individual insects were pinned and submerged in a 1% sodium chloride solution contained in a wax-lined dissection tray prior to dissection. Dissections were made using BRI surgical super fine micro scissors (Biomedical Research Instruments, Inc. Silver Spring, MD) under a stereomicroscope (Meiji model EMZ-TR, Japan). A transverse cut was made across the scutellum and hemelytra exposing the tergal surface of the abdomen, which was then excised to expose the internal anatomy.

Ovaries and spermathecae, or testes were removed and placed onto individual microscope slides (25 x 75 mm. VWR, Radnor, PA) mounted with a 1% sodium chloride solution to prevent desiccation before the addition of a cover slide (22 x 22 mm. Fisher Scientific, Pittsburgh, PA). An optical microscope with a video-camera attachment (Meiji model ML2000 microscope [Japan] with Hitachi D.S.P color video camera model VK-C370, Japan) was used to observe development status of eggs in the ovaries, and presence of sperm in the testes and spermathecae. The spermatheca is highly sclerotized and had to be mechanically ruptured with pressure applied to the coverslip, and then viewed under phase-contrast microscopy to identify the presence of sperm. To confirm presence or absence of sperm within female spermathecae (Figure 2.1), contents of the testes (Figure 2.2) were compared to the expelled liquid of the spermathecae. Approximately 20 live adult insects were selected by hand from the three collection locations and were dissected each week. More specimens were examined for egg development during later weeks. In total, 552 females and 476 males were dissected.

Data analysis. Percent of females storing sperm and percent with observable egg development were pooled and analyzed on a monthly basis. Percentage data were subjected to a Chi-squared test ($\alpha = 0.05$) to determine if there were any significant differences between the total numbers of females storing sperm, and females storing sperm on a successive monthly basis, as this would indicate when mating resumed post-winter (SPSS Inc.). The same statistical method was use to compare the monthly numbers of collected males and females.

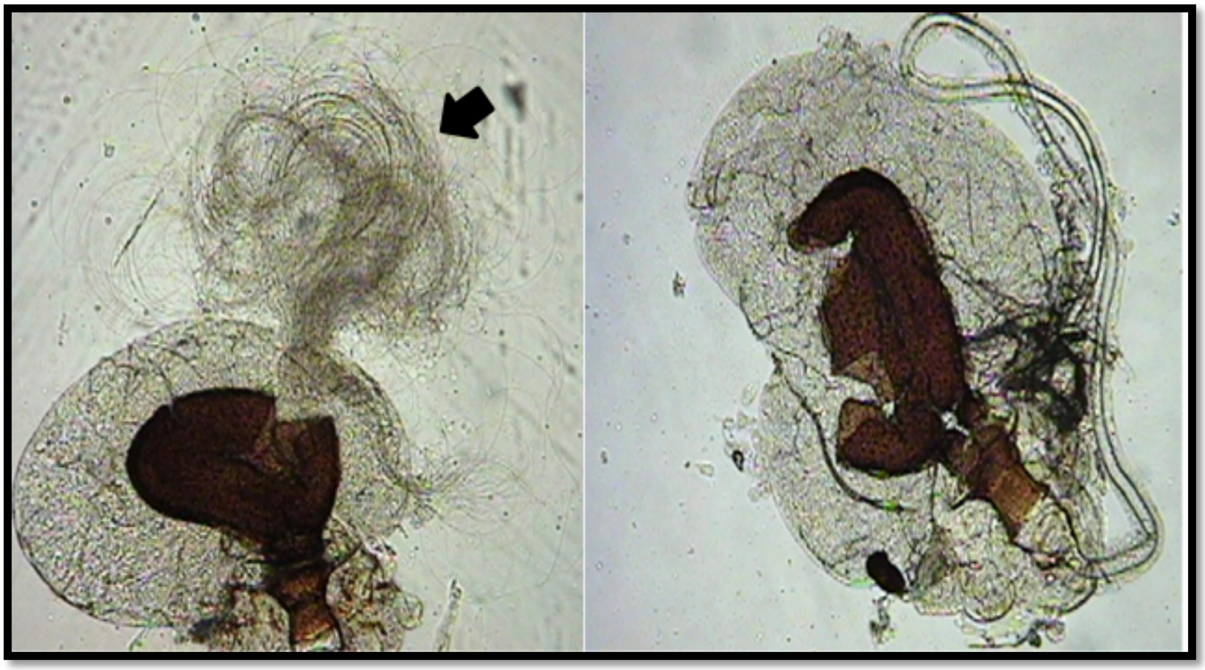

Figure 2.1. Ruptured spermatheca containing sperm (indicated by arrow on left) and spermatheca without sperm (right) (viewed under 100x).

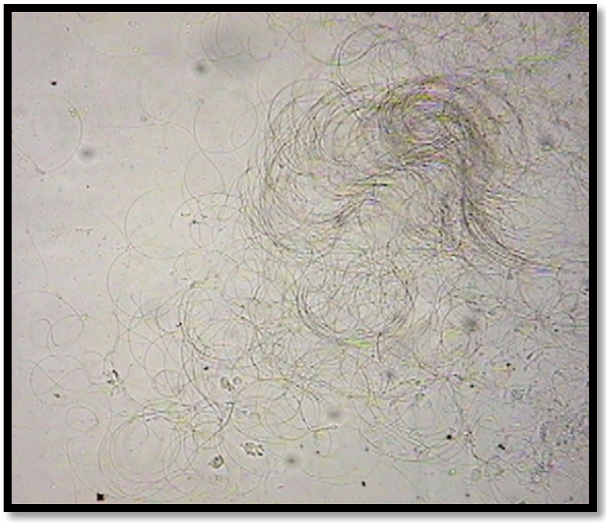

Figure 2.2. Sperm from male testes (viewed under 100x).

Results

Dissections of spermathecae and testes. Sperm that was expelled from both female spermathecae (Figure 2.1) and male testes (Figure 2.2) appeared as long, thin lance-like filaments. Sperm motility was observed in sperm that was expelled from both male and female reproductive organs, however proportions of live/dead sperm could not be assessed, and no differences could be distinguished between the head and tail regions, or any other fine details of the sperm morphology.

The percentages of females storing sperm on a pooled monthly basis from September 2013 through March 2014 are shown in Figure 2.3. Overall, 15% of females stored sperm in their spermatheca during overwintering months, indicating that they had mated before overwintering. The average percentage (varying between 9%-23% in individual samples) of females storing increased gradually from pre-wintering (Sept. 13%), to preparatory overwintering months (Oct. and Nov. 14-15%), with minor fluctuations during overwintering months (Dec. through early Mar., 13-17%), and finally spiked post-overwintering by the end of March (36%). The X^2 test showed no significant difference between females storing sperm before entering winter (Sept., 2013) through overwintering samples (middle Mar., 2014) ($X^2 = 1.135$; df = 6; $P = 0.980$), indicating that no copulation occurred during wintertime, and the adults were reproductively dormant. The difference became strongly significant when pre-winter (Sept., 2013) samples were compared to post-winter (end Mar., 2014) samples ($X^2 = 10.976$; df = 1; $P = 0.001$), indicating the occurrence of post-winter copulations.

All the dissected males had sperm in their testes, indicating that they were capable of mating immediately in the spring. We were not able to differentiate mated from virgin males.

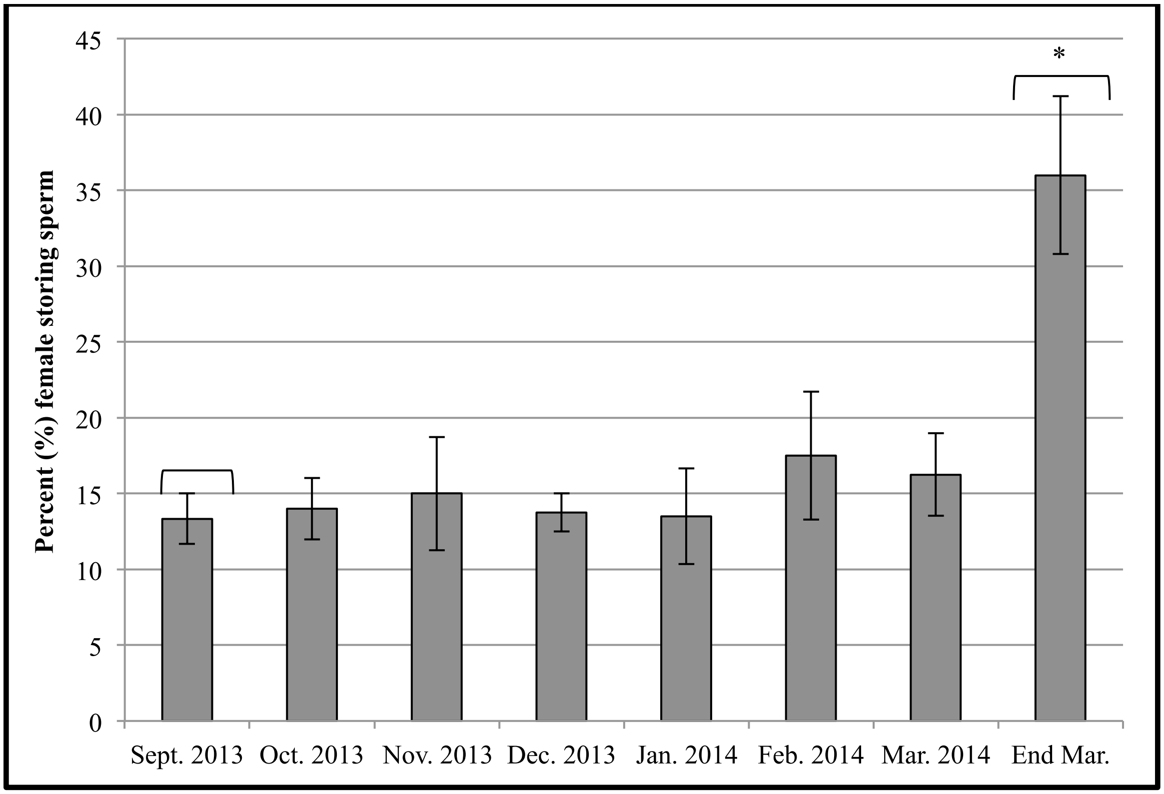

Figure 2.3. Average monthly percentage (± SE) of females storing sperm before, during, and after dormancy. The Chi-squared test indicated that the percent of females storing sperm from Sept. 2013 to Mar. 2014 did not significantly differ ($X^2 = 1.135$; df = 6; $P = 0.980$). There was a significant difference when pre winter females (Sept. 2013) were compared with post-winter (End Mar. 2014) ($X^2 = 10.796$; df = 1; $P = 0.001$) (indicated by brackets over bars) females, indicating mating had occurred. Asterisk (*) indicates significant difference ($P < 0.005$).

Egg development in females. Female kudzu bugs have a pair of ovaries, each composed of seven ovarioles branching from the oviduct (Figure 2.4). During winter, females had little development in their ovarioles with no evidence of oocyte formation from September 2013 through middle February 2014. Before the onset of post-winter mating, or feeding in the spring, oocytes began developing as indicated by the formation of the blastoderm and vitelloengensis (Figure 2.4). Developing oocytes in overwintering females were first observed within the ovarioles on February 26, 2014, and continued until the last collection date on March 27, 2014. By then, approximately 54% of the previously mated females (n=13) contained eggs of later developmental stages that appeared ready for fertilization.

Developing eggs were also observed in collections of females between February 26 and March 27, 2104, though at a much lower rate (18%), in females (n=79) that lacked stored sperm in their spermatheca, indicating that the oocytes develop before fertilization. The more developed oocytes in mated females may suggest that stored sperm promote oocyte development.

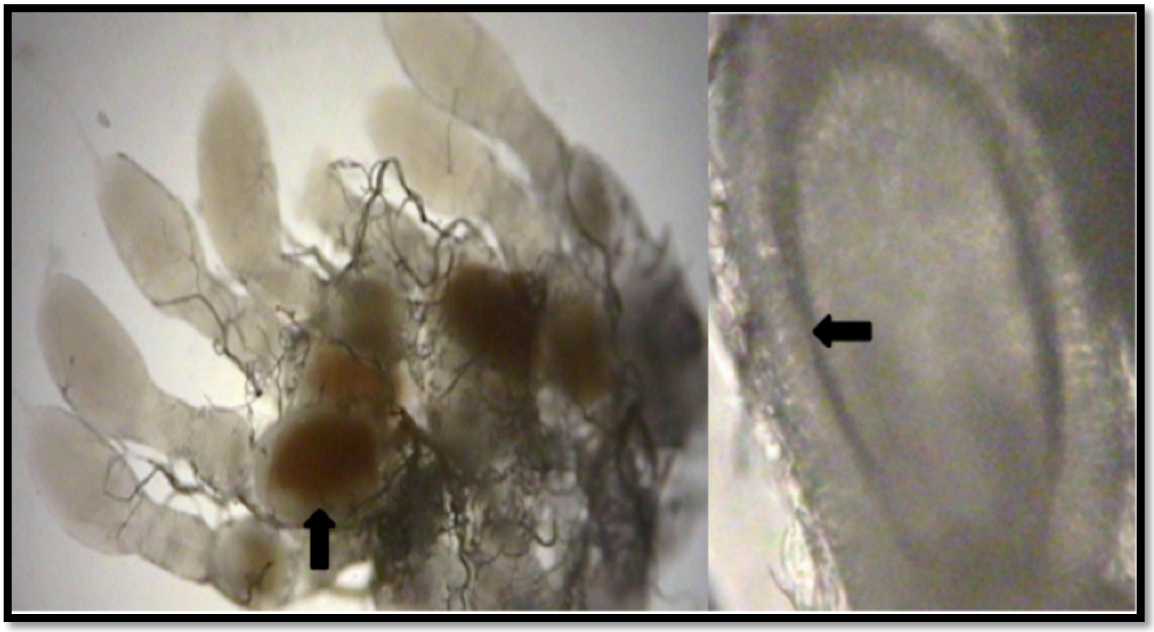

Figure 2.4. Oocyte development: arrows indicate vitellogenesis (left) and blastoderm formation (right) (pictures taken 02 Mar. 2013; viewed under 100x and 400x).

Sex ratio. The numbers of overwintering males and females are shown in Figure 2.5. The M:F ratio was close to 1:1 before overwintering. In subsequent months, the ratio slightly decreased and females were continually collected from field sites in greater quantity than males, accounting for > 50% of the collections each month. The lowest male ratio was observed in February 2014, a month that had 8 d of below freezing temperatures (Hu, 2014). The last collection at the end of March 2014 had a M:F ratio of 1:1.53. A X^2 test showed a significant difference in the overall numbers of male and female collected from October 2013 to March 2014 ($X^2 = 136.116$; df = 1; $P < 0.001$). The difference in the male and female numbers between pre-winter and onset of post-winter mating ($X^2 = 33.884$; df = 1; $P < 0.001$) was highly significant. The M:F ratio decline during the overwintering period suggests that winter survival of females was greater than that of males.

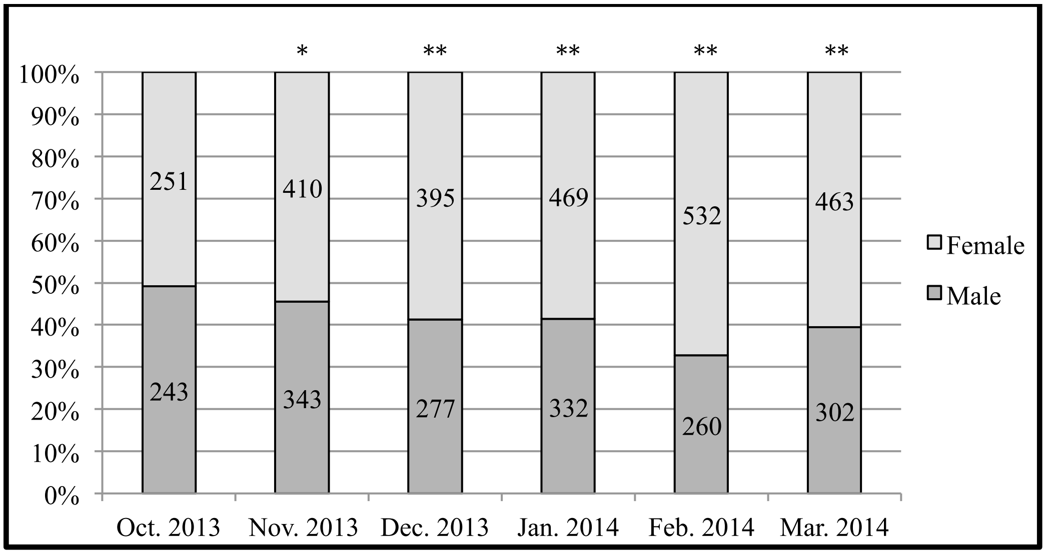

Figure 2.5. Number and proportion of male and female *M. cribraria* collected per month. Asterisks above a bar indicate significant differences in numbers of females and males for that month (*, $P<0.05$; **, $P<0.001$) according to a X^2 test.

Discussion

Pre-wintering copulation. About 15% (9-23%) of female kudzu bugs had mated prior to entering winter dormancy. The relatively consistent percentages during winter months indicate there was no copulation at that time. Kudzu bug females copulated, received and stored sperm in their reproductive tract. This finding helps explain the observations of October egg laying prior to seeking overwintering sites (Zhang et al. 2012), and the November egg laying of the field collected females that were transferred from an overwintering aggregation to laboratory rearing conditions (authors observations). The findings also corroborate our hypothesis that adults do not enter a strong cold-season diapause, but rather a reproductive dormancy that can be broken with a period of warmer cold-season weather.

Copulation prior to overwintering is not uncommon in insects that overwinter in the adult stage (Denlinger 1985, Tauber et al. 1986). This behavior is particularly common in Hemipterians, such as *Orius* spp. (Kobayashi and Osakabe 2008), various Nabidae (Kott et al. 2000, Roth and Reinhardt 2003), and the stinkbugs *Nezara viridula* (L.) and *Menida scotti* (Puton) (Kiritani et al. 1966, Koshiyama et al. 1994, 1997a, 1997b, Jones and Sullivan 1981). This study is the first report on pre-winter mating and the overwintering of mated females in reproductive dormancy in the family Plataspidae, and in particular *M. cribraria*.

Sperm storage and egg development. Pre-winter mated female kudzu bugs can successfully overwinter with their ovaries at the pre-vitellogenic stage of development, and can store viable sperm in their spermatheca for up to seven months. After winter sperm retained in the female spermatheca appeared to be as active as sperm within male testes after spring activation, suggesting that sperm remains viable after long cold storage. The percentage of females storing sperm was consistent during the overwintering period. Females that were mated pre-winter had observable oocyte development on 27 February 2014, which indicates that adult dormancy was terminated on or near that date. Developing eggs in unmated females indicates that maturation of oocytes occurred prior to fertilized, and independent of mating; however, mating may stimulate oocyte development and subsequent oviposition of fertilized eggs, as is common in other Heteropterans (Soares et al. 2011). The observations of egg development show that females undergo a rapid post-overwintering reactivation period in which oocyte development abruptly begins and mating resumes. It may additionally indicate that females are able to release and utilize stored sperm to fertilize eggs at a time pivotal for maximizing subsequent instar development and success in spring.

Questions remain whether the previously mated females belong to the previous spring generation or the summer generation, whether or not they need to re-mate in spring to stimulate reproductive events after winter dormancy, or if the prolonged storage of up to seven months decreases the quality of the sperm and reproductive ability of females. Of the insects that overwinter as mated adults, many have a capacity to maintain viable sperm during winter for several months and use the stored sperm for successful fertilization of eggs after spring activation without re-mating prior to oviposition in spring, and without decreasing the reproductive ability of females (Taylor 1984, Kott et al. 2000, Ceryntgier et al. 2004, Kobayashi and Osakabe 2008, Leong et al. 2012, Awad et al. 2013). Future studies should explore the fecundity of pre-winter mated females vs. post-winter mated females, egg hatch rate, and sex ratio in this insect.

Male reproductive physiology appeared to remain active throughout dormancy as all of the males dissected from September 2013 through March 2014 contained active sperm within their testes. While this is a rare occurrence in Heteroptera (Saulich and Musolin 2011) it has not gone unobserved. The male stinkbug, *Menida scotti* (Puton), likewise remains physiologically active throughout winter and may even be capable of copulating during these times (Koshiyama et al. 1993). However dormancy and reproductive physiology of male insects during diapause are much less understood and have received far less attention than in female insects (Pener 1992). Proportions of viable sperm in males and females and the ability of females to utilized stored sperm for fertilization were not determined through this study, therefore future studies should address these questions.

Sex ratios. The overwintering male-to-female sex ratio gradually decreased from ≈1:1 before entering overwintering to 1:1.53 after overwintering, which was similar to the sex ratio of 1:1.84 observed by Zhang et al. (2012) in early spring flight interception traps. The largest proportions of females were obtained in samples from February 2014, the coldest month with 8 d below freezing point. In many insects including some stinkbugs, males donate nutrients in their ejaculate to females during pre-winter copulation. Such nutrients may increase female overwinter survival yet reduce the survival of mated males (Koshiyama et al. 1996, Shapiro 1982, Boggs and Gilbert 1979, Kobayashi and Osakabe 2008, Leong et al. 2012, Zhu et al. 2013). It has additionally been speculated that pre-winter copulation may stimulate lipid accumulation and increase cold hardiness, or change behaviors in females to better survive winter conditions (Kobayashi and Osakabe 2008, Zhu et al. 2013). In this study, there was no sufficient evidence indicating that the increasing female ratio was a result of higher survival of mated females because the increase in the percentage of mated vs. unmated females was not statistically significant; however, it may be explained as a cost of pre-winter copulation for males. Yet trade-offs such as nutrient contents, cold hardiness and longevity of mated and virgin males and females remain to be investigated. It is likely that the percentage of females mated prior to overwintering, the overwintering survival, the viability of sperm and its storage in female spermathecae, varies between years depending on the temperature and differences in microclimatic conditions.

In summary, our study demonstrates several previously unreported aspects of the life cycle of *M. cribraria*. These new findings may represent important mechanisms involved in the invasiveness and rapid spread of this species throughout the southeastern U.S. Pre-winter mated females potentially increase the invasion of new feeding habitats without the need to mate again in the spring, enabling them to begin spring migration earlier than unmated females and to be the first to exploit feeding hosts in new geographical locations. It is possible that like in many other insects, mating prior to overwintering is adaptive because sperm storage by females indicates they can disperse, lay fertilized eggs, and successfully colonize new habitats when there is no access to mates (Socha 2010, Leong et al. 2012). This is especially important for kudzu bug adults that have greater dispersal capabilities and high female overwinter survival rates. Additionally, the smaller numbers of overwintering males available for copulation in spring may limit the accessibility of sperm for fertilization. Females that contain sperm from a previous autumn mating may be able to overcome this obstacle (Härding et al. 2008). Most importantly, this strategy indicates that females might be able to establish subsequent populations via founders effects, which would aide in understanding the rapid spread and invasiveness of this species.

CHAPTER 3

FIRST-GENERATION *MEGACOPTA CRIBRARIA* (F.) (HETEROPTERA: PLATASPIDAE) ARE NOT RESTRICTED TO THE WILD LEGUME KUDZU, *PUERARIA MONTANT* LOUR. (MERR.) (FABLES: FABACEAE)

Abstract

The biology of *M. cribraria* (F.) is not well understood in its new North American distribution, especially its development on alternative developmental or reproductive host plants. Therefore we investigated whether overwintered *M. cribraria* could directly feed and oviposit on other legume species and subsequently complete the first-generation on these species in a no-choice greenhouse assay. Overwintered *M. cribraria* successfully oviposited on mung bean (*Vigna radiata* (L.) R. Wilczek), black-eyed pea (*Vigna unguiculata* (L.) Walp), butter bean (*Phaseolus lunatus* L.), soybean (*Glycine max* L. Merrill) and kudzu (*Pueraria montana* (Lour.) Merr. variety *lobata* (Willd.) Maesen and S. Almeida). There were no significant differences in the number of days to oviposition, number of eggs and egg masses deposited, or in the hatch rates on the different legume species. First-generation *M. cribraria* developed on all legume species except black-eyed pea in ≈ 55.6 d with little variation in developmental time of the instars. Mean developmental days of the first, second, and fourth instars were consistent ≈ 7.0 d, while the third and fifth instars took longest to develop (≈ 12.3 d). There were significantly more first instars

molting to second instars on kudzu, mung bean, and soybean than on butter bean. Mortality from egg to adult was greatest on black-eyed pea (100%), followed by butter bean (90%), kudzu (68.33%), mung bean (55%), and soybean (45%). These results indicate that kudzu is not an obligatory host for *M. cribraria*.

Introduction

Megacopta cribraria (F.) is an invasive agricultural and residential pest species that was first detected in the United States in 2009 (Eger et al. 2010). Since then, it has quickly expanded its U.S. distribution and is now found in 13 southeastern states (http://www.kudzubug.org/distribution_map.cfm). This herbivorous insect feeds on plant phloem by probing stems, petioles, leaves, and pods of various plant species (Eger et al. 2010, Zhang et al. 2012, Sieter et al. 2013). The preferred hosts of *M. cribraria* are kudzu, *Pueraria montana* (Lour.) Merr. variety *lobata* (Willd.) Maesen and S. Almeida, and soybean, *Glycine max* (L.) Merrill (Tayutivutikul and Yano 1990, Li et al. 2001, Wang et al. 2004, Xing et al. 2008). It is considered a serious agricultural pest of soybeans in many Asian countries (Ishihara 1950, Wang et al. 1996, Wu and Xu 2002). *Megacopta cribraria* undergoes five nymphal instars lasting ≈ 24-56 d in its native range, before eclosing into the adult (Eger et al. 2010).

In the southeastern U.S., *M. cribraria* is thought to complete two-generations per year (Zhang et al. 2012). Depending on location, overwintering adult *M. cribraria* may spend up to 6 mo without food when sheltered in overwintering sites (authors observations), and become active once temperatures increase in spring. Overwintered adults will either stay in kudzu patches or disperse to alternative hosts (Zhang et al. 2012) such as soybean, where they can cause tremendous damage (Seiter et al. 2013).

Overwintered adult *M. cribraria* feed on multiple leguminous and non-leguminous plants (Zhang et al. 2012, Hu and Carroll 2012), yet they display a restrictive reproductive host range (i.e. able to develop from egg to adult) which is limited to legumes, namely kudzu (Zhang et al. 2012, Medal et al. 2013), soybean (Zhang et al. 2012, Del Pozo-Valdivia and Reisig 2013) pigeon pea, *Cajanus cajen* L., black-eyed pea, *Vigna unguiculata* L., lima bean, *Phaseolus lunatus* L., and pinto bean, *Phaseolus vulgaris* L. (Medal et al. 2013) in the southeastern U.S. This biological trait, a broad host range yet limited reproductive range, is common of many native and invasive Pentatomoidea (review by Panizzi 1997), and is most likely the result of differences in adult and nymphal characteristics such as mobility (Panizzi et al. 1980, Schumann and Todd 1982), nutritional demands (Kehat and Wyndham 1972, Kester and Smith 1984), and stylet morphologies of adults and nymphs (see Moizzuddin and Ahamd 1975).

Because this insect overwinters in or near kudzu patches (Waldvogel and Alder 2012), which are usually available before cultivated soybean crops (authors observations), it was anecdotally thought that *M. cribraria* must initially feed on kudzu to gain essential nutrients before dispersing to other feeding and reproductive host plants. However Smith (2013) and Seiter et al. (2013) reported that overwintered *M. cribraria* could infest early-planted soybeans, and Del Pozo-Valdivia and Reisig (2013) suggested that the first-generation is able to bypass kudzu and develop on soybean. Previous studies have not, however, demonstrated in-full if kudzu is necessary in the diet of *M. cribraria*, since adults that produced eggs or nymphs for these studies had been reared on or initially fed kudzu (Zhang et al. 2012, Medal et al. 2013, Del Pozo-Valdivia and Reisig 2013).

To investigate if first-generation *M. cribraria* can develop on alternative host plants in the absence of kudzu, a no-choice greenhouse assay was conducted utilizing starved overwintered adults before the onset of feeding in spring.

Materials and Methods

Plants and insects. Mung bean, *Vigna radiata* (L.) R. Wilczek, black-eyed pea, *Vigna unguiculata* (L.) Walp, (http://www.nuts.com, Cranford, NJ), butter bean, *Phaseolus lunatus* L., (Alabama farmers cooperative), and soybean ('93Y92', DuPont-Pioneer, Des Moines, IA) were grown from seed beginning 10 February 2014, while kudzu was grown from field collected root crowns (Lee Co., Auburn, AL). Seeds and root crowns were placed in 3.7 L black plastic pots containing Sunshine Pro Premium Potting Soil (SunGro Horticulture Canada Ltd., Seba Beach, AB). Plants were grown and maintained under natural light conditions, 23 ± 3^{o}C, 50-70% RH, and provided with water as needed in a greenhouse on the Auburn University campus (Lee Co., Auburn, AL). Four plants of each legume species were grown. Overwintered adult *M. cribraria* were collected from tree bark and leaf-litter near a kudzu patch at Park Town Creek, Auburn, AL (Lee Co.) (32°36'32.80" N, 85°28'30.83" W), and an area of fragmented forest adjacent to Route 14 near E. Glenn Ave. (Lee, Co., Auburn, AL) (32°36'32.80" N, 85°30'02.66" W) on 19 March 2014, before migrating to feeding plants.

No-choice experiments. On 21 March 2014, one of each legume species was placed inside a 24.5 x 24.5 x 63.0 cm cage (Bugdorm^{TM}, BioQuip Products, Rancho Dominguez, CA) and 10 pairs of field-collected adult *M. cribraria* (10 males: 10 females) were introduced to the cages. Adults were allowed to feed on their respective legume plant as needed, and were removed after

25 d. Oviposition occurred ≈ 21.5 d after adult introduction. Plants were then inspected and the number of egg masses and individual eggs per mass were counted. Upon hatching neonate nymphs were allowed 24 h to ingest the endosymbiont capsule contents, at which time they displayed wandering behavior from the egg mass, and only 20 neonates were selected to remain on their respective host plant per replicate (n = 60 nymphs per replicate). Three of the same caged legume species were considered one replicate. Egg masses occurred on both the mesh cage material or on the plant itself. If instars were on the cage they were transferred to the plant leaves with a fine haired paintbrush (size 3/0, Royal Brush Mfg. Merrillville, IN). The hatch rate and days required for completion of each nymphal instar were recorded every 2 d. When plants reached maturity (every ≈ 20 d), the same plant species in a vegetative growth stage was placed inside the cage and nymphs were encouraged to wander to the new plant. Once the remaining nymphs moved to the vegetative plant, the mature plant was removed. At the completion of the experiment the total days required for development from egg to adult, and nymph to adult mortality were determined. On butter bean three replicates were used until the fourth instar, then the remaining instars (n= 9) were transferred, due to low survivorship in the three replicates, to a single caged butter bean for the duration of the experiment. Observational results are given past the fourth instar for this plant.

Data analysis. Data were subjected to an analysis of variance (ANOVA) (SPSS, Inc. v. 22, Chicago, IL), and means separated using Tukey's Honestly Significant Difference (HSD) procedure ($P = 0.05$) when appropriate. A Chi-squared ($\alpha = 0.05$) test was used to determine whether there were significant differences in total numbers of adults or in numbers of males and females produced by each legume species, and for rates of nymph to adult mortality on the five legume plants.

Results

Oviposition. In the no-choice assays, it took an average of 21.5 ± 0.41 d for oviposition to occur, and the duration from initial introduction until oviposition of adult *M. cribraria* did not differ significantly among the five legume species (df = 4, 10; F = 0.235; *P* = 0.912). There were no significant differences in the number of egg masses (df = 4, 10; F = 0.951; *P* = 0.474) (Figure 3.1) or numbers of individual eggs (df = 4, 10; F = 0.647; *P* = 0.642) (Figure 3.2) laid on the five legume species. The duration of the egg stage (9.3 ± 0.21) did not differ significantly among the legume species (df = 4, 10; F = 1.170; *P* = 0.381) (Figure 3.3).

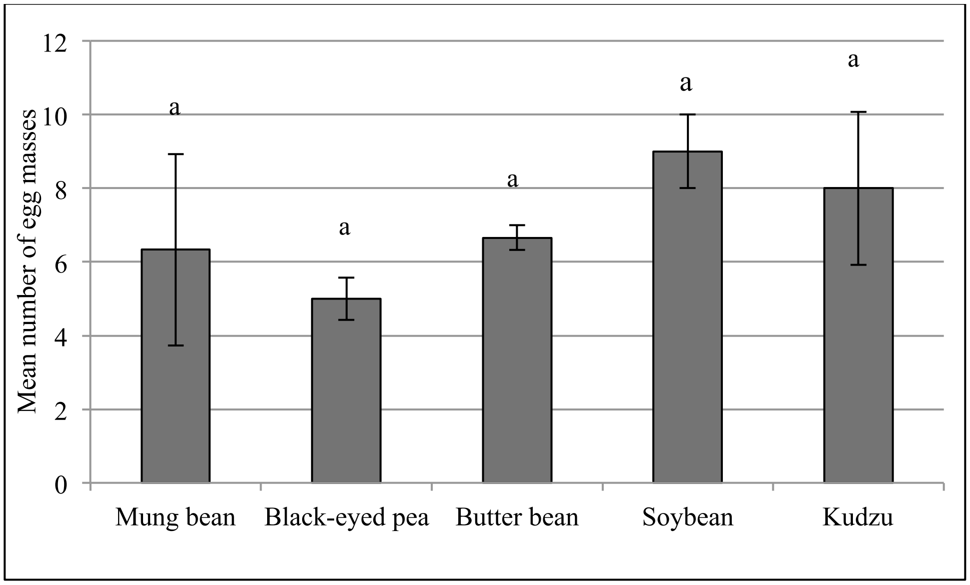

Figure 3.1. Mean number (±SE) of egg masses per host plant. Different letters on bars indicate a significant difference at a *P* = 0.05 using Tukey's HSD test.

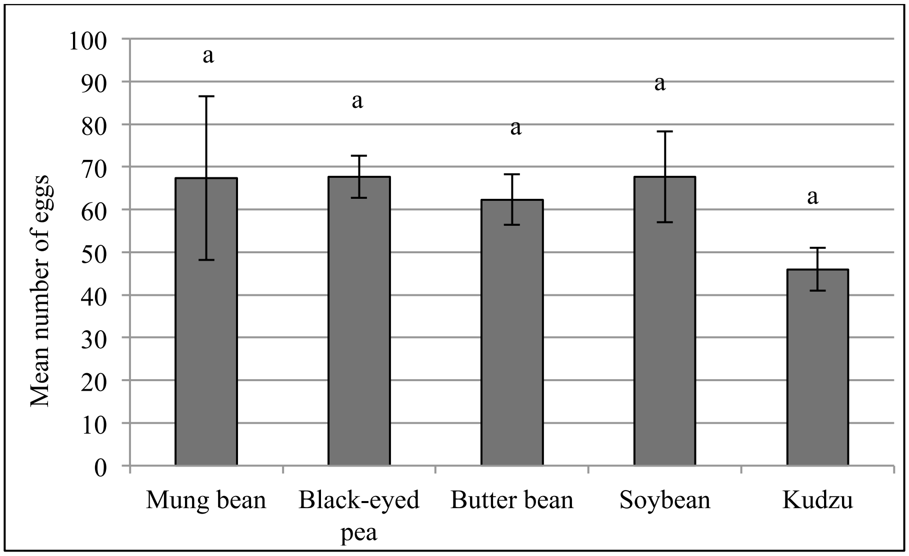

Figure 3.2. Mean number (± SE) of eggs per host plant. Different letters on bars indicate a significant difference at a *P* = 0.05 using Tukey's HSD test.

Duration of instars. Total hatch rate was greatest on soybean (68.2%) followed by kudzu (52.0%), butter bean (51.3%), black-eyed pea (33.6%), and mung bean (23.9%). However there were no significant differences in the hatch rates among the five legume species (df = 4, 10; F = 0.761; *P* = 0.574) (Table 3.1). No first instar (n= 60) successfully molted to second instars on black-eyed pea. After restricting the number of neonates to 20 per plant (n= 60 per complete replication) the mean duration of the first instar nymph (6.3 ± 0.67 d) did not differ significantly among the other four legume species (df = 3, 8; F = 0.167; *P* = 0.916), nor was the duration of second instar (7.1 ± 0.23 d) significantly different (df = 3, 8; F = 2.867; *P* = 0.104). However there were significantly greater mean numbers of first instar nymphs molting to second instar nymphs on mung bean (19.0 ± 1.0), soybean (14.7 ± 3.0), and kudzu (19.6 ± 3.3) than on butter bean (5.3 ± 2.4) (df = 3, 8; F 11.139; *P* < 0.005) (Table 3.1). Mean developmental days of the third instar took significantly longer on butter bean (20.6 ± 0.67) than on mung bean (11.3 ±

1.76), soybean (10.0 ± 1.15), and kudzu (10.0 ± 0.00) (df = 3, 8; F = 21.697; *P* < 0.005) and only 5 third instars (10.0%) molted to the fourth instar nymphs on butter bean. However there were no significant differences in the mean number of third instar nymphs on mung bean, butter bean, soybean, or kudzu (df = 3, 8; F = 3.438; *P* = 0.72) (Table 3.1). There was no significant difference in mean developmental days for fourth instar nymphs on mung bean (8.0 ± 1.15 d), soybean (10.0 ± 2.0), and kudzu (5.0 ± 1.0) (df = 2, 6; F = 3.00; *P* = 0.125) or any significance in the mean number of fourth instars nymphs among the these three legume species (df = 2, 6 F = 0.675, *P* = 0.544) (Table 3.1). Lastly, development of the fifth instar took significantly longer on kudzu (18.3 ± 1.0 d) than mung bean (11.0 ± 0.00 d), but development days on soybean (14.3 ± 0.9 d) was not significantly different between kudzu and mung bean (df = 2, 6; F = 6.50; *P* = 0.031) (Figure 3.3) (Table 3.1). Transition of *M. cribraria* from egg to adult was completed in 49-55 d on mung bean, followed by kudzu (51-60 d) and soybean (55-59 d) (Fig. 4), averaging 51-58 d among the three bean species (Table 3.2) Mean developmental time from egg to adult did not differ significantly among mung bean (52.3 ± 1.8), soybean (57.7 ± 1.3), and kudzu (56.7 ± 1.3) (df = 2, 6; F = 1.855; *P* = 0.236) (Figure 3.4).

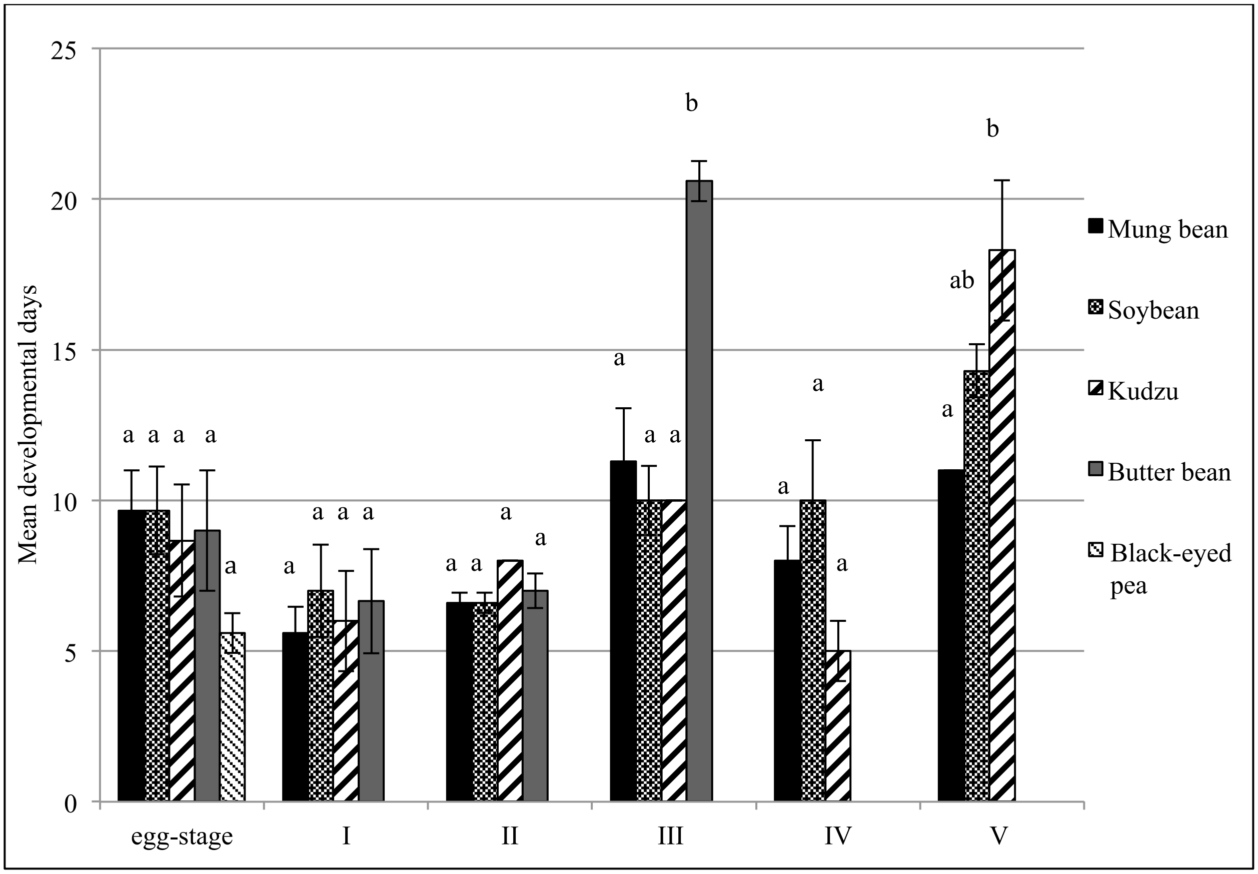

Figure 3.3. Mean development days (± SE) of each instar (I-V) reared on mung bean, soybean, kudzu, butter bean, and black-eyed pea. Different letters on bars indicate a significant difference at $P = 0.05$ using Tukey's HSD test.

Table 3.1. Mean (± SE) numbers of egg masses and individual eggs, mean hatch rate, and mean number of first, second, third, fourth and fifth instars, and adults. Due to low survivorship in the three replicates of butter bean, raw numbers are given past the third instar (indicated by asterisks [*]). Different letters within a column indicate a significant difference at a $P = 0.05$ using Tukey's HSD.

	Legume species				
	Mung bean	Black-eyed pea	Butter bean	Soybean	Kudzu
No. egg masses	6.3 ± 2.6 a	3.0 ± 0.58 a	6.6 ± 0.3 a	9.0 ± 1.0 a	8.0 ± 2.1 a
No. egg eggs	67.3 ± 19.2 a	67.6 ± 8.5 a	62.3 ± 5.9 a	67.3 ± 10.7 a	42.6 ± 11.5 a
Hatch rate (%)	23.9 ± 13.9 a	33.6 ± 17.2 a	51.3 ± 20.0 a	68.2 ± 9.8 a	52.0 ± 11.0 a
No. first instars	22.6 ± 0.7 a	21.7 ± 9.8 a	29.7 ± 10.3 a	45.3 ± 6.8 a	23.3 ± 8.0 a
20 neonates transferred					
No. second instar	19.0 ± 1.0 b	0	5.3 ± 2.4 a	14.7 ± 3.0 b	19.6 ± 3.3 b
No. third instar	12.6 ± 4.0 a	0	3.3 ± 1.2 a	12.0 ± 3.1 a	16.6 ± 3.3 a
No. fourth instar	10.3 ± 5.2 a	0	9.0*	11.3 ± 3.5 a	12.3 ± 2.8 a
No. fifth instar	10.0 ± 5.5 a	0	9.0*	11.3 ± 3.5 a	11 ± 3.5 a
No. Adults	9.0 ± 5.7 a	0	6.0*	11.0 ± 2.3 a	6.3 ± 3.5 a

Table 3.2. Pooled mean developmental days (± SE) of the egg stage and the five nymphal instars (I-V) of *M. cribraria* on mung bean, butter bean (not included in third, fourth, or fifth instars, or adult analysis, indicated by asterisks [*]) black-eyed pea, soybean, and kudzu.

Mean developmental days (± SE) of *M. cribraria* stadia						
Egg-period	I	II	III	IV	V	Total
8.6 ± 0.68	6.3 ± 0.67	7.1 ± 0.23	10.0 ± 0.44*	7.7 ± 1.0*	14.6 ± 1.3*	55.6 ± 1.3*

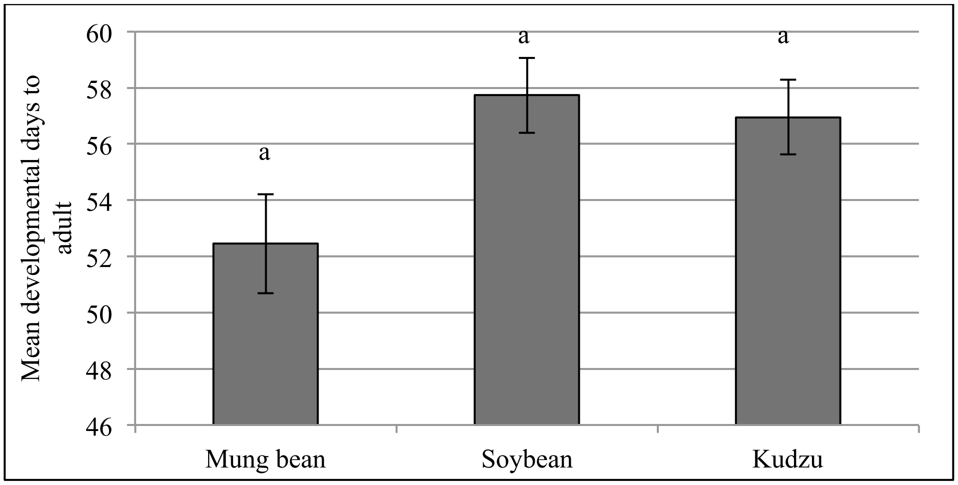

Figure 3.4. Mean developmental days (± SE) from egg to adult *M. cribraria*. Different letters on bars indicate a significant difference at a $P = 0.05$ using Tukey's HSD test.

Adults. There were no significant differences in the mean number of adults that developed on kudzu (6.3 ± 3.5), soybean (11.0 ± 2.3), and mung bean (9.0 ± 5.7) (df = 2,6; F = 0.268; P = 0.774) (Fig. 5) (Table 2) or the total number of *M. cribraria* adults reared on these legume plants ($X^2 = 3.747$; df = 2; $P = 0.154$) (Figure. 3.5). There were no significant differences between the number of males or females produced on mung bean ($X^2 = 0.333$; df = 1; $P = 0.564$), butter bean (X^2= 0.200; df = 1; $P = 0.655$), soybean (X^2= 0.030; df = 1; $P = 0.862$), and kudzu (X^2= 1.316; df = 1; $P = 0.251$).

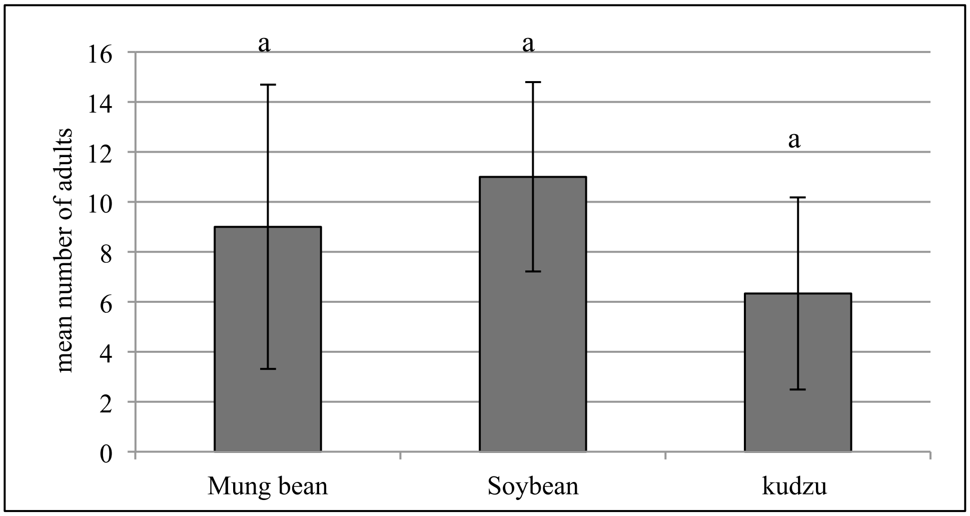

Figure 3.5. Mean number (± SE) of *M. cribraria* developing to adult stadia. Different letters on bars indicate a significant difference at a $P = 0.05$ using Tukey's HSD test.

Mortality. Pooled percent morality from nymph to adult was greatest on black-eyed pea (100%), followed by butter bean (90.0%), kudzu (68.33%), mung bean (55.0%), and soybean (45.0%). The overall Chi-squared test showed a significant difference ($X^2 = 29.905$; df = 4; $P < 0.01$) in nymph to adult mortality among the legume species. Mortality was significantly greater on black-eyed pea than kudzu ($X^2 = 6.095$; df = 1; $P = 0.014$), mung bean ($X^2 = 13.065$; df = 1; P < 0.005), and soybean ($X^2 = 20.862$; df = 1; $P < 0.005$), but did not differ from butter bean ($X^2 = 0.526$; df = 1; $P = 0.468$). On butter bean, mortality was significantly greater than on soybean ($X^2 = 5.535$; df = 1; $P = 0.020$), and mung bean ($X^2 = 8.448$; df = 1; P = 0.004), but did not differ from kudzu ($X^2 = 3.063$; df =1; P = 0.080). Mortality on kudzu was only significantly greater than soybean ($X^2 = 4.681$; df = 1; $P = 0.030$). Mortality on mung bean was not significantly different from soybean ($X^2 = 1.000$; df =1; $P = 0.317$).

Discussion

Previous experiments (Zhang et al. 2012, Medal et al. 2013, Del Pozo-Valdivia and Reisig 2013) have used adult *M. cribraria* that were either reared on kudzu and first instars transferred to selected legume species, or adults that were collected in the field after feeding on kudzu. However our study utilized overwintering *M. cribraria* that were starved and deprived of kudzu for approximately 6 mo before experimentation in no-choice greenhouse assays. Overwintered adult *M. cribraria* oviposited on all the caged legume species, including those that were not suitable alternative hosts for the first-generation, an observation that is consistent with reports from other researchers (Medal et al. 2013, Zhang et al. 2012) on *M. cribraria*. The hatch rate did not differ significantly on any of the five legume plants, indicating that there was no effect of host plant on this life-stage parameter. First-generation adults were able to develop on soybean, kudzu, and mung bean, and in one replication of butter bean. The successful development on mung bean represents a new host record in the U.S.

The lack of development of adult *M. cribraria* on black-eyed pea, and the successful development on mung bean, contradicts those of Medal et al. (2013). They found that *M. cribraria* could be reared from egg to adult on black-eyed pea, but not on mung bean. However the result of our experiments agrees with Zhang et al. (2012) in that there was no adult development on black-eyed pea, yet they did not test mung bean. Differences in developmental success on different plants may be because Medal et al. (2013) used field-collected adults after the availability of kudzu, or variety of black-eyed pea used in their experiments.

In its native Asian distribution, 24-56 d are needed for *M. cribraria* to transition from egg to adult on various host species (Eger et al. 2010). In this study, *M. cribraria* developed 5-9 d slower on soybean when compared to no-choice experiments by Del Pozo-Valdivia and Reisig (2013). This result is likely a difference in rearing conditions and soybean variety.

Mortality was significantly greater on both black-eyed pea and butter bean suggesting that these two legume species are not suitable alternative reproductive hosts of *M. cribraria*. However, mung bean and soybean are adequate alternative reproductive hosts for *M. cribraria*, as mortality was lower on soybean then on mung bean and kudzu. Furthermore, soybean had a significantly lower mortality rate compared to kudzu, suggesting that soybean is a more suitable reproductive host for *M. cribraria*. The high rates of mortality (> 45.0%) observed in the no-choice experiments could have been induced by restricting nymphal *M. cribraria* to small cages preventing their access to shade or of declining host plant quality. Researchers have previously had difficulties rearing this insect in controlled environments (Zhang et al. 2012, Del Pozo-Valdivia and Reisig 2013).

Mean developmental time of the first, second, and fourth instars were consistent among mung bean, soybean, and kudzu at ≈ 7.0 d for each instar, however the third and fifth instars were slower to develop than the aforementioned stadia, lasting ≈ 10.0 d and ≈ 14.6 d respectively on mung bean, soybean, and kudzu. Slower development of the fifth instar has been recorded previously (Tayutivutikul and Yano, 1990), however there are no previous reports of slower development of the third instar. During this instar, *M. cribraria* nymphs undergo a physiological change developing functional dorsal abdominal scent glands opening through ostia on tergites 3-4 and 4-5 (Moizuddin and Ahmad, 1975). A consequence of this morphological change may be the result of a trade-off between nutrient reallocation to favor the secondary development of defensive features over gaining body mass (Tollrian and Harvell 1999, Steiner and Pfeiffer 2007, Brönmark et al. 2012).

Although mortality > 90%, a small number of *M. cribraria* nymphs (n= 5) were able to successfully develop into adults after 59 d in one replicate of butter bean. Noteworthy is the developmental time of the third instar, which took an average of 21 d to molt to fourth instars (Fig. 3). This result indicates that there is most likely a plant factor inhibiting or delaying nymphal development. This may be due to plant nutritional quality and elements like nitrogen, which are known to retard the development of immature insects (Matton 1980, Scriber and Slansky 1981). Research should further investigate what these plant factors are that significantly delayed the development of third instar *M. cribraria*.

While this study was limited to no-choice greenhouse experiments, it indicates that kudzu is not an obligatory host for this insect. However feeding on kudzu and/or other supplementary plant species, or host switching from adult to nymph, may benefit the overall development of *M. cribraria.* For instance, supplemental feeding on legumes that are high in lipids, such as soybean, have been shown to increase starvation tolerance and reproductive fitness in the southern green stinkbug, *N. viridula* (L.) (Panizzi and Hirose 1995), additionally host switching from nymph to adult *N. viridula* has been indicated to increased fitness (Panizzi and Saraiva 1993). Perhaps *M. cribraria* displays similar benefits and/or trade-offs from this behavior, and subsequent investigations should address how host switching and supplementary feeding may affect certain biological and life history traits of *M. cribraria*.

Previous reviews (Panizzi et al. 1997) have emphasized the importance that nutritional ecology plays in the life cycles of Pentatomoidea of agricultural concern. Few studies have examined the effects of host plant and reproductive host plant switching has between stages and on adults of subsequent generations. Further research should address these topics, since a better understanding of host and reproductive plant use, the sequence in which these plants are used, and the dispersal from which wild to cultivated crops takes place (Panizzi et al. 1997) could improve control methods against *M. cribraria* in the southeastern U.S.

CHPATER 4

STRONGYGASTER TRIANGULIFERA (DIPTERA: TACHINIDAE) AS A PARASITOID OF ADULTS OF THE INVASIVE *MEGACOPTA CRIBRARIA* (HETEROPTERA: PLATASPIDAE) IN ALABAMA

The kudzu bug, *Megacopta cribraria* (F.) (Heteroptera: Plataspidae), also known as the kudzu bug, is native to Asia. Before its initial detection in the U.S. in October 2009 (Eger et al. 2010), species of the family Plataspidae were not known in the Western Hemisphere. In its native range, *M. cribraria* is not regarded as a significant pest (Ruberson et al. 2013). However, in the U.S., its rapid dispersal, explosive population growth, and severe damage to soybean and other legume crops, as well as its offensive odor, has elevated its status from an urban nuisance to a serious legume crop pest throughout the Southeast. Adults and nymphs extract plant nutrients from the vascular tissues of host plants. Whereas kudzu, *Pueraria montana* Lour. (Merr.) variety *lobata* (Willd.), and soybeans, *Glycine max* (L.) Merr., are its preferred hosts, adults have been collected from numerous legume and non-legume plants (Gardner et al. 2013). This can be largely attributed to its adaptability, high mobility, the relative lack of natural enemies, availability of reproductive host plants and feeding hosts, as well as a lack of fully-developed and tested management strategies. As of 19 August 2013, it was confirmed in 11 states and all 67 counties in Alabama (unpubl. data, W.A. Gardner, http://www.kudzubug.org).

On 10, 13, and 18 July 2013, the authors collected via beat sampling approx. 300 *M. cribraria* adults from early-planted (01 April 2013 planting date) soybeans on the campus of Auburn Univ., Auburn (Lee Co.), AL (32°35’23.34 N 85°29’19.01 W). These overwintered adults were collected to determine female reproductive development; however, dissections of the collected adults under a stereomicroscope (Meiji EMZ-TR, Meiji Techno, Japan) revealed a solitary endoparasitoid dipteran larva. The authors observed dipteran larvae at various developmental stages (1st to 3rd instar) in the abdomen near the reproductive structures. Larvae appeared to affect oocyte development in *M. cribraria* females, and both males and females showed tissue damage proximate to the reproductive structures. Feeding also resulted in damage to the adult’s circulatory system evident by hemolymph inside the abdominal cavity.

In total, 214 (107 F; 107 M) adult *M. cribraria* were dissected. The overall rate of parasitism was 5.14% regardless of gender, with the parasitism rate in females of 9.34%, which was 10X greater than the parasitism rate (0.93%) in males. The size of the various larval instars observed ranged from 0.7 mm to 3.5 mm in length, with the 3.5 mm length being the oldest stage (3rd instar) (Figure 4.1).

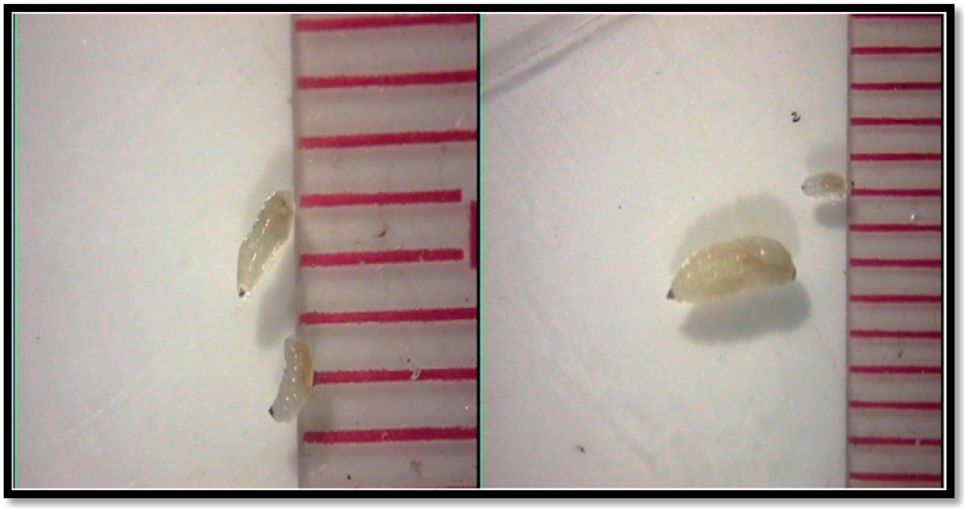

Figure 4.1. *Strongygaster triangulifera* larval instars removed from M. cribraria adults (graduations on scale = 1 mm).

The remainder of the collection (n = 86) was maintained at room temperature (25°C, 65% RH) in glass jars and provided with tender soybean stems and leaves. The transparent glass jars allowed for observation of the 3rd -instar larvae exiting the host body to pupate on the bottom of the jars or attached to the underside of soybean leaves. Adult tachinid flies emerged 10 d after pupation and ranged from 4.3-5.0 mm (mean: 4.66, SE: 0.09; n = 7) in body length (Figure 4.2).

Adults were identified as *Strongygaster* (= Hyalomyodes) *triangulifera* (Loew) 1863 (Diptera: Tachinidae: Tachininae: Strongygastrini). Specimens were deposited in the Insect Museum of Auburn University (voucher #116 - 2013) and ARS museum (voucher # USNM ENT 00,039,875).

Figure 4.2. *Strongygaster triangulifera* adult reared from *M. cribraria* adult.

Strongygaster triangulifera is a small parasitic fly that is widely distributed in North America (Reeves and O'Hara 2004). This New World fly typically parasitizes only adult insects, with a few reports of larval parasitism viewed as abnormal occurrences (Thompson 1954). It is most frequently reported as a parasitoid of adult Coleoptera with host species in more than 10 families (Guimarães 1978, Gerding and Figueroa 1989, Purrinton et al. 1990, Reeves and O'Hara 2004), but it is also reported as having a broad host range including representatives of Dermaptera, Hemiptera, Lepidoptera, Orthoptera (Arnaud 1978, Kevan et al. 1988), Hymenoptera (Shima 1999), and Pentatomidae of Heteroptera (McPherson et al. 1982).

This is the second report of parasitism by an indigenous North American tachinid fly species of *M. cribraria* adults in its expanded U.S. range. Ruberson et al. (2013) reported recovery of the tachinid *Phasia robertsonii* (Townsend) from a single adult in 2012 in Tift Co., GA. *Strongygaster triangulifera* has several characteristics as a generalist parasitoid that warrant further evaluation for its utilization as a naturally occurring or augmented biocontrol agent in a management program of *M. cribraria* in the southeastern U.S.

CHPATER 5

DISCOVERY OF *PARATELENOMUS SACCHARALIS* (DODD) (HYMENOPTERA: PLATYGASTRIDAE), AN EGG PARASITOID OF *MEGACOPTA CRIBRARIA* F. (HEMIPTERA: PLATASPIDAE) IN ITS EXPANDED NORTH AMERICAN RANGE

Megacopta cribraria F. (Hemiptera: Plataspidae), commonly known as the kudzu bug or bean plataspid, was first discovered in the Western Hemisphere in October 2009 (Eger et al. 2010). The plataspid quickly spread from the 9 northeastern Georgia counties in which it was initially confirmed into 383 additional counties in Alabama, Florida, Georgia, Mississippi, North Carolina, South Carolina, Tennessee, and Virginia by the end of 2012 (Gardner et al. 2013). Subsequent reports show that the insect has now been confirmed in 4 additional states – Delaware, Kentucky, Louisiana, and Maryland – and the District of Columbia, bringing the total number of confirmed states to 12 (W.A. Gardner, unpubl. data).

Ruberson et al. (2013) reported that several existing generalist predators and a single entomogenous pathogen had been recorded as attacking *M. cribraria* in its expanded range in the southeastern U.S. They also reported a tachinid, *Phasia robertsonii* (Townsend), parasitizing a single adult *M. cribraria* in 2012, but no parasitism of eggs or immatures was observed in their 2010 and 2011 surveys in Georgia. Golec et al. (2013) discovered *Strongygaster triangulifer* (Loew) (Diptera: Tachnidae) parasitizing individual adults (mean parasitism = 5.14%; n = 214) collected from soybean, *Glycine max* (L.) Merr., in Auburn, AL, in April 2013. Ruberson et al. (2013) concluded that parasitism of *M. cribraria* eggs did not appear to be occurring in its expanded North American range based on the lack of egg parasitoids reported in their study as well as the lack of egg parasitism observed by Zhang et al. (2012) in their 2010 study in northeastern Georgia.

Furthermore, research conducted at the USDA-ARS National Biological Control Laboratory in Stoneville, MS, confirmed that *M. cribraria* eggs were not attacked by 11 species of native egg parasitoids (8 from Pentatomidae and 1 each from Coreidae, Reduviidae, and Rhopalidae) plus 4 species of exotic egg parasitoids being assessed for the brown marmorated stink bug, *Halyomorpha halys* Stål, host range project (W.A. Jones, unpubl. data).

On 30 May 2013, 2 *M. cribraria* egg masses with several abnormally dark-colored eggs were collected by Joni L. Blount (UGA) from kudzu, *Pueraria montana* Lour. (Merr.) variety *lobata* (Willd.), growing on the University of Georgia Campus in Griffin (Spalding Co.). Microscopic examination of the masses showed that some of the eggs were a reddish color, others were a charcoal gray color, and others were the normal salmon color. The egg masses were placed in a single No. 1 Gelatin Capsule (Eli Lilly and Company, Indianapolis, IN) and held at ambient room conditions. Two weeks later, 3 small hymenopterans were discovered in the gelatin capsule with the egg masses. Microscopic examination of the egg masses confirmed that the wasps had emerged from 3 of the previously discolored egg capsules. An egg mass of 22 eggs collected 2 July 2013 from kudzu in the same vicinity exhibited the same coloration and subsequently yielded 18 parasitoids.

In June 2013, egg masses oviposited by *M. cribraria* adults in soybean growing on the Auburn University Campus in Auburn (Lee Co.), AL, were observed with a gray coloration. On 17 July 2013, 4 egg masses (mean number eggs per mass ± SD = 22.25 ± 1.89) were collected by Julian R. Golec (Auburn Univ.) at that location, placed in a Petri dish, and maintained at 25°C in an environmental chamber. Forty-eight wasps emerged from 3 of the 4 egg masses on 23 July 2013 with parasitism rates of 95.4% (n = 22), 95.2% (n = 21), and 52.4% (n = 21). Eleven egg masses were subsequently collected from the same location on 5 August 2013, with all but 1 of those masses being parasitized (84.8% egg parasitism, overall). Microscopic examination of the parasitized eggs showed that the grayish coloration was caused by the either black coloration of the developing parasitoid within the cream-colored host egg capsule or discoloration of the lining of the egg capsule (Figure 5.1). This grayish-coloration is distinctive for parasitism and serves as an aid in identifying parasitized eggs in the field.

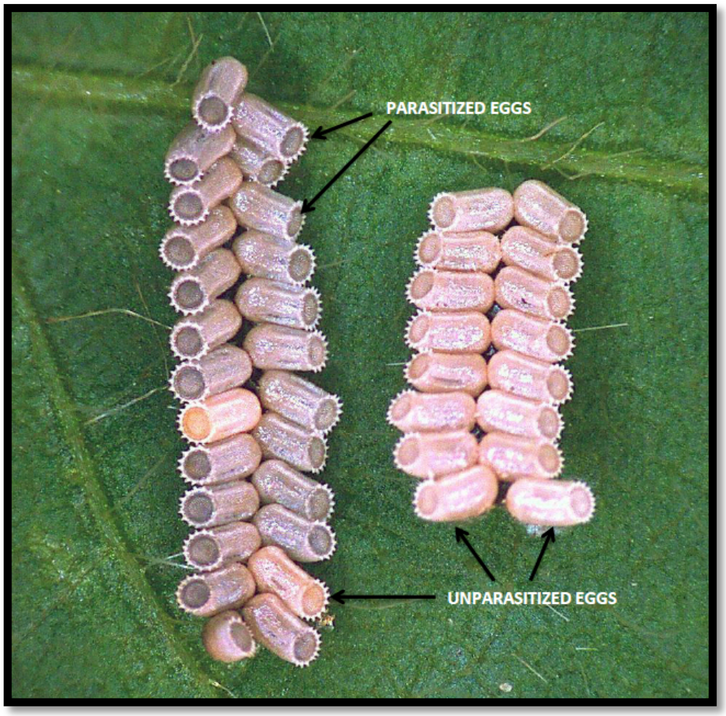

Figure 5.1. Eggs of *Megacopta cribraria* parasitized (gray coloration) and unparasitized (salmon coloration) by *Paratelenomus saccharalis* (Image by J. Golec, Auburn Univ.).

Jannelle Couret (Emory Univ.) observed adult parasitoids under magnification probing and ovipositing into *M. cribraria* eggs collected from kudzu on and near the Emory University campus in Dekalb Co., GA (metropolitan Atlanta), in late June. Parasitized eggs were maintained in Petri dishes in environmental chambers. Adult parasitoids were later observed emerging from the egg masses and, specifically, from the gray-colored eggs. The gray eggs that did not hatch were dissected to find parasitoid larvae and adults that had not successfully emerged.

Egg masses were collected from 5 locations in Dekalb Co. on 24 July 2013. These masses were transported to the Department of Entomology Laboratory on the UGA Griffin Campus where numbers of eggs per mass were counted and the masses were placed individually into 2-dram glass vials. The vials were stoppered with cotton and placed in an environmental chamber maintained at 28°C with 70 - 80% RH and on a 12:12 h photoperiod. Vials were examined periodically for parasitoid emergence. In total, 91 egg masses were collected from those 5 sites on that date with a mean (±SD) number of eggs per mass of 13.3 ± 5.1. Seventy-eight of those 91 masses (85.7%) were eventually confirmed as parasitized. Parasitism rates averaged 69.0% (n = 159), 77.9% (n = 144), 72.0% (n = 507), 66.7% (n = 310), and 55.9% (n = 109) at the individual collection sites.

Ten egg masses (21.2 ± 7.0 eggs per mass) were collected on 23 July 2013 from soybean growing on the UGA Bledsoe Research Farm in Pike Co., a county adjacent to Spalding Co. where the parasitoid was initially discovered. All but 1 of those masses was confirmed as parasitized with an overall 47.5% level of egg parasitism.

Microscopic comparison of the adult parasitoids from the Dekalb Co. sites (Emory Univ.) with those from the UGA sites in Spalding and Pike counties indicated all were the same species. Specimens from these sites were identified by Walker A. Jones (USDA-ARS) as *Paratelenomus* *saccharalis* (Dodd) (Hymenoptera: Platygastridae) using the taxonomic key of Johnson (1996); they also appeared identical to specimens from a colony of *P. saccharalis* maintained in quarantine at the USDA-ARS National Biological Control Laboratory in Stoneville, MS. Voucher specimens from Georgia and Alabama sites were sent to Matthew L. Buffington and Elijah J. Talamas of the Systematic Entomology Laboratory at the National Museum of Natural History (Smithsonian Institute, Washington, DC); Talamas confirmed the identification as *P. saccharalis* using the key of Johnson (1996). Images accompanying confirmation of the identity were provided by E.J. Talamas (Figure 5.2).

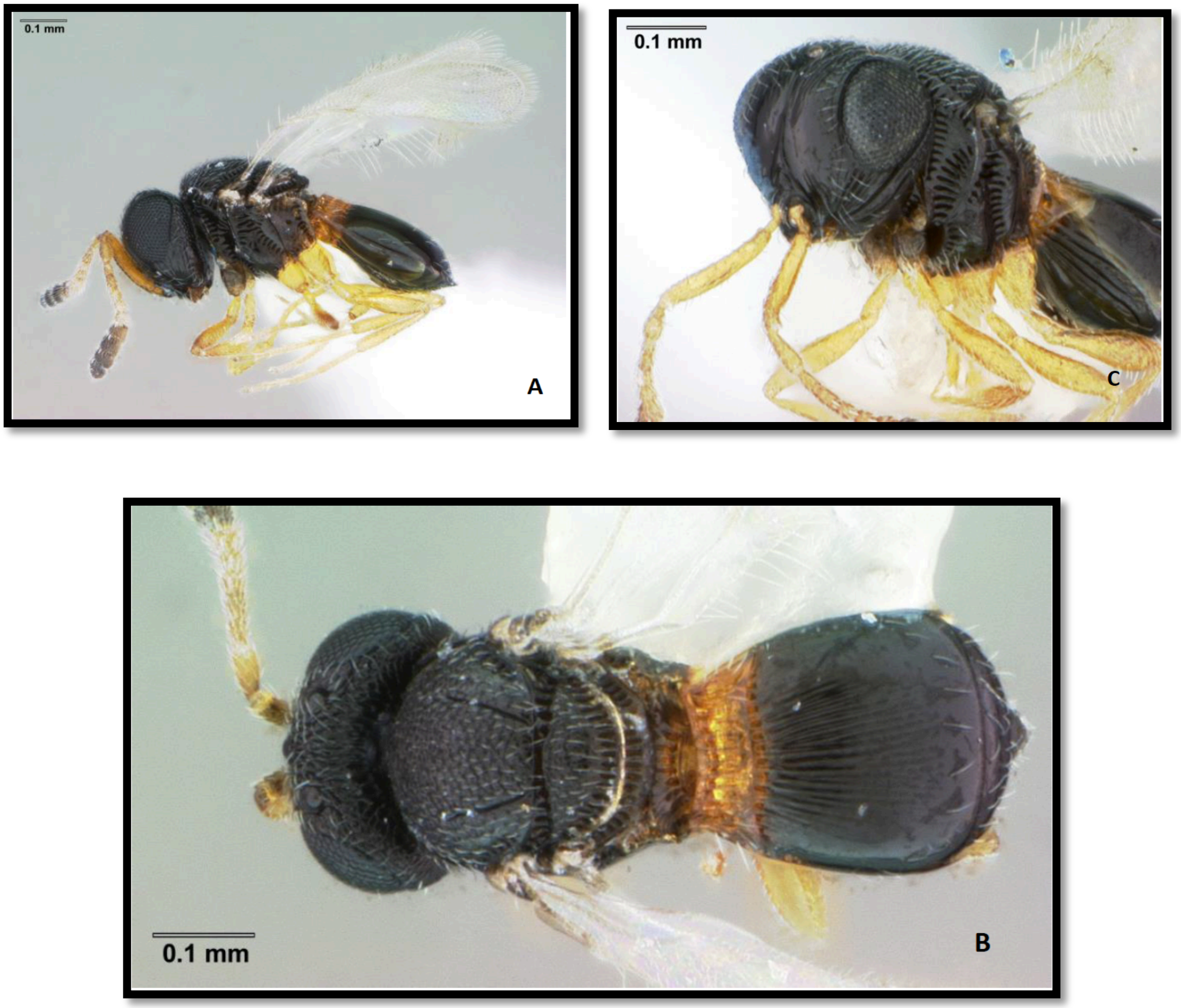

Figure 5.2. *Paratelenomus saccharalis* adult from lateral (A), dorsal (B), and frontal (C) views

(Images by E. Talamas, USDA-ARS)

Paratelenomus saccharalis is widely distributed in the Eastern Hemisphere (Ruberson et al. 2013), but this is the first report of this parasitoid in the Western Hemisphere. Ruberson et al. (2013) further noted that known hosts of *P. saccharalis* are restricted to the family Plataspidae. The only known hosts of *P. saccharalis* were listed as *M. cribraria*, *M. punctatissimum* (Montandon), and *Brachyplatys subaeneus* Westwood in Asia, and *Coptosoma scutellatum* (Geoffrey) in Italy. Given this level of host specificity, *P. saccharalis* would not be expected to occur in the Western Hemisphere prior to 2009 because the first known occurrence of any members of the family Plataspidae in the New World was in October 2009 with the discovery of *M. cribraria* in Georgia (Eger et al. 2010). Yet, since these initial independent discoveries of the parasitoid in Alabama and Georgia, *P. saccharalis* has been confirmed parasitizing M. cribraria eggs in 17 additional counties in Georgia, 8 additional counties in Alabama, and 1 county in Mississippi (unpubl. data).

Paratelenomus saccharalis was identified as a candidate for importation and release against *M. cribraria* in its expanded range in the U.S. because of its host specificity, wide geographic distribution in its native range, and its close biological association with *M. cribraria* (Ruberson et al. 2013). Walker A. Jones and Richard M. Evans have been assessing the host range of the parasitoid in quarantine (USDA-ARS National Biological Control Laboratory) for over 2 years and, based upon their data, prepared a petition to USDA-APHIS for release of the parasitoid.

The introduction and sudden appearance of this parasitoid in the expanded *M. cribraria* range might never be determined. Its appearance over a wide geographic area and at relatively high parasitism rates soon after its discovery are also puzzling. It is highly likely that the establishment of the parasitoid occurred after *M. cribraria* was established. Perhaps parasitized eggs were introduced and even dispersed on shipments of plants or nursery stock or on fresh food products. Regardless, this is not the first report of an inexplicable appearance of an egg parasitoid following the accidental introduction of a plataspid. Beardsley and Fluker (1967) reported the establishment of *Coptasoma xanthogramma* (White) (Hemiptera: Plataspidae) in September 1965 as the first plataspid discovered in Hawaii. They also reported that no parasitoids and only few predators were attacking the introduced pest in its expanded range in Hawaii. Notes from the 1968 meeting of the Hawaiian Entomological Society (1969) indicate that a parasitoid, presumably *Trissolcus* sp. (Hynenoptera: Platygastridae), was found parasitizing *C. xanthogramma* eggs at several locations and on multiple islands in early 1968. Interestingly, the reported appearance of the parasitized eggs, percent parasitism, and the developmental timing of the parasitoid are consistent with those reported by Tagaki and Murakami (1997).

CHPATER 6

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Kudzu Bug Control in Residential Areas: Frequently Asked Questions (FAQ)

The kudzu bug is an invasive pest species from Asian and was first reported in Georgia in 2009. In late 2010 it spread to Alabama, and by the summer of 2014 it had been reported from all 67 counties in the state. Since its accidental introduction it has been confirmed in more than 14 southeastern states. First considered a nuisance pest in residential areas, it is posing a much greater threat than previously thought. In Asian countries, it has been a serious pest of soybean and vegetable beans. Here in the United States, it not only cause yield loss of soybeans, but also poses threats to international trade of agricultural products to Central America, and is an urban nuisance pest. Here are some of the most frequently asked questions concerning kudzu bug control around homes and in urban settings.

Why are there so many kudzu bugs on my home in spring and fall? During the fall kudzu bugs will seek out protected areas to spend the colder winter months. In spring, the warm weather wakes the bugs up from their overwintering sites and they will begin flying in search of food plants. Additionally, they are attracted to light colors such as white and yellow. Unfortunately this means they may aggregate on or nearby your home, on you, or your vehicle, and may accidently wander into your home. March-April and September-October present the most common times that homeowners will encounter this insect.

What should I do if kudzu bugs enter my home? Kudzu bugs can enter your home through various ways such as holes in window and door screening, or through gaps under doors. The best way to prevent this from happening is to secure your home; make sure that cracks/holes are well sealed, windows are tight, and any opening to the outside is screened. If insects do enter your home do not squish them, as they will emit a foul odor and can stain fabrics.

- Get a vacuum cleaner and secure pantyhose to the vacuum tube with a rubber band, this will allow you to suck the bugs up and dispose of them.
- If you have a shop vacuum you can fill the canister with hot soapy water and suck them up that way.

What do they eat and why they are seen on many garden plants and yard ornamentals? Kudzu bugs are leguminous plant feeders. The primary hosts, or named reproductive hosts, are kudzu vines and soybeans. This means that they will have to feed on the primary hosts to be able to produce eggs. However they are capable of feeding on a wide range of leguminous and non-leguminous plants. This is particularly true in early spring and late fall when the preliminary hosts are unavailable but they are desperately in need of storing reserves for overwinter, or are hungry enough to non-selectively eat tender plants to obtain energy before host plants become available.

Do they cause damage or kill garden vegetables, fruit trees, and landscape plants? Kudzu bugs suck out the sap of plants, and can cause severe yield loss to soybeans. They don’t chew holes or eat leaves. The effects of their feeding on vegetable beans and peas can range from discolored spots to the death of the plants. Their feeding on non-legume plants is often minor, and discolored spots are barely visible.

How can I control kudzu bugs in my garden and outside my home? If kudzu bugs begin to show up outside your home or in you garden, chemical control is not recommended on home gardens or residential structures, due to the potential for run off and their toxicity. If bugs begin to aggregate on your home you may control them mechanically via:

- Washing them off your home with a high-powered hose or soap-water.
- In the fall, remove plant debris around your home and garden in order to prevent the bugs from overwintering near your home.
- Eliminating nearby kudzu patches with the use of herbicides or mowing.
- Place buckets of warm soapy water beneath garden plants and knockdown bugs from plants into the buckets, then dispose of them.
- You may delay the planting of vegetable plants past the critical time of kudzu bug emergence.
- Since these bugs are attracted to lighter colors, you may set up a white-panel trap on the edge of your property. Use a piece of white poster board and cut it in half, attach the two halves by cutting a line up the middle of the two piece and put them together. Place a bucket with soap-water underneath the panel trap and you may deter some

bugs from landing on your home (Figure 6.1).

Figure 6.1. White panel trap used to catch adults
(S. Horn and L. Hanula 2011).

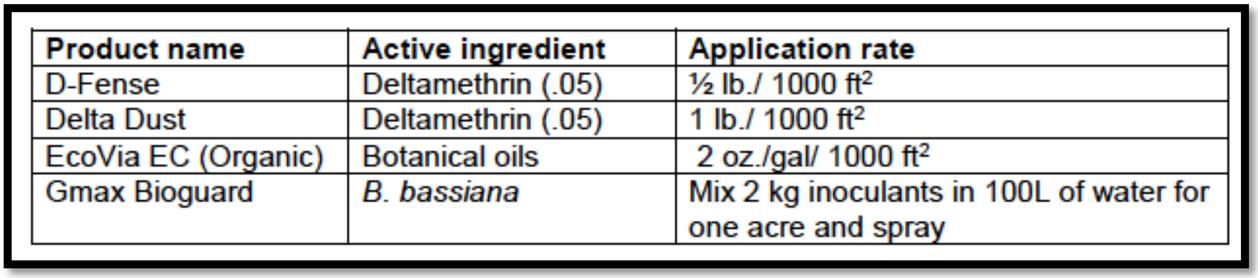
Do they bite or transfer disease? They don't bite like mosquitoes; they don't sting like wasps and bees. The most significant impact they have on humans is the pungent odor they discharge when disturbed. Your skin will be stained orange if you are in touch with the odor emission. They are not known transfer disease.

Will killing kudzu around my house eliminate my kudzu bug problem? Controlling kudzu near your house will likely reduce the large number of kudzu bugs seen in spring, summer, and fall. This should be a community-wide effort to eradicate kudzu patches. However, kudzu bugs are strong fliers and regardless of whether or not there is kudzu near by, they may still end up on or near your home.

Will treating Kudzu bugs with insecticides get rid of them? Spraying insecticides discourages these bugs temporarily, but does not provide lasting control. Large scales spraying of house siding or plants that are not in the bean family is not recommended and will not get rid of these insects. Kudzu bugs tend to migrate in spring and fall and infestations come in waves. Many frequent applications of insecticides, or other treatment methods will only help to reduce bugs currently in the area. More or less, the Kudzu bug is here to stay, we have to learn how to manage them come spring and fall.

What insecticides can I use if I have to kill Kudzu bugs? If you treat kudzu bugs with chemicals, it is best to apply these directly to kudzu patches in the landscape. Any product using an active ingredient ending in –thrin should be effective against the kudzu bug. However, synthetic pyrethroids are toxic to bees and beneficial insects that naturally help to keep pest populations in balance. So timing of application is critical. Never use harmful chemicals on your home or garden vegetables. There are many insecticides labeled for kudzu bug control, but most are for agricultural crops not for home gardens.

Table 6.2. Examples of common insecticidal products available at local retail garden supply stores. Always read and follow all direction on the product label



Product name	**Active ingredient**	**Application rate**
D-Fense	Deltamethrin (.05)	½ lb./ 1000 ft^2
Delta Dust	Deltamethrin (.05)	1 lb./ 1000 ft^2
EcoVia EC (Organic)	Botanical oils	2 oz./gal/ 1000 ft^2
Gmax Bioguard	*B. bassiana*	Mix 2 kg inoculants in 100L of water for one acre and spray

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Kudzu Bug Control in Soybeans: Frequently Asked Questions (FAQ)

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First recognized as a nuisance pest when discovered in Georgia during the fall of 2009, the kudzu bug has become a serious pest of soybeans. Initial reports indicated that this invasive insect is capable of reducing soybean yields by nearly 60%. Yield loss due to kudzu bug feeding has been observed to reduce pod size, seeds per pod, and seed weight. Control of the kudzu bug in agricultural settings is possible and begins with proper insect identification, attention to economic thresholds, and timing of chemical treatments. As this insect begins to migrate to soybean in the coming the months, below are a few frequently asked questions concerning kudzu bug control in soybeans.

Where do kudzu bugs tend to aggregate initially in soybean fields? When kudzu bugs first encounter a soybean field, they will aggregate abundantly on the outside rows, or edges of the field. As the season progresses they will move toward the center of the field.

When will I find kudzu bugs in soybean fields? Adults have been observed entering soybean fields as early as May in South Carolina (V1-V3 growth stage). Once adult insects are observed in fields, they will persist there throughout the growing season. Nymphs are normally first encountered in June and last through September.

What is the recommended scouting technique? Sweep netting (15" diameter net) or visual inspections of the crop are the proper scouting techniques. Make sure to sample the entire field and not just crop borders or edges.

Do kudzu bugs reduce plant height when they infest seedling soybeans in large numbers? Yes, kudzu bugs feed on plant sap and thus reduce the amount of nutrients flowing through the plant. This will reduce the plants vigor. Additionally, high kudzu bug populations have been indicated to be able to reduce soy yield by 60%. However no damage to seeds has be seen.

How will kudzu bug feeding affect soybean plants? Kudzu bugs feed by inserting their mouthparts into the plant stems, and they will suck the plant sap. This removes vital nutrients from the plants, reduces plant vigor, and can induce the growth of black sooty mold leading to various secondary plant problems.

Are early season soybeans more susceptible to kudzu bug infestation? Field observations show that kudzu bug numbers tend to be greater in early-plant soybeans than in later planted soybeans.

Do all pyrethroids provide at least 80% control shortly after application? Not all, but many pyrethroids provide high mortality after application, usually 2-5 days after treatment. Pyrethroids that initially give 80% control or better of kudzu bugs include products containing bifenthrin (Brigade, Discipline, Fanfare), gamma-cyhalothrin (Declare), lambda-cyhalothrin (Karate Zeon, Silencer), and zeta-cypermethrin (Mustang Max). Additionally, carbaryl (Sevin) and acephate

(Orthene 97) also initially give better than 80% control of kudzu bugs (Fig.1).

How and when should I treat my field? The Alabama Pest Management Handbook (2014) recommends treating fields before first bloom when kudzu bugs reach a density of 5 adults per plant across the entire field. The suggested treatment threshold once plants begin to bloom is 10 adults per sweep net when sweeping across two rows, or when one nymph per sweep is collected. If immature kudzu bugs are easily and repeatedly found on petioles and main stems during visual inspections of the canopy, treatment is likely warranted. Border row applications of recommended insecticides have, in some cases, slowed the movement of the kudzu bugs into the center of fields. However, re-application of insecticides may be needed if applied before or during kudzu bug migration into soybean fields. Kudzu bugs continued to migrate into soybean fields at Prattville, AL through the third week of July in 2013. Make sure insecticide applications thoroughly penetrate the canopy as these insects feed on the stems and petioles of the plant.

Are adults or nymphs more susceptible to chemical treatment? Both adults and nymphs are susceptible to insecticides.

Will one application of an effective insecticide provide season long control when applied at least one month after soybeans emerge? No, these insects will migrate into soybean fields from other feeding sites, and their migration to fields may last as long as two-to-three months. You should only apply an insecticide in response to a kudzu bug infestation when the pest density reaches the economic threshold. When treating multiple times make sure to change the insecticide and the active ingredient to prevent insecticide resistance.

When do population peak in soybean fields? Adult and nymphal peak are usually seen in September (corresponding to ~R5), while eggs tend to peak in August (~R2)

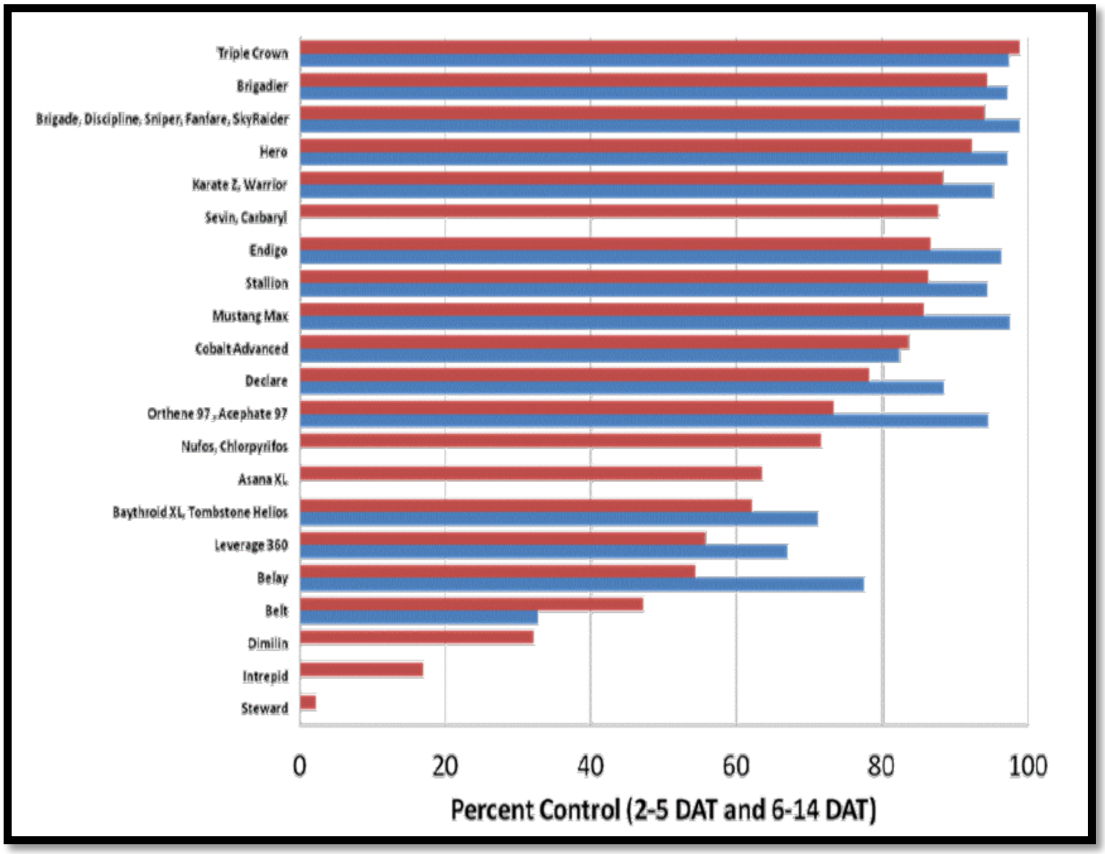

Figure 6.2 Insecticide Efficacies

CONCLUSIONS AND DIRECTIONS FOR FURTHER RESEARCH

The work completed through this thesis has reported on several previously unknown aspects of the overwintering biology and physiology of the kudzu bug. It showed that both females and males stored sperm throughout winter dormancy, that both virgin and pre-overwinter mated females could develop oocytes post-dormancy before feeding, or in the case of virgin females, without having had received sperm, and that the there was significantly more females collected throughout the overwintering months. Furthermore these findings give support to those by Jenkins and Eaton (2011). They determined that the current population of *M. cribraria* in the U.S. was established from a single female lineage, thus indicating that the female who was responsible for the subsequent establishment of the insect throughout the southeastern U.S. was likely mated and storing sperm while in transit to North America. It was additionally determined through no-choice greenhouse assays, that the overwintering generation could feed and oviposit on alternative host plants, and the first generation could develop from egg to adult on soybean and mung bean. This suggesting that kudzu is not an obligate host plant for the kudzu bug. Furthermore, field surveys lead to the discovery of two locally existing parasitoids of the kudzu bug. While this research has helped to answer some fundamental questions about certain biological and physiological traits of the kudzu bug that may have aided in the rapid spread of this insect in the U.S., it has certainly created many other questions that the author hopes subsequent researchers will investigate. The author offers some suggestions for further research in the next paragraphs.

In regards to the second chapter, some of the questions that remain currently unanswered are; what are the proportions of live-to-dead sperm within overwintering female spermathecae, does the proportion change throughout the course of the overwintering months, and does this prolonged storage of sperm have any effect on post-winter fecundity, hatch rate, or sex ratio compared to the spring and summer generations, and do females need to re-mate post-winter, or can they successfully utilize stored sperm to fertilize eggs.

While it was determined that the overwintered generation kudzu bugs are able to develop on the alternative host plants, soybean and mung bean, indicating that kudzu is not necessary for reproductive development, they may in fact benefit from host switching. Adult kudzu bugs do demonstrate host switching in the field, which can be seen by overwintering adults feeding on various plants around or within kudzu patches just after emerging from dormancy (unpublished data available on some wild host species), then switching to kudzu when it becomes available, or switching from kudzu to soybean as the cultivated crop becomes available. Host switching is a particularly common feature of other Heteroptera of economic concern and have been indicated to increase insect fitness (see Panizzi et al. 1997). Future investigations should address such questions, in particular the effects, if any, of host switching between the adult to nymph or nymphal to adult from kudzu to soybean and vise-versa.

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