EFFECTS OF HOST PLANT SHIFT ON LIFE HISTORY AND FITNESS OF THE KUDZU BUG *MEGACOPTA CRIBRARIA*

by

JAMES THOMAS MURPHY

(Under the direction of Patricia J. Moore)

ABSTRACT

The kudzu bug, *Megacopta cribraria*, is an introduced pest of leguminous plants that was first detected in the American southeast in 2009. Previous studies have sought to quantify the life history of this insect, but there is little insight in the literature concerning life history traits of the bivoltine population as it shifts hosts midsummer. Understanding the life history is essential to the control of any new invasive. This is especially true for *M. cribraria* as it is both an urban and agricultural pest in its introduced range. In a greenhouse study, we found that host plant and generation have an effect on life history parameters of kudzu bug (e.g. fecundity, body size, flight ability, and maternal oviposition choice). A subsequent field study demonstrated similar trends, indicating that oviposition and rearing on kudzu and soybean impact fitness differentially and influence distinct phenotypes during the annual active period of kudzu bug.

INDEX WORDS: *Megacopta cribraria*, flight mills, morphometrics, triglyceride assay, fitness, life history, invasive species, kudzu, soybean

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DEDICATION

I dedicate my thesis to my grandparents; whose care and guidance has led me from the shores of Carolina Bay, onward to Death Valley, and now through The Arch to continue my journey.

I also dedicate it to my mother, father, and sister. I owe them a debt of intense gratitude for the incalculable amount of love, support, and encouragement they've bestowed upon me

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CHAPTER 1

INTRODUCTION

Preface

Megacopta cribraria is a new invasive to the southeastern United States. As with any new invasive species, in order to effect integrated management strategies it is crucial to understand as many aspects of the organism's behavior and life history. This project seeks to fill in existing gaps in the literature with regard to the voltinism, host preference, development, and life history *M. cribraria* in order to better inform management strategies and allow policy makers and growers to have access to a comprehensive body of literature to protect their interests and inform their choices as the kudzu bug (*M. cribraria*) becomes increasingly established in its new range.

Definitions of Life History Terms

For the purpose of clarity and to establish the driving impetus of the following thesis, the authors have decided to define life history terms used in the *a priori* structuring of the studies as well as *a posteriori* discussion of results and implications. In the first issue of the 51st volume of *The Quarterly Review of Biology* (1976), Stephen C. Stearns cheekily defines 'fitness' as "Something everyone understands but no one can define precisely". In this thesis any references to 'fitness' will be defined as a measure of reproductive success as approximated by our chosen parameters: female potential fecundity (gross number of eggs laid per female per trial), body size

(correlated by proxy measures of notum and scutellum dimensions), flight ability (total distance flown), and available energy stores (as estimated by triglyceride content of abdominal tissue).

Another term of interest is 'maternal effect'. Similar to fitness, maternal effect is nebulous in its exact definition, and may be interpreted differently depending on the design of a given study. Generally it is defined as a measurable impact on the phenotype of an individual that is dependent on the phenotype and environmental conditions of its mother, and independent of its own genotype and environmental conditions. Precisely defining developmental cut-offs and identifying genetic markers of maternal effect is an entire field of genetics and behavioral study; therefore, we define maternal effect as any measurable impact on the previously mentioned fitness parameters of offspring that is independent of host plant treatment, but is dependent on the host plant of its mother as determined by statistical analysis.

Impacts of Host Plant on Fitness – Insect Evolution and Development

Host choice and preference of insects is known to be influenced by numerous environmental factors, interpreted variously as visual, olfactory, and gustatory cues across orders including but not limited to: Lepidoptera (Yoshida et al. 2015), Coleoptera (Gray et al. 2015), and Hemiptera (Schroeder et al. 2014). It is well established in the literature that plants and herbivores exert strong selective pressures upon each other (Schoonhoven et al. 2005). Plants have evolved physical defenses including glandular trichomes as well as a large diversity of chemical defenses (Fritz and Simms 1992; Schoonhoven et al. 2005; Wise and Rausher 2013). In response, herbivores have evolved mechanisms to cope with these defenses. This is achieved primarily by escaping detection by host plants, or alternatively suppressing plant defenses (Schoonhoven et al. 2005; Kant et al. 2008; Sarmento et al. 2011). The ability to subvert host plant defenses is particularly important for polyphagous herbivores, which can feed and

reproduce on a diverse range of plant families. (Schoonhoven et al. 2005). Conversely, monophagous species are specialized on a few closely related plant species likely equipped with similar defenses. For polyphagous species that may frequently be exposed to new hosts, colonizing a new host plant species often involves tolerating or resisting defenses specific to the new host plant. Therefore, quick response to diverse and possibly strong selection is critical for success. Responses of herbivore populations to environmental changes, such as a host plant shift, form a continuum spanning success (adaptation) to failure (local extinction). This raises an interesting question: how and by which mechanisms can populations of polyphagous herbivores adapt and exploit many host plant species? Addressing this question is not only exciting from an evolutionary perspective, but can also provide crucial information for the management of crop pests.

Many established theoretical models of plant/insect relationships assume that natural selection has fully optimized the ability of phytophagous insects to select food plants (Thompson, 1988; Courtney & Kibota, 1990; Mayhew, 1997). Logically it would seem that whether a host plant is chosen or an egg is placed at a feeding site by the ovipositing female (yielding no possible choice), phytophagous insects should select plants that promote the growth of feeding stages and the survival of all stages that feed and shelter on plants thereby maximizing their fitness and reproductive potential. Though this appears to be a simple relationship, it is also highly reductionist in scope and ignores many important components of the large and stochastic system of plant and insect interactions. Testing for and evaluating these interactions has proved similarly complex and prompted many authors to propose methodologies to standardize the measurement of fitness (Singer, 1986; Thompson, 1988; Courtney & Kibota, 1990; Jaenike, 1990). Regardless of the particular system, designing experiments to examine fitness tradeoffs

requires a comprehensive understanding of the biology of the insect and plant(s), natural enemies, and especially the environment. In spite of the impossibility of understanding a system such as this with full consideration for all variables, the relationship between host selection and offspring performance has been investigated for many insects (Mayhew, 1997), generally with the understanding that a completely comprehensive methodology is either impractical or unnecessary for addressing the questions brought up by a study.

In the face of such adversity, efforts must still be made to understand the system as best as possible given the limits on scope and overall resolution of the impacts various parts have on the whole. To evaluate and quantify adaptation of herbivore populations to a given host plant species, an established methodology is to compare their fitness components (e.g., life-history traits: fecundity, body size, lifespan, etc.) on a chosen plant species with those displayed by other herbivore populations that have evolved on a different plant species (Kawecki and Ebert 2004). It is expected that coevolved genotypes will have a higher fitness on their established host plant species than do genotypes from a different host plant species. However, this relationship and its potential impacts becomes much less clear when considering two potential hosts of seemingly equal fitness.

Kawecki and Ebert (2004) recommend rearing subsequent generations on different host plants as a viable method to complement research on natural (wild caught) populations. This approach allows researchers to establish putative causality of evolutionary processes and may also elucidate adaptation to individual host plants (Kawecki et al. 2012a,b; Magalhães and Matos 2012), which may prove more difficult with natural populations due to additional confounding variables. Unfortunately, experimental evolution literature is lacking and no two protocols will work the same for different taxa to study the adaptation of herbivores to new host plant species

(Gould 1979; Fry 1990; Bolter and Jongsma 1995; Agrawal 2000; Fricke and Arnqvist 2007; Magalhães et al. 2007; Kojima et al. 2010; Fox et al. 2011; Fellous et al. 2014). Moreover, studies that do use this approach are limited in both the number of traits measured and by the fact that plasticity and dispersal processes were usually not simultaneously analyzed. Herein, we attempt to fill in some of these gaps in the literature using our chosen insect *Megacopta cribraria* (F.).

Megacopta cribraria - Taxonomy and Systematic Status

As of this writing, the family Plataspidae contains a reported 560 species and 59 genera (Davidova-Vilimova and Stys 1980, Henry 2009). It is nested within the superorder Pentatomoidea (shield bugs, chust bugs, and stink bugs), which in turn resides within the order Hemiptera. According to the literature, *Megacopta cribraria* was first described by Fabricius in 1798 as *Cimex cribrarius*. Since then, several related genera have been described, including *Tetyra*, *Thyreocoris* and *Coptosoma*, before eventually being revised into the genus *Megacopta*. *Megacopta cribraria* is commonly called the kudzu bug, a name that was officially designated at the 2014 meeting of the Entomological Society of America in Portland, Oregon. Previous unofficial common names include bean plataspid, globular stink bug, and lablab bug. It was originally identified in the United States using established morphological markers (Eger et al. 2010, Ruberson et al. 2010), and later confirmed by mitochondrial and nuclear DNA sequencing of the primary and secondary endosymbionts (Jenkins and Eaton 2011) as well as genetic study of its cytochrome oxidase I sequence (Jenkins et al. 2011). Genetic testing indicated a significant amount of similarity between *M. cribraria* and a congener, *M. punctatissima* (Montandon). It is likely that the two species are conspecific (Eger et al. 2010, Hosokawa et al. 2014) and may in fact be synonymous (Yang 1934, Davidová-Vilímová 2006).

External Morphology

Megacopta cribraria is distinct in its appearance from other North American Pentatomoidea. Its sub-spherical shape and pronounced body curvature separate it visually from other members of the superfamily. Family Pentatomidae exhibits complex folding of the hind-wing under the scutellum (Schuh and Slater 1995). Eger et al. (2010) created a key to families of Pentatomoidea in America north of Mexico that includes *M. cribraria* to make species-level diagnosis easier. Two key characteristics used in the diagnosis of Plataspidae (including *M. cribraria*) from other Pentatomoidea are as follows: **1)** two segmented tarsi on all legs and **2)** an enlarged scutellum covering the majority of the abdomen and protecting the wings. Other general morphological characters are as follows: 3.5-6.0 mm in overall body length and ochre brown to olive green body coloration with darker contrasting punctuations on the dorsum (Figure 1.1) (Eger et al. 2010).

Figure 1.1. Dorsal view of adult female *M. cribraria* (Photo by J. E. Eger, Jr. 2010).

Sex determination is based on three diagnostic characters: the color pattern of the abdominal sternites, the concavity of the terminus, and finally the appearance of the genitalic capsule. The sternites of females are lighter in color than males, with a distinct area of dark coloration restricted anteromedially. Male sternites are instead entirely dark in coloration with almost no light coloration laterally (Figure 1.2). Females have a convex terminus, whereas males are concave or blunted in comparison. Finally, the genitalic segments of the male are similar in shape to a crescent moon with a round central protrusion. This is in contrast to those of the female, which are triangular (Figure 1.3).

Figure 1.2 Ventral view of female (left) and male (right) *M. cribraria* illustrating dimorphic coloration of sternites (Photo by Julian Golec, Auburn University 2014).

Figure 1.3 External structure of genitalic capsule of male (left) and female (right) *Megacopta cribraria* (Photo by Julian Golec, Auburn University 2014).

Like other Pentatomoidea, kudzu bugs have evolved scent glands used in the secretion of both defensive substances and attractant pheromones (Kitamura et al. 1984, Aldrich 1988). Nymphal *M. cribraria* possess dorsal abdominal scent glands that function primarily for defensive purposes (Vilímová and Kutalova 2011) with secretions emitted from ostia located between tergal segments 3-4, 4-5, and 5-6 (Ahmad and Moizuddin 1975b, Schuh and Slater 1995). Following eclosion adult *M. cribraria* develop additional ventral metathoracic scent glands. The secretions from these glands have been identified as a combination of several molecules including undecane, dodecane, tridecane, pentadecance, decenyl acetate, octenal, decenal, and oxo-hexenal (Baggini 1966, Kitamura 1984).

Geographical Distribution

Megacopta sp. is a new invasive of the United States, with a natural distribution including reports 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 is the first reported Plataspidae on the North American continent (Eger et al. 2010). The closely related *Coptosoma xanthogramma* (White) (Hemiptera: Plataspidae) has been reported from the Hawaiian Islands (Beardsley and Fluker 1967), but has not been subsequently reported on the mainland. Since 2009; at present, *M. cribraria*, has 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. (Figure 1.4; www.kudzubug.org 2015).

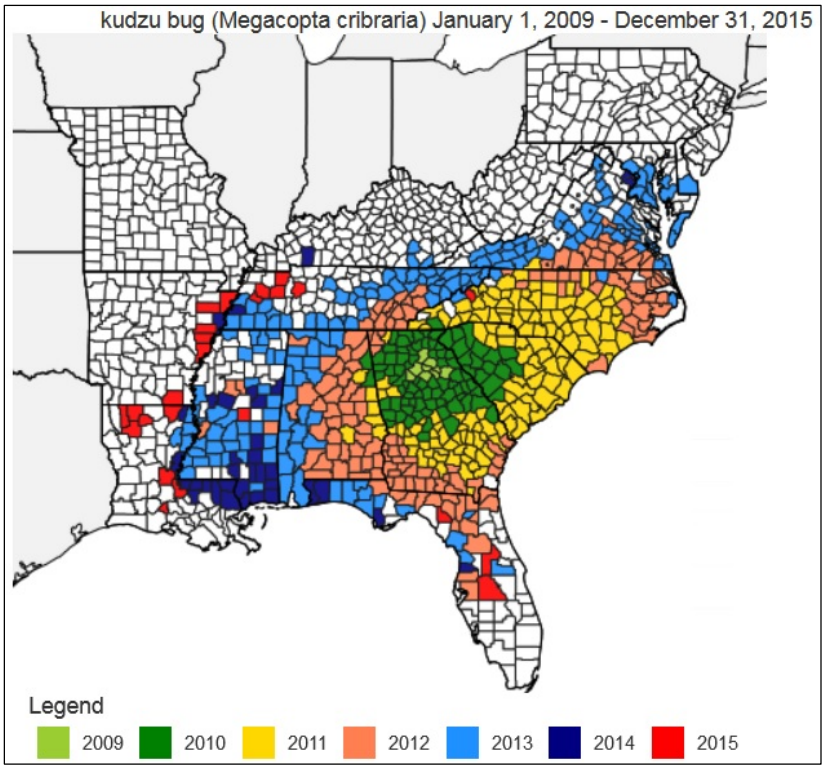

Figure 1.4 Current introduced range of *Megacopta cribraria* in the United States (Wayne Gardner, University of Georgia 2015).

Life Cycle and General Biology

M. cribraria undergoes hemimetabolous development like all other Heteroptera. Following oviposition, it undergoes 5 nymphal stadia (Figure 1.5) before eclosing into its adult stage over a period ranging from 25 to 56 days in its native Asian range (Eger et al. 2010). Instars may be readily discerned from one another by measuring body size, observing coloration of the body, noting scalloping of the lateral margin, monitoring development of the wing pads, and counting setae (Moizuddin and Ahmad 1975). Determination of each instar may be achieved by measuring width between the eyes and body size as described by Zhang et al. (2012).

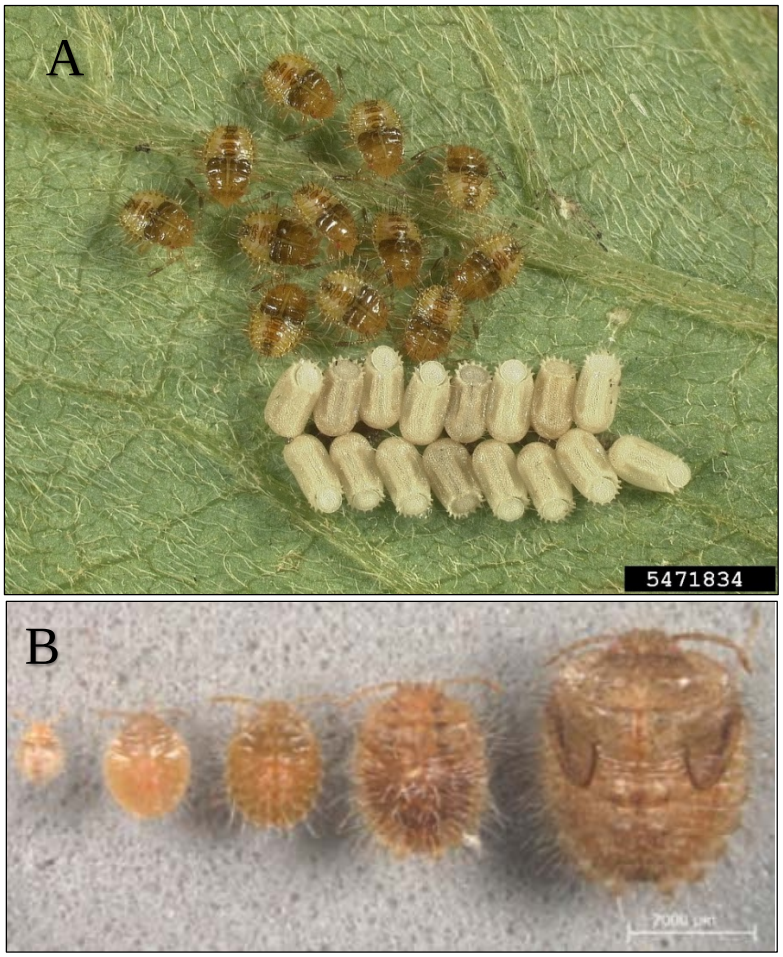

Figure 1.5 A: Newly emerged *Megacopta cribraria first instars* (Photo by J. E. Eger, Jr. 2010) **B:** development of *Megacopta cribraria* nymphs from first instar (left) to fifth instar (right) (Photo by Zhang et al. 2012).

Adult longevity differs between subsequent generations, and there is disagreement in the literature as to the actual lifespan of adults. Zhang and Yu (2005) reported that first generation adults live 1.5-3 months in a bivoltine population of *M. cribraria* from China, whereas the second generation lives 9-10 months. These differences may be due to the overwintering dormancy of the second-generation adults and therefore different physiological and ethological profiles for each generation.

Mating occurs after adults emerge from overwintering in early spring. Males and females engage in large mating aggregations, wherein 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 before joining their genitalia to mate (Figure 1.6). Coupling has been reported to sometimes last in excess of 10 hours in lab studies, though sperm transfer is regularly completed within 2-4 hours (Hoskawa and Suzuki 2001). Prolonged copulation by males may serve to guard the female and in turn prevent re-mating of females by other males (Parker 1970, Simmons and Siva-Jothy 1998), a common and well established reproductive behavior seen in insects (Alcock 1994). Following copulation an incubation period of approximately 4-7 days is necessary (Ahmad and Moizuddin 1977, Srinivasaperumal et al. 1992, Hosokawa and Suzuki 2001), after which females will deposit egg masses in parallel rows (Figure 1.6).

Figure 1.6 Adult *Megacopta cribraria* arranged in copula on soybean leaf.

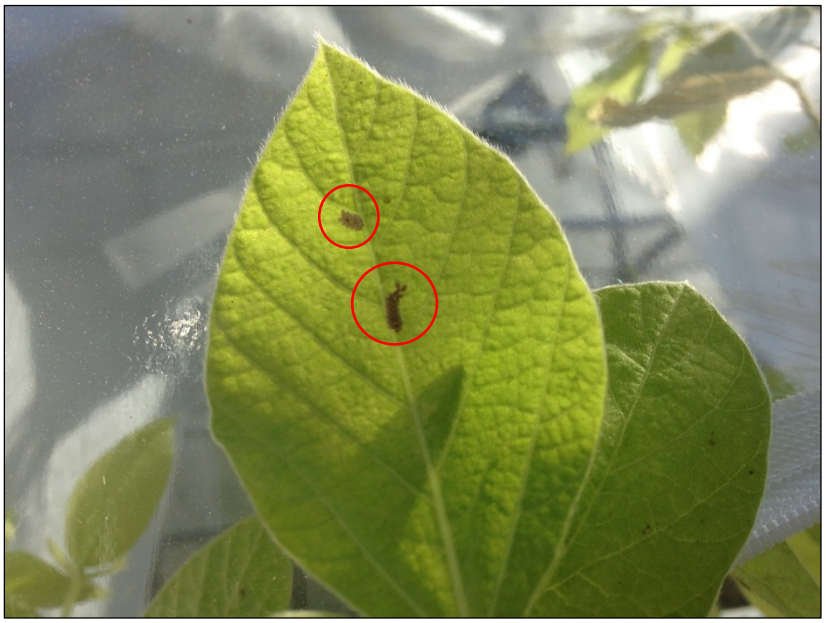

Figure 1.7 Two *Megacopta cribraria* egg masses oviposited on soybean (circled in red).

The literature indicates that females preferentially 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), but are also known to oviposit on both leaf and stem material of other host plants (Figure 1.7). During egg deposition, the female simultaneously places small, brown-colored endosymbiont capsules underneath the egg mass. These capsules, comprised primarily of frass, contain two essential obligate bacterial endosymbionts: the primary gamma-proteobacterium, *Candidatus Ishikawaella capulata*, and the secondary alpha-proteobacterium, *Wolbachia* (Fukatsu and Hosokawa 2002, Hosokawa et al. 2007, Zhang et al. 2012).Three to five days post-oviposition, first instars emerge (Ahmad and Moizuddin 1977, Sprinivasaperumal et al. 1992) and ingest the bacteria contained in the capsules before dispersing to obtain plant food. These bacteria are sequestered in the midgut and are essential for proper digestion of host plant material, development, and reproduction in the Plataspidae (Fukatsu and Hosokawa 2008, Hosokawa et al. 2010). Without the vertical transfer of these symbiotic bacteria, development of the insect is significantly retarded and immature mortality increases

drastically (Müller 1956, Hosokawa et al. 2007) with development past first instar dropping off precipitously or ceasing totally.

Annual Voltinism

In the United States, adult *M. cribraria* exhibit three peaks of activity annually (Figure 1.8). The first occurs from late March through early May, when adults emerge from overwintering to break diapause. The second occurs in late June to August, after first generation adults emerge. The final peak occurs as second generation adults migrate from feeding sites to overwintering in late September to early October (Zhang et al. 2012). Immature development is prolonged, and instars often overlap with one another during the time between peaks. Instar I-IV are collected from late April through late June. Instar V are more readily collected in 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). Seasonal activity of *M. cribraria* occurs with near uniformity throughout its range. However, its voltinism differs between its introduced range compared to native regions. It is reported as univoltine in Southern Japan (Hibino and Itô 1983), bivoltine in south-central Japan and Thailand (Tayutivutikul and Yano 1990), and trivoltine in eastern China (Wang et al. 1996). In warmer climates, *M. cribraria* may be active throughout the entire year (Tippeswamy and Rajagopal 1998a) indicating that ambient temperature may play an important role in its developmental biology. In its introduced U.S. range throughout the southeast, it is currently reported as a bivoltine species (Zhang et al. 2012). This reported phenology correlates in latitude with native regions where it exhibits a similar voltinism.

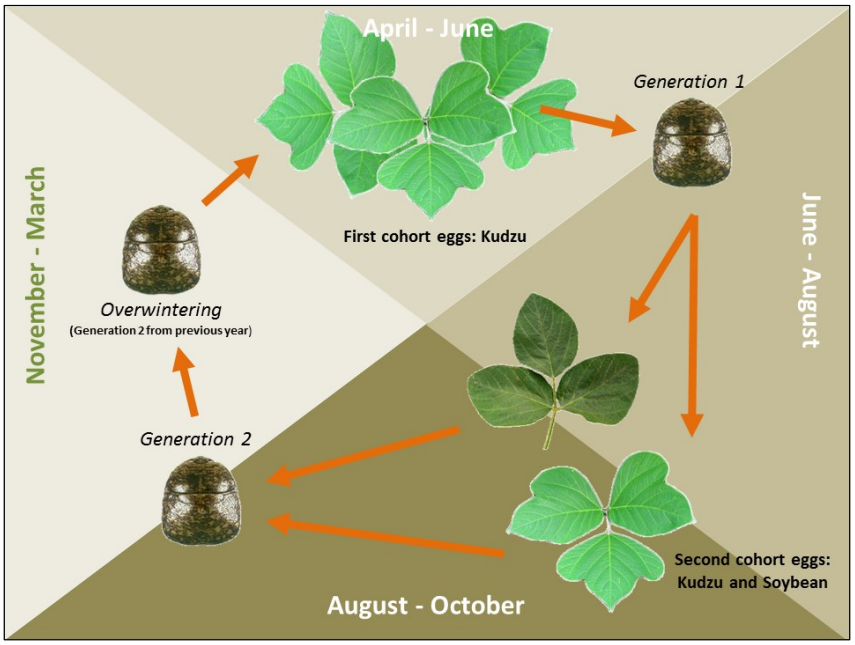

Figure 1.8 Generalized annual voltinism of *Megacopta cribraria*

Agricultural Pest Status

Megacopta cribraria, like other Plataspidae preferentially feeds on legume species (Schaefer 1988) and poses a threat to the production of legume crops throughout the Old World (Yang 1934, Hasegawa 1965, Lal 1980, Ren 1984), as well as the New World (Ruberson et al. 2012). However, a review by Eger et al. (2010) reported that the bean plataspid is not limited to these species for feeding. It has an apparently broad host range, including 20 leguminous (Fabaceae) and 14 non-leguminous species spanning 14 plant families in its native range. *M. cribraria* preferentially feeds on new plant growth, where it extracts photosynthate from the phloem (Zhang et al. 2012). It can commonly be found feeding along plant petioles, leaves, pods, stems, and flowers (Zhang et al. 2012, Seiter et al. 2013b) of a wide range of plants. Damage resulting from the feeding activity is characterized by purple spots that progress to form large black necrotic regions (Thippeswamy and Rajagopal 2005). Extensive feeding can also result in defoliation (Chaterjee 1934) and mortality of the host plant. *M. cribraria* produces honeydew as

a result of feeding on phloem that may potentiate secondary plant issues including black sooty mold that in turn negatively impacts 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) (Figure 1.7). In India it is commonly reported to attack the lablab bean *Dolichos lablab* (L.) (Ahmad and Moizuddin 1975b, 1977; Thippeswamy and Rajagopal 1998, Thejaswi et al. 2008).

Figure 1.9 Dense Aggregations of adult *M. cribraria* on soybean in Athens, GA in early July.

Due to the recent invasion of the United States by *M. cribraria,* research is currently being undertaken to determine whether its host range is similarly broad in its introduced region. In a recent study by Zhang et al. (2012) *M. cribraria* was found to feed on 10 forest legume species and non-selectively oviposit on 8 species, although adults developed only on soybean.

The development of the insect was not possible on other plants during the study, indicating that *M. cribraria* can adopt additional plant species for a nutrient source and survive for an extended period of time on their respective feeding plant, but cannot successfully develop. 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 reported 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.

Economic Importance

Megacopta cribraria is a well-documented pest of soybean throughout its native distribution, with impacts on crop production reported from India (Thippeswamy and Rajagopal 2005), Japan (Ishihara 1950, Kobayashi 1981, Takasu and Hirose 1985, Kono 1990, Hosokawa et al. 2007, Kikuchi and Kobayashi 2010), and China (Wang et al. 1996, Wu and Xu 2002, Xing et al. 2008). Its impact on growth and yield of the bean ranges from minor to significant (Eger et al. 2010). In China, studies have reported yield reductions up to 50% resulting from untreated infestation (Wang et al. 1996). Lablab bean, *Lablab purpureus* (L.), an edible bean grown throughout India and the Middle East, *M. cribraria* has been reported by numerous authors 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). Feeding activity has resulted in reductions in seed yield between 9-44% (Thippeswamy and Rajagopal 1998). In the United States, the *M. cribraria* has become disruptive and economically damaging to the cultivation of soybean (Ruberson et al. 2012). *M.*

cribraria was observed in soybean fields on plants of early vegetative growth stages in Georgia and South Carolina during the 2010 growing season (Seiter et al. 2013). Limited studies have been conducted on the effects of *M. cribraria* feeding since, and have demonstrated that the insect is capable of severely negatively impacting both growth and yield. Preliminary studies by Greene et al. (2012) reported *M. cribraria* infestation and feeding reduced crop yield in untreated fields by 47%, with average yield loss observed at 18%. Another study by Seiter et al. (2013b) demonstrated that peak densities were capable of yield by nearly 60%. Additionally, feeding stress on the plants decreased seed weight, and adversely impacted pod size and number of seeds per pod of soybean.

2011 was the first year yield losses were reported from *M. cribraria*. According to a report by the Midsouth Entomologist (Musser et al. 2012), a total of 31,349 bushels were lost per acre at cost of pesticide treatment plus loss of yield totaling US $988,034 throughout the southern U.S. From 2011 to 2012 (Musser et al. 2013), there was an additional 366,600 acres reported as infested *M. cribraria*, coinciding with 61,000 more acres necessitating treatment with insecticides. In total, this resulted in 24-fold increase in areas contaminated with the *M. cribraria*, and a 6.4-fold increase in acres needing treatment to mitigate its invasion.

Megacopta cribraria has increased its range annually and so it is a paramount concern for soybean growers both in the Southeast as well as the rest United States to have up to date and accurate information regarding the proper identification and control of kudzu bug. Soybean ranks as the second most grown crop in the U.S., behind corn (www.epa.gov). In 2014, the U.S. produced nearly 4 billion bushels of soybeans, and exported approximately 45% (1.790 billion bushels) internationally that same year. The total value of the crop in 2014 was reported as US$40 billion dollars (www.soystats.com).

Agricultural Control Strategies

Soybean growers in Georgia during the 2010 and 2011 growing seasons undertook field trials of insecticides utilizing a number of different chemical formulations. These trials revealed that formulations containing acephate, bifenthrin, cyhalothrin, zeta-cypermethrin, or carbaryl offered greater than 80% control 2-5 days after treatment. In the same trials, trade name insecticides including Hero, Brigade, Karate+Orthene, Endigo, Brigadier, Discipline, and Sevin demonstrated at least 90% control 2-5 days after treatment (Roberts and Whitaker 2012). The results of these trials were used to draft guidelines on managing *M. cribraria* in soybean fields throughout the introduced range of the insect, and have been incorporated into the University of Georgia (2013) Pest Management Handbook, which recommends the use of nine different insecticides in three classes; neonicotinoids, organophosphates, and pyrethroids. In its native distribution control of field infestations of *M. cribraria* can be effected successfully by the application of a number of broad-spectrum insecticides including: chlorpyrifos, beta-cypermethrin, deltamethrin, beta-cypermethrin methamidophos, and sumicidin. Adults and nymphs show differing levels of vulnerability, but when applied with care for the life stage and overall ratio of individuals these chemicals have been to control populations well in soybean (Wang et al. 1996, Zhang and Yu 2005). Insecticide treatments should be applied in response to the presence of adult and immatures, and only when scouting results in exceeding the economic threshold of 5 adults per sweep (Roberts 2013) or 1 nymph per sweep (Reisig and Bacheler 2012).

Adult Infestations typically begin as aggregations localized to field edges and move infield as over the course of the season (Seiter et al. 2013b). Migrating adults often arrive at field edges first, so border rows should be given priority for insecticide applications early in the

season. Subsequent applications should focus on where the insects have since aggregated. 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) are limited in scope by the number of trials and growing seasons conducted, the chemistries selected by the authors successfully control kudzu bug in soybean. However, chemical controls often end up as acute solutions to a chronic problem as is the case with kudzu bug. Due to high population densities and continual invasion over the growing season there is often a need for multiple insecticide applications during the active season (Roberts and Whitaker 2012), representing a significant financial and time investment on the part of the grower. In addition, the trade name insecticides used in these trials are broad-spectrum in nature and may negatively impact populations of beneficial arthropods after their application thus making control of *M. cribraria* solely through the use of chemicals uneconomical and potentially environmentally harmful. For example, the recently discovered parasitoids *Paratelenomus saccharlis* and *Strongygaster triangulifera* are sensitive to broad spectrum insecticidal treatments. Research completed on other members of the former's family (Hymenoptera: Platygastridae) shows sensitivity to deltamethrin (Rauno et al. 2010), chlorpyrifos, and *Bacillus thuringeiensis* var. *kurstaki* (DiPelTM) (Amaro 2013), while fenoxycarb (Grenier and Plantevin 1990, 1991) appears to be toxic to members of the latter's family (Diptera: Tachinidae). The use of insecticides must be timed to coinicide with adult migration, and insecticides should be used conservatively towards the beginning of summer to avoid nontarget impacts on beneficial arthropods.

Anecdotal evidence communicated by researchers in the field (Gardner 2014, Toews 2014) indicates that wild populations of kudzu bug are also susceptible to significant mortality

by infection with the entomopathogenic fungus *Beauvaria bassiana* (Bals.-Criv.) Vuill. (1912) (Figure 1.9). *B. bassiana* is the asexually reproducing form of *Cordyceps bassiana* (Li et al. 2012), and is currently undergoing evaluation for its control potential on bedbugs (*Cimex lectularius*) and malaria transmitting mosquitoes (*Anopheles* spp.) (Barbarin et al. 2012, McNeil 2005). If sufficient control potential is exhibited in either species, future studies could be undertaken to quantify efficacy on *M. cribraria* in agricultural and urban applications.

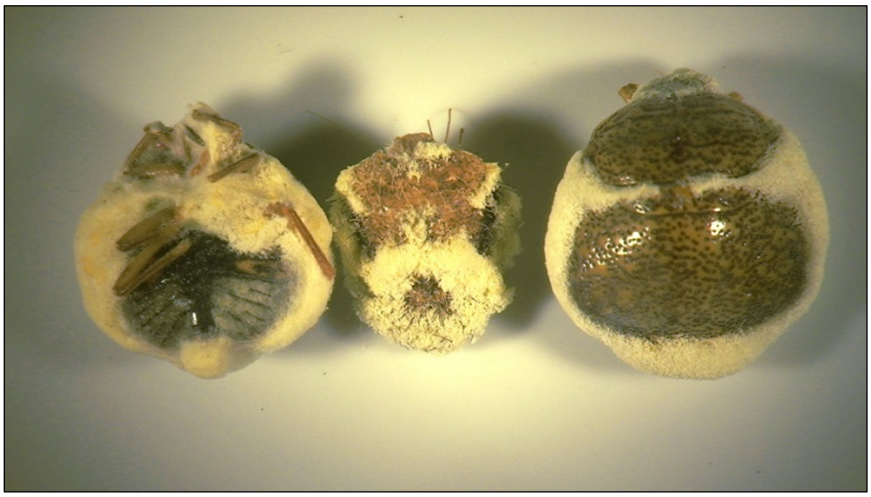

Figure 1.10 Kudzu bug adults (left, right) and a nymph (center) encrusted with *B. bassiana* fruiting bodies

Project Objectives

Megacopta cribraria was first detected in the U.S. in Georgia during October of 2009. Since then it has expanded to a currently confirmed distribution spanning 13 southeastern states and is continuing its spread northward and westward annually. Upon its initial discovery, it was only thought to be a minor urban nuisance, but in the successive years since it has demonstrated its potential to be a serious agricultural pest with important economic and ecological ramifications. Currently, there is a distinct lack of literature concerning life history and behavioral characteristics that have enabled the establishment, dispersal, and explosion of population size in

its new North American range. This project has the proximal goal of this project is to gain a comprehensive understanding of the fitness tradeoffs induced by rearing on different host plants of. The long-term goal is to use the information gathered to aide in the development of IPM programs to control infestation and spread kudzu bug. This project consists of three objectives.

1. Develop a protocol suitable for measuring flight capability of adult female *Megacopta cribraria* using a modified version of the flight mill apparatus and software to evaluate dispersal as a function of host plant switching.
2. Conduct host preference and rearing trials in a controlled greenhouse environment to investigate differential impacts of *M. cribraria* development on kudzu (*Pueraria montana* var. *lobata*) and soybean (*Glycine max*) by measuring key fitness characteristics: immature development time, adult female fecundity, body size, triglyceride content, and flight ability.
3. Investigate the traits listed in Objective 2 over the course of a second growing season in the field, to eliminate confounding variables and cage effects present due to the limitations of a greenhouse study.

Given these three objectives we present the following hypotheses:

1. Soybean and kudzu are unequal in quality as hosts for development of *M cribraria*, driving the annual dispersal from kudzu to soybean each summer in their introduced range. Rearing on both hosts will produce adults of unequal fitness as measured by our chosen parameters.
2. Switching host plants across generations (spring to fall) and developing on a different host than the one chosen for oviposition will result in a measurable difference in the fitness of nymphal and adult *M. cribraria*.

3. Development and overall fitness of nymphal *M. cribraria* is dependent on maternal investment at a level greater or equal to the variety of host plant available and will present in a measurable maternal effect.

CHAPTER 2

FLIGHT MILL ASSEMBLY AND DATA COLLECTION PROTOCOL

Introduction:

Flight mills are important tools for investigating the dispersal ability of insects under controlled conditions. Flight behavior is often essential for insect dispersal, migration, and host plant colonization. Quantifying the environmental influences on flight behavior can be especially useful for important pest insects and invasive species to inform management strategies. Recent studies of agriculturally important insects including brown marmorated stink bug (*Halyomorpha halys*), peach fruit moth (*Grapholita molesta*), and Colorado potato beetle (*Leptinotarsa decemlineata*) (Lee and Lesky 2015, Ishiguri 2004, Alyokhin, 1999) have investigated the impact of modifying ambient conditions on flight ability using flight mills in lab and field settings.

Several laboratory techniques have been developed for the study of insect flight behavior (Hardie 1993, Reynolds 2002). These range from simple static tethering (Davis 1986, Dingle et al 1980) to sophisticated devices that allow greater freedom of movement for the tethered insect (Gatehouse et al. 1980). To date flight chambers (Grace et al. 1988; Kennedy et al. 1963 & 1974; Laughlin 1974) represent the devices allowing the highest level of freedom of flight in controlled conditions. This technique has two major drawbacks: it is difficult to use for the study of large insects and the manual procedure of data collection is time consuming.

Flight mills represent one of the most common and affordable techniques for the study of insect flight under laboratory conditions (Krell et al. 2003, Liu et al. 2011, Wang et al. 2009). This technique is preferable to static tethering because it offers moving stimuli (Dingle 2014),

but it differs from a free flight behavioral response (Blackmer et al. 2004, Riley et al 1997, Taylor et al. 2010). Some aspects of the flight behavior on the mill and in the wild are similar (Gatehouse et al. 1980, Cooter 1993) so despite some limitations, flight mills represent a viable option to investigate questions regarding the occurrence of particular flight behavior responses, as is the case of migratory flight type. Also, flight mills are easier to construct than wind tunnels or flight chambers and the data collection can easily be automated. Thus, researchers interested in flight behavior often find that flight mills are the best choice, but should be aware of the potential limitations to the method. Here, a flexible and customizable flight mill design is presented for researchers that have chosen to utilize flight mills to investigate flight behavior.

Several authors describe alternative flight mill designs. In general the main part of the flight mill system, (i.e. the pivoting mill's arm), is simple in construction. Less straightforward is the electronic part of the flight mill system, which allows the recording of the data. Dealing with electronic circuits design can be challenging, especially for the entomologist or the behavioral ecologist lacking in background knowledge of electronics. Some authors describe a complicated or out of date electronic circuit component in their flight mill design (Chambers et al. 1976, Clarke et al. 1984, Resurreccion et al. 1988, Taylor et al. 1992), or the description of the electronic part of the flight mill is missing (Bruzzone et al. 2009, Schumacher et al. 1997). Other designs describe mechanically complicated actographs, which are more intricate, but can help investigators to undertake more complex behavioral observations (Gatehouse et al.1980).

In this paper a design for a flight mill that is both simple to build and relatively inexpensive in cost of materials for the study of tethered flight in insects is described. Together with the extremely simple electronic component, the design has a number of advantages. The flight mill is designed to be used in the constrained spaces typically available in the standard

insect ecology laboratory. The structure is made out of transparent acrylic plastic so that a single light source can evenly reach every individual in separate chambers of the mill. Given the transparency of the material and small size, the flight mill can be used in an incubator for standardized light and temperature conditions. Finally, the entire structure can be assembled and disassembled easily and, once disassembled, it can be stored in a small space. Another advantage to the design of the structure is that the flight mill can be modified to allow the study of insects of different size and using different revolution distances.

This flight mill has been used to collect data on insects as diverse in size and shape as milkweed bugs, *Oncopeltus fasciatus* (Attisano et al. 2013), burying beetles, *Nicrophorus vespilloides*, and in the case of this particular study kudzu bugs, *Megacopta cribraria*. The flight mill design also allows for high throughput required for studies requiring large sample sizes. Data can be collected using 8 simultaneous channels for each of the data loggers used so that a high number of individuals can be analyzed simultaneously and large numbers of samples can be handled in the same day. Two or more flight mills can also be arranged in tandem for increased data collection. No expensive software is needed to record and visualize the data and the custom written script for the data analysis can be modified following the specific needs of the experimental design. Flight response is highly variable in different insect species. Thus, before conducting a full flight mill experiment, preliminary tests on the flight response of the focal insect model are recommended. These will provide an understanding of the extent of the behavioral variation in flight response, which will be used to fine tune aspects of the flight analysis such as recording time or flight speed range.

Protocol:

1) Construct the flight mill

1.1) ***Construct the acrylic plastic support structure***:

1.1.1) Cut 3 mm thick transparent acrylic sheets into the two outside vertical walls, the one central vertical wall and the five horizontal shelves as specified by the design shown in Figure 2.1.

1.1.2) Assemble by inserting the shelves (Figures 1 and 2; HS) into vertical walls (Figures 2.1 and 2.2; OW and CW) to form the support structure (Figure 2.2A).

1.1.3) Strengthen the structure by inserting polystyrene columns at the external corners at the back of the device (Figure 2.2A and Figure 2.2C). If required, glue short pieces of right-angled edge-protectors along the central vertical wall junctions to provide additional support for the horizontal shelves.

1.2) ***Construct the pivoting arm assembly***:

1.2.1) Glue a 5 cm length of 1 cm diameter plastic tubing into the top center of each cell. Glue a 2 cm length of 1 cm diameter plastic tubing into the bottom center of each cell, making sure the top and bottom tubing in each cell is aligned. Using hot glue, affix two 10 mm x 4 mm N42 neodymium magnets to the end of each support, forming the magnetic bearing for the mill's arm.

1.2.2) Insert an entomological pin into a 20 μL pipette tip and fix in place with hot glue. Position the pin such that both ends extend out of the pipette tip to form the armature of the flight mill.

Note: During the flight trials, the top of the pin is held in place by the top set of magnets. The bottom set of magnets is to maintain the armature in a vertical position, allowing it to revolve around its axis.

1.2.3) Cut a 24 cm length of 19 gauge non-magnetic hypodermic steel tubing. Using hot glue, affix the center point to the top of the pipette tip from step 1.2.2. Bend one end of the tubing at 2 cm from the end to an angle of 95°, leaving a long arm of 12 cm from the center point and a short arm with a 10 cm radius from the center to the bend (Figure 2.2B).

1.2.4)

Note: The radius length can be varied in order to accommodate different revolution distances.

1.3) ***Set up the IR sensor and data logger***:

1.3.1) Fix the IR sensors to the eternal sides of each cell using reusable adhesive putty, allowing the sensor to extend into the cell through the openings cut into the external vertical wall supports (Figure 2.2C).

1.3.2) Connect the IR sensors to a data loggers through a very basic electronic circuit built on a solderless breadboard (Figure 2.3). Connect two resistors of 180 Ω and 2.2 kΩ respectively on the input and output of the IR connection on the breadboard (Figure 2.3A,B). Place the resistors

in alternate rows along the breadboard to minimize drops in the voltage signal during recording from multiple sensors (see Figure 2.3C).

2) Flight trials

2.1) ***Tether insects to the flight mill arm indirectly through an insect pin***:

2.1.1) Place a small foil flag at the end of the unbent end of the pivoting arm to maximize disruption of the IR beam in the sensor and to act as a counterweight.

2.1.2) Depending on the insect's size and cuticle area available for attachment, attach the experimental insect to an insect pin with reusable adhesive putty or non-toxic skin glue. If necessary, anesthetize the insect by either chilling or with CO_2.

2.1.3) Mold a small amount of adhesive putty around the rounded tip of an entomological pin and cover it with a drop of non-toxic skin glue. Gently apply on the pronotum area and wait 5-10s until the glue is dry.

Note: The procedure in step 2.1.3 is suited for insects with hard (beetle, bugs) or soft (wasps, flies) cuticle. Insects with hairy cuticle (moths, butterflies) will need to have the hair gently removed with a very fine paintbrush before tethering.

2.1.4) Insert the pin with the insect attached into the bent end of the pivoting arm assembly.

2.1.5) After the flight test has ended, remove the tethering with fine forceps.

Note: Data logger set up and acquisition has been optimized as follows for the specific equipment listed in the materials table and should be adjusted for use with alternative equipment.

2.2) ***Initiate a recording session with the freely available WinDAQ Lite software***:

2.2.1) Download and install the free software WinDAQ Lite (see equipment list).

2.2.2) Open the instrument hardware manager, select the data-logger from the pop-up list and press "Start Windaq". A new window will open and the input signal from each sensor will be shown.

2.2.3) Select the desired sampling frequency at which the data-logger reads and displays the sensor’s output.

Note: The sampling frequency will depend on the insect's flight speed, however sampling frequencies ranging between 30-45 Hz will be fast enough to capture the flight of small-medium sized insects.

2.2.4) Press Ctrl-F4 to start a recording session. Select the destination path of the recording file in the first pop-up window. Choose the appropriate length of time to record flight for the particular insect and experiment. Define recording time in the second pop-up window. Once the recording time is elapsed press Ctrl-S to finalize the recorded file.

2.3) ***Check quality of the recording***.

2.3.1) Open the recorded flight track and select a voltage channel. Press Ctrl-T to open a pop-up window with the voltage statistics for each channel.

2.3.2) Ensure that no large drops in minimums value resulted from voltage drops across the circuit (Figure 4). Discard any channels in which the difference between the channel average and minimum voltage is greater than 0.1 V.

2.4) ***Save the file in a *.CSV format***: Go to File > Save as and in the pop-up window select "Spreadsheet print (CSV)". In the "Spreadsheet Comments" pop-up window select "Relative Time" and deselect all the other options. Click OK to save the file.

3) Analysis of flight data using Python 3.4.x

3.1) ***Install the latest Python 3.4.x version***. Save the scripts standardize_peaks.py and flight_analysis.py (supplementary materials) onto the desktop.

3.2) ***Standardize and select the peaks in the recorded signal as follows***:

3.2.1) Right-click on the standardize_peaks.py icon. Select "Open with IDLE".

3.2.2) In Lines 18-19, specify the threshold values around the mean voltage used to perform the standardization of the voltage signal for each channel.

Note: The default values are set to deliver a fine tune signal standardization, but the user can define any desired threshold according to the value of the mean voltage for each channel. These can be found in the voltage statistics window (see step 2.3).

3.2.3) In line 45, type the path to the folder in which the recorded *.CSV file is saved.

3.2.4) In line 91, type the path to the folder in which you wish to record the *.TXT peak file.

3.2.5) In line 61 and line 72, specify the number of channels needed. Add or delete channels by deleting the # at the beginning of line 61-63 and 72-74 up to a maximum of 8 channels.

3.2.6). Save the file and launch the script by pressing F5.

3.2.7) Enter the name of the *.CSV file (with any additional sub-folders) in to the pop-up window and press return to save a new *.TXT file with the standardized signals in the specified folder.

Note: Depending on the number of channels used n, this file contains n+1 columns: the first column is the relative time of the sampling event, the other n columns represent the base and peaks events from the n channels used for the recording. A value of 0 represents the base voltage, while a value of 1 represents a peak derived from the passage of the flag through the IR sensor.

3.3) ***Analyze the flight track using the standardized file***: Edit the flight_analysis.py script to accommodate the user experimental conditions:

3.3.1) Right click on the flight_analysis.py icon. Select "Open with IDLE".

3.3.2) In line 39 and line 80 adjust the length of the circular flight path according to the arm radius.

3.3.3) If required, activate an optional speed correction loop by deleting the # in lines 50-52. Change speed value accordingly.

3.3.4) In line 77 and line 85, edit the speed threshold and the time gap values to correct for false speed readings in the flight track and account for very short time gaps occurring between two consecutive long uninterrupted flying bouts.

3.3.5) In line 198, specify the total recording time in seconds. Change the value ranges in the output lines from line 287 onwards.

Note: The default ranges can be modified according to the user experimental requirements. In order to do so, all the numerical values inside the function (included the ones in the variable name, for example in the variable “flight_300_900”) need to be changed to the desired value.

3.3.6) In line 248 type the path to the folder in which the *.txt standardized file is saved.

3.3.7) Specify the number of channels. Add or delete channels by adding or deleting a # at the beginning of lines 257-259, lines 270-272 and lines 279-281 up to a maximum of 8 channels.

3.3.8) In line 304 type the path to the folder in which you wish to save the output files.

3.3.9) Once all the user settings are specified, save the file and launch the script by pressing F5.

3.3.10) Enter the name of the *.TXT file to analyze (with any additional sub-folders) in the pop-up window and press return.

Representative Results:

Figure 5 shows representative examples of the type of graphs that can be obtained using the scripts described in the previous section. Flight data were obtained from experimental work conducted in the Department of Zoology at the University of Cambridge using *N. vespilloides* as model (Attisano, *unpublished data*). Two young unmated males of about 20 days of age were tethered to the flight mills and placed in controlled environmental conditions of 14:10 L:D and 21°C. The beetles were left in the flight mill for 8 consecutive hours and the flight activity was recorded. The on screen analysis and the graphic output make it possible to resolve individual differences in flight activity patterns. For example, the first male (Figure 2.5A) showed a strong flight activity within the first hour of recording, characterized by high speed and continuous flight that lasted about three hours. This prolonged activity phase is characterized by a gradual decrease in speed from about 1.6 m/s to about 1 m/s which after the initial flying bout, the individual showed an almost periodical pattern of relatively short flight bouts about 10-15

minutes duration each. The second male showed a very different flight pattern with flying bouts that never exceeded the duration of 15-20 minutes (Figure 2.5B). In this individual the flight activity is characterized by a wide spread of flying bouts in the first 4 hours of recording, after which its activity becomes almost periodical. This individual also presented very low flying speed that only occasionally exceeded 0.4 m/s.

Another representative example was obtained using a different insect model, the milkweed bug *Oncopeltus fasciatus*. Data were collected during a study on the migratory behavior and physiological response to food stress in milkweed bug females (Attisano et al. 2013). In this study the recording time was set to one hour in order to characterize females as migrants or residents. These behavioral types are characterized by an "all or nothing" response. Migratory females engage in sustained and continuous flights usually lasting for few hours, while resident females never show flight activity longer than few minutes. Thus, a migrant female will show a flight pattern like in Figure 2.6A, while a resident female will be characterized by a movement pattern like the one in Figure 2.6B.

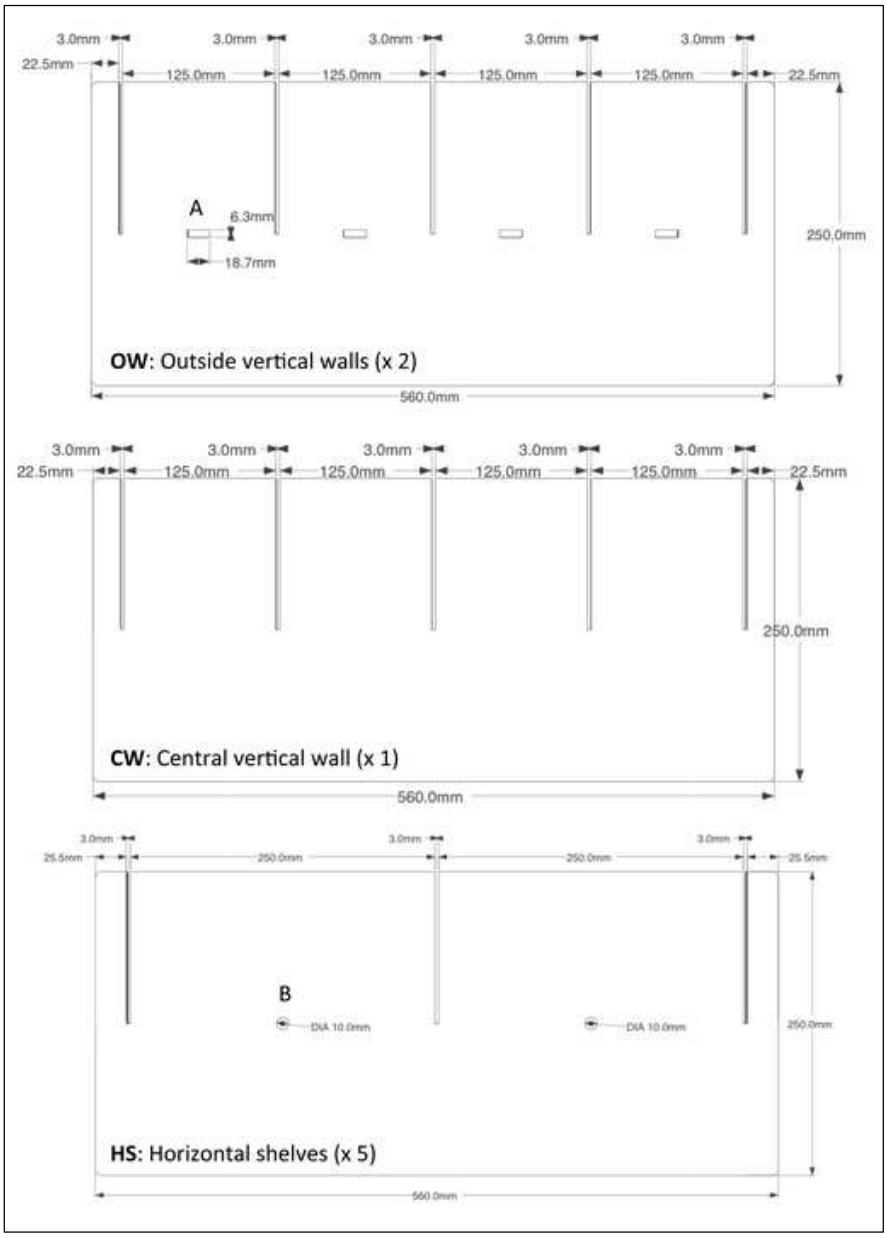

Figure 2.1: Design configuration for acrylic plastic support structure. The acrylic plastic support structure for the flight mills is constructed from three different components. There are two outside vertical walls (OW) containing both slots for the shelves and an opening to accommodate the IR sensors (A). There is a single central vertical wall (CW) with slots for the shelves. And there are 5 horizontal shelves (HS) with slots for the walls. The magnetic pivot is glued to the horizontal shelves at position B.

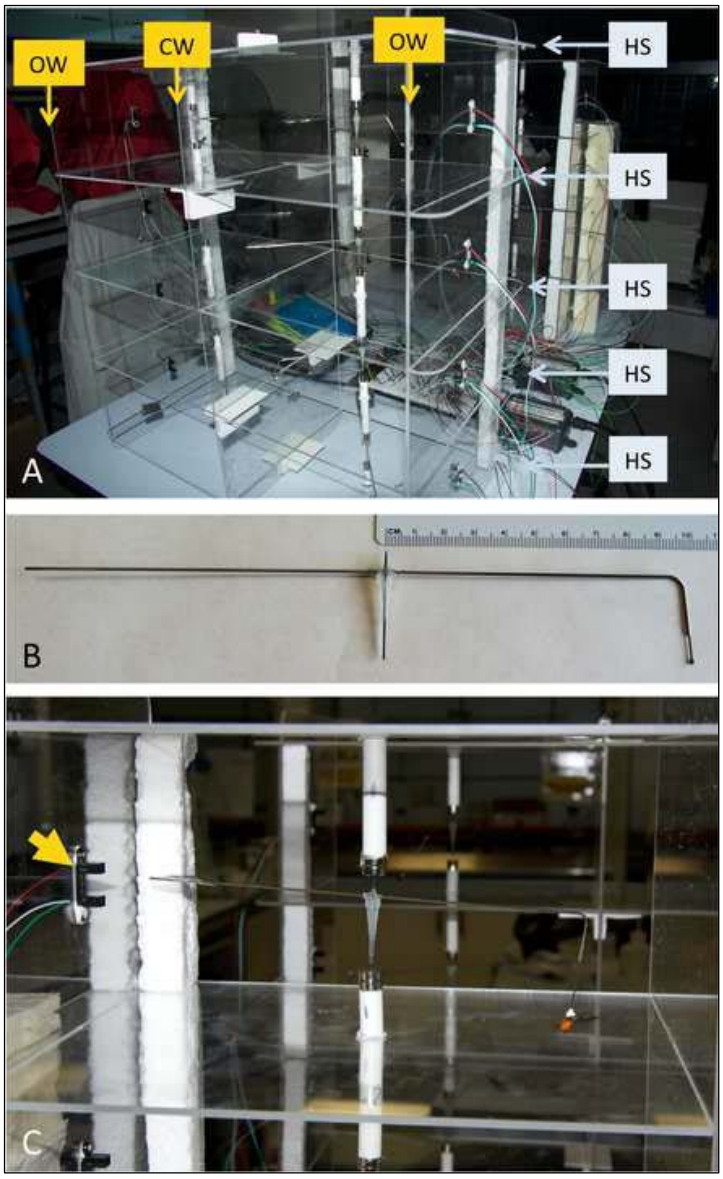

Figure 2.2: Assembled flight mill. (A) The acrylic plastic support structure is assembled by sliding the five horizontal shelves (HS) into the slots in the two outside walls (OW) and the central wall (CW), resulting in a structure with 8 individual cells each containing a magnetic pivot and an IR sensor, allowing for 8 individuals to be flown at the same time. (B) The pivot arm to which the insects are tethered can be constructed to accommodate a variety of sizes and morphologies of insects. (C) As the tethered insect moves the pivot arm suspended between the magnets, the foil flag at the other end of the arm activates the IR sensor (arrow).

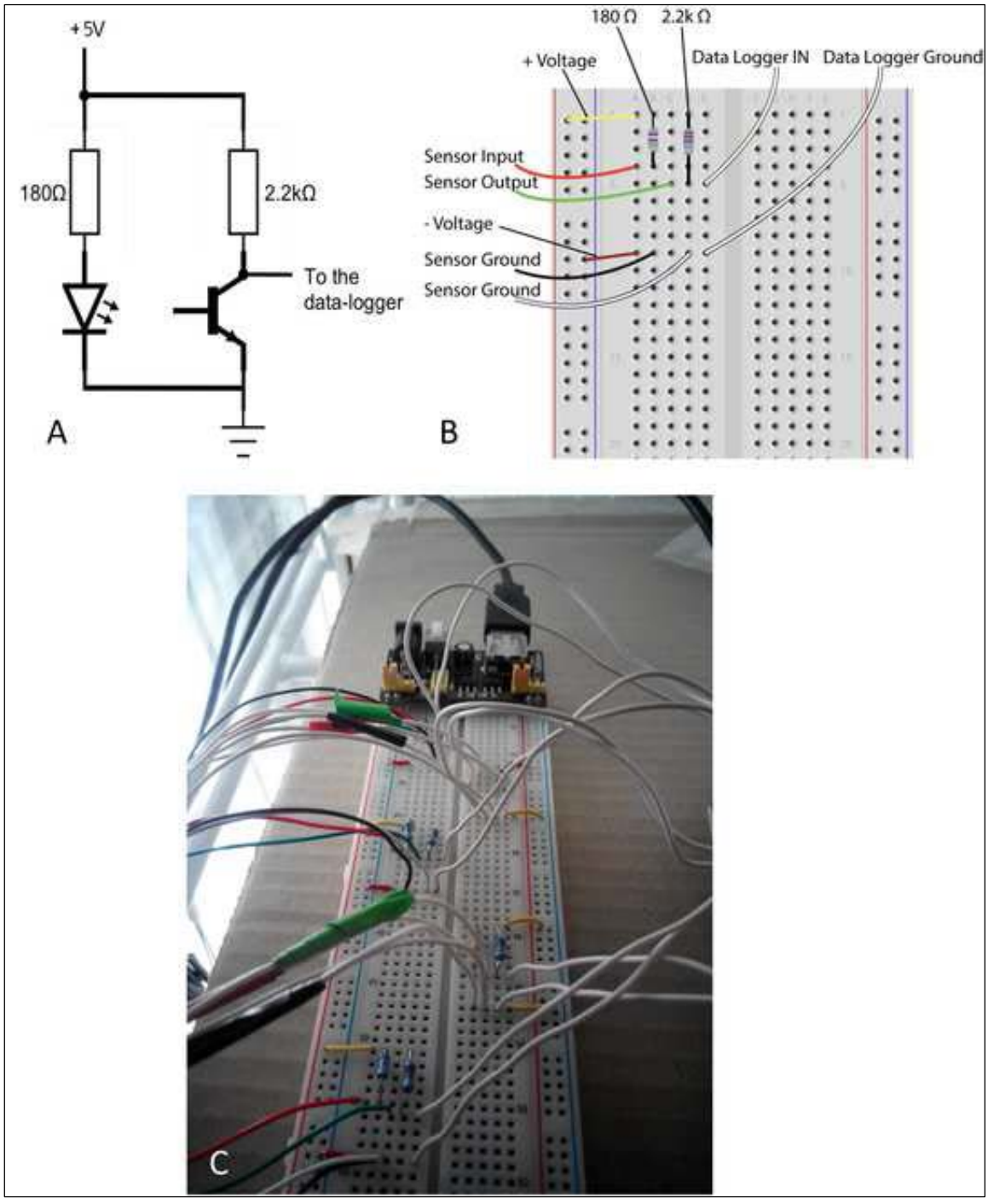

Figure 2.3: Design of the circuit connecting the IR sensors to the data logger. **A:** A simple circuit connects input from the IR sensor to the data logger. **B:** Each data logger can be powered and connected to the data logger via a solderless breadboard using the diagram. **C**: Multiple sensors can be connected to the single data logger using the same breadboard.

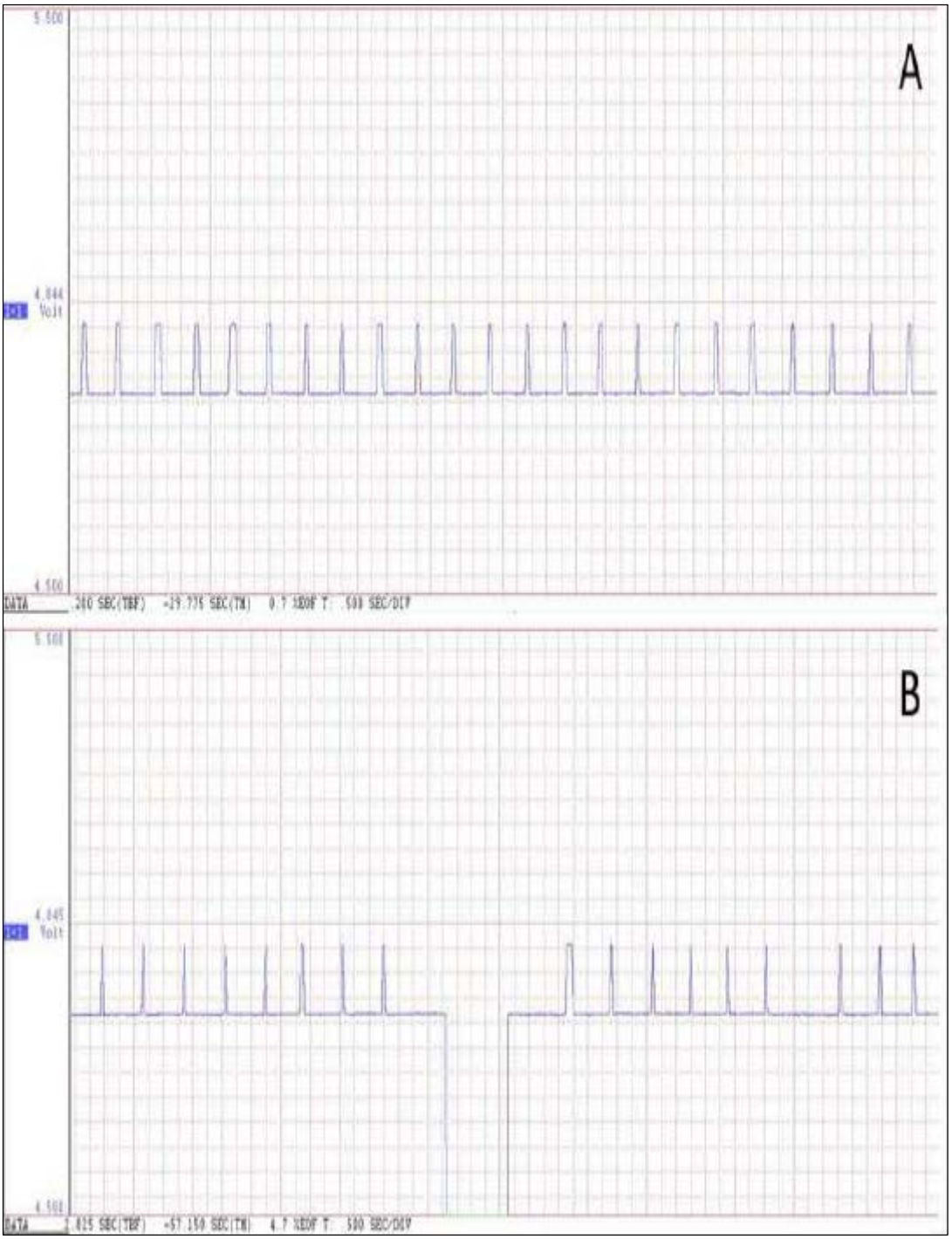

Figure 2.4: Examples of recorded flight events. Voltage peaks represent complete revolutions of the flight mill's arm. **A:** A high quality recording of a flight event with no voltage drops in the recorded signal. **B:** A flight event with a voltage drop in the recorded signal.

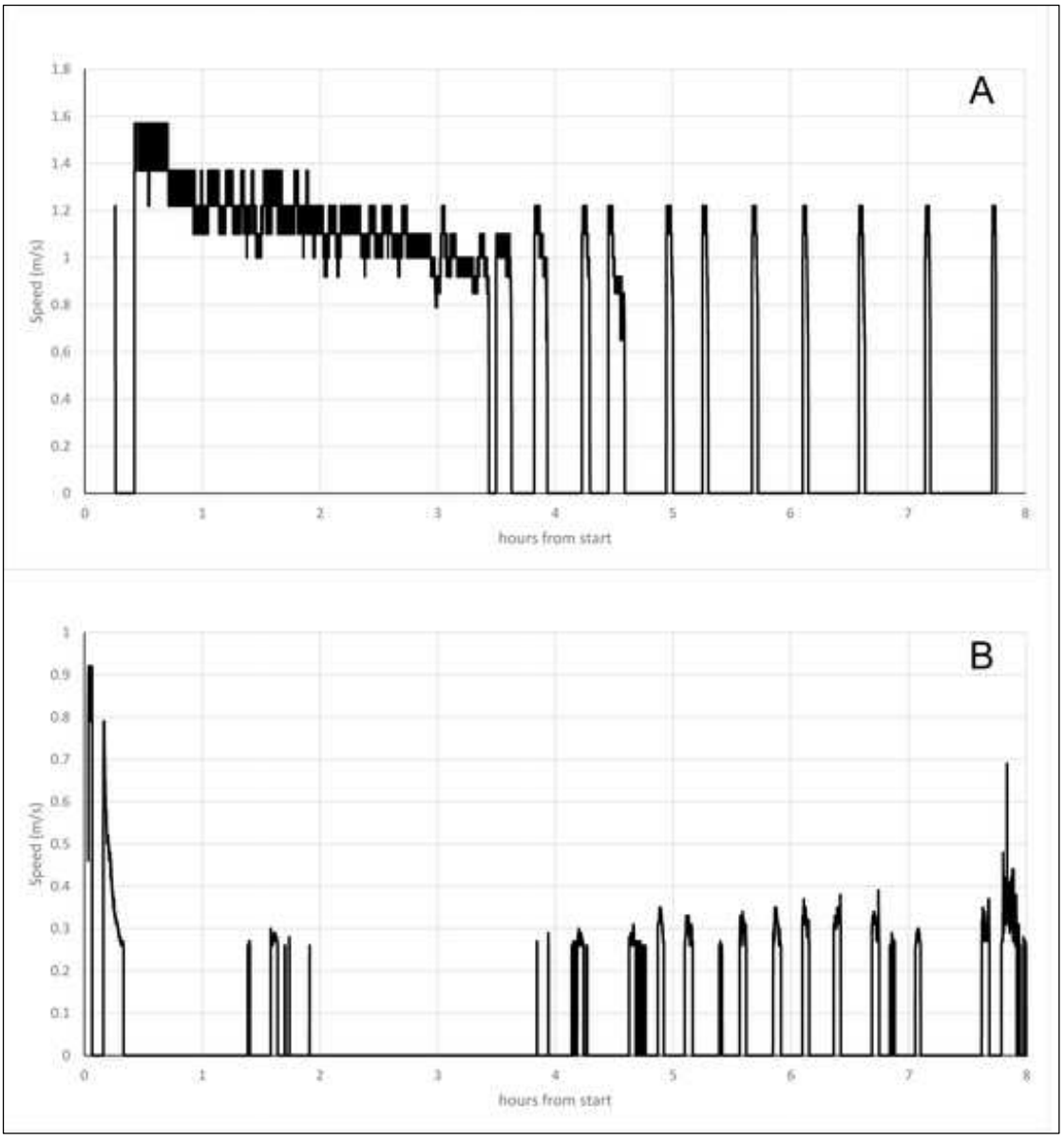

Figure 2.5 Representative flight data from the burying beetle *Nicrophorus vespilloides*. Individual variation in flight behavior is easily recognized in the flight recordings. **A:** One individual flew continuously for about three hours after the start of the trial and then flew periodically at high speed throughout the rest of the trial. **B**: The behavior of the individual is different in that this beetle flew only sporadically throughout the trial and never flew at the high speeds seen in the individual in panel **A** (note the difference in scale on the Y axis).

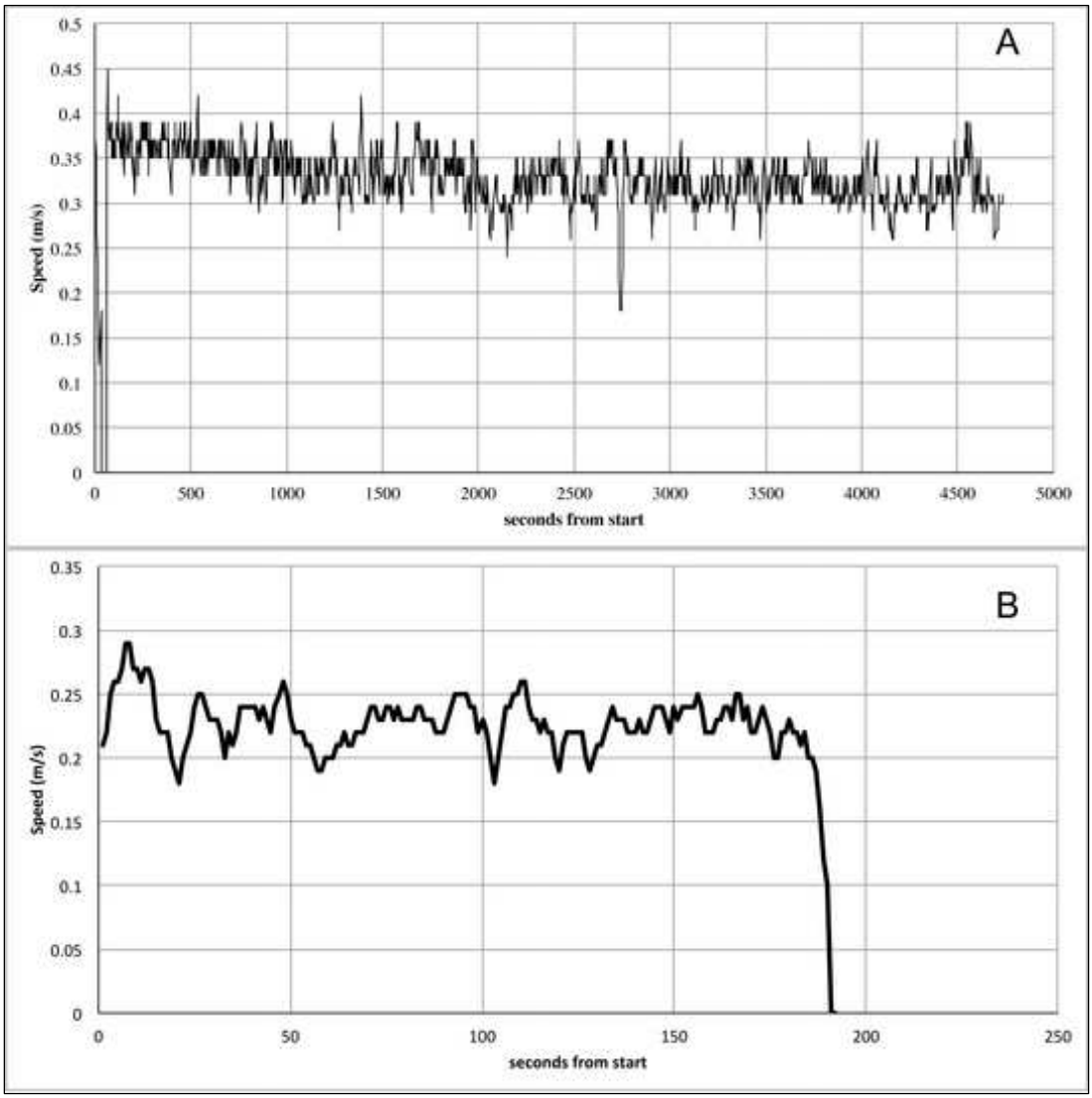

Figure 2.6: Representative flight data from the *O. fasciatus*. Two different patterns of behavior are clearly observed between the flight data recordings. **A:** This recording is typical of the type of flight behavior seen in migratory individuals. Migratory individuals fly at a relatively steady speed over long periods of time. **B:** The behavior in Panel A is contrasted with the typical flight behavior of a resident individual. Residents fly at lower speeds and flight bouts only last a short time (note the difference in scale on the X axis for A and B).

Discussion:

An affordable, flexible, and adjustable flight mill design.

Insect flight behavior is of interest to a range of scientists, from those interested in the basic behavior of insects under variable environments to specialists in integrated pest management who needing to understand how conditions influence the propensity of a pest species to disperse, as is the case in our study. Flight behavior can be studied by various methods that range from flight "treadmills" and wind tunnels that approximate field conditions to static tethered flight devices. Tethered flight mills, like the one presented here, are limited in that certain aspects of flight, such as changes in altitude, cannot be measured. However, tethered flight mills do allow insects to fly uninterrupted and thus allow researchers to quantify parameters such as speed, distance and periodicity of flight and correlate these parameters with environmental conditions, physiology, and morphology.

The flight mill presented here is designed to allow researchers without specialized knowledge of electronics to build and use a tethered flight mill in order to study flight behavior in insects. One advantage of this design is that the overall cost of the flight mill is low compared to other designs. The overall cost can be maintained well below $300 US. The plastic acrylic sheets are the most costly item. The second advantage is that the flight mill is adaptable for the limited controlled condition workspaces available in many laboratories, as opposed to a specialized wind tunnel. The use of 3 mm thick transparent acrylic plastic sheets means that the structure is both transparent, to allow easy observation of the insects, and also light weight, enabling the flight mill to be moved to the appropriate location for flight trials. The stacked configuration of the flight mill cells maximizes the number of samples run while minimizing the footprint of the device. Further, the device can be easily disassembled for storage. Additionally,

the flight mill was designed to allow for large numbers of individuals to be sampled relatively easily. Each flight mill contains 8 cells, enabling researchers to record flight activity of multiple individuals simultaneously. Attaching insects indirectly to the pivoting arm through an insect pin allows for individual insects to be placed in and removed from the flight mill rapidly. Finally, the data recording electronics is simple and easy to use, with freely available software for data analysis. Once assembled, the flight mill uses simple IR sensors to record flight activity. The passage of the foil flag at the end of the arm through the infrared beam allows each revolution of the arm to be recorded. The rate of revolution allows data like speed, distance traveled, total flight time and patterns of flight to be recorded as input into a data-logger.

The flight mill is able to be adapted for a number of different types of insects. The use of hypodermic steel tubing for the pivoting arm is more effective than other options, such as wooden sticks or drinking straws because, even though heavier, the drag produced is reduced by the narrow diameter, allowing even small insects to be flight-tested. Recently, small pieces of optic fiber have been used in a flight mill for small insects (Martini et al. 2014). The bent ending of the arm can be glued to the armature at different angles relative to the support axis in order to position the experimental insect in its natural flight orientation. In the design presented, in which the radius is 10 cm in length, the entire distance traveled in one revolution is 62.8 cm. Removing the central vertical wall will allow an alternative configuration of the flight mill in which the arm radius can be doubled in length to accommodate larger insects and revolution distances up to 1.20 m. In this case, stronger magnets are recommended to accommodate and stabilize longer mill's arm.

As stated throughout, the flight mill design is flexible and adaptable for the insect species of interest and researchers are able to customize it for their particular needs. This includes not

only the physical needs of the insect, including parameters such as size, power, structure of the cuticle, but also biological differences among species. One potential drawback to all flight mills is that the lack of tarsal support "forces" the insects to fly, perhaps to exhaustion. While this is true in some species, for example, we observed the automatic flight response with our milkweed bug trials, it is not true for all the insects we have tested (for example *N. vespilloides*). However, even with the automatic response, we never observed insects flying to exhaustion or death, in part because of the recording time we chose to accommodate the biology of the insects. Thus, it is important to do preliminary observations on the insect of interest to understand its behavior in the flight mill in order to optimize data collection. An additional, well-known issue with flight mills, is that inertia can maintain motion even after the insect has stopped actively flying. The script provided accounts for the misreadings due to inertia of the flight mill, characterized by rapid decrease in flight speed and increasing distances between peaks. The script "flight_analysis.py" discards these "false peaks" and constructs a new signal for analysis. The user can choose the speed threshold for correction, as explained in the notes provided in the script.

A 5 V power source is enough to obtain a readable voltage signal, however a power unit with variable output voltage can be used as power source to allow the power input to be varied and thus optimize working voltage for each sensor. Such a solution can also help to increase the visualization quality of peak signals in the software's recording interface. The sensor’s output is shown in the software interface as formed by a base and peak voltages where the base voltage represents the lowest output voltage from the sensor at rest (when the IR beam is not interrupted) while the peak voltage is the rise from base voltage that occurs when the IR beam is interrupted as the arm travels through the beam. An input voltage of 5 V provides a rise of around 100 mV

while increasing the input to 7 V increases the peak's rise to 300 mV allowing for a clearer discrimination of base and peak voltages. The size of the chosen solderless breadboard determines how many flight cells can be accommodated. In order to minimize drops in the voltage signal during recording from multiple sensors, it is recommended to place the resistors in alternate rows along the breadboard (see Figure 2.3C).

Customizable signal standardization and analysis scripts written for the open access programming language Python.

The standardization and analysis of the voltage signal are conducted by using custom written scripts in Python, which is a free, widely used general-purpose and high-level programming language. The end user can easily customize the scripts to work with own specified settings. The customization is achieved by simply changing numerical values or variable names. Notes on how to customize the parameters can be found within the scripts themselves. The default values in the scripts are set to deliver a fine tune signal standardization, but the user can define any desired threshold according to the value of the mean voltage for each channel. In the flight analysis script, the function flying bouts from line 105 calculates the duration in seconds of longest and shortest flying bouts, the percentage of time spent in flight over the total recording time and the number of flying bout events of a specified duration range. The ranges can be modified according to the user experimental requirements. In order to do so, all the numerical values inside the function (included the ones in the variable name, for example in the variable "flight_300_900") need to be changed to the desired value. The number of ranges and their duration simply depends on the user's specification. The script will print on screen the results of the analysis for each channel. These include: average flying speed, total flight time, distance travelled, shortest and longest flying bouts and flight composition. Additionally, the

script returns a *.DAT file for each channel and saves it in the output folder specified by the user. Each *.DAT file contains two columns: the first one represents the relative time of the peak event, the second is the detailed speed variation between two successive peak events. This file can be imported in Excel or R to produce a graph of the speed variation over time and visualize the flight activity patterns. An appendix containing the complete Python scripts used for peak standardization (*standardize_peaks.py*) and flight analysis (*flight_analysis.py*) is included with this thesis.

In conclusion, these results demonstrate that this flight mill design can be easily and successfully implemented to gather data for behavioral studies looking at flying activity patterns in different insect models. Such data can be used to investigate individual variation in movement patterns as dependent for example on physiology and morphology. This can offer great insights into the underlying physiological and morphological traits determining individual variation in movement patterns like foraging or migratory activity, which ultimately affects population as a whole. The detailed speed variation over time can be used in combination with detailed physiological and morphological measurements, offering a tool to study patterns of resource consumption or effects of variation in body part morphology on the flight activity.

CHAPTER 3

FITNESS TRADEOFFS INDUCED BY DEVELOPMENTAL HOST AND ANNUAL GENERATIONS IN THE KUDZU BUG *MEGACOPTA CRIBRARIA* (HEMIPTERA: PLATASPIDAE)

Introduction:

The kudzu bug *M. cribraria* (F.) (Hemiptera: Heteroptera: Plataspidae) is a relatively new introduced agricultural pest native to Southeast Asia. First observed in the American Southeast in 2009, as of 2014 it has spread north to Maryland, and west to Louisiana. Its current distribution closely matches the established invasive range of kudzu (*Pueraria lobata*), which it uses as a host plant in the early spring. It has demonstrated strong invasive potential and is capable of significantly reducing crop yields if left untreated, with yield loss of up to 47% in soybean reported (Ruberson et al. 2012). In the United States it is bivoltine with an initial emergent population rousing from overwintering refugia such as pine bark or dried plant matter, usually adjacent to food sources. There are two subsequent generational peaks over the summer and into early fall. Once cultivated soybean emerges from the soil, a portion of the kudzu bug population will leave kudzu to infest soybean, though the reason for this shift remains unclear.

Host switching of oligophagous insects like *M. cribraria* can be driven by necessity. For example, if a primary food source is depleted or represents a high risk of encounters with natural enemies (Schmitz el at. 1997) the net fitness benefit of switching hosts may outweigh the inherent costs associated with the switch (i.e. energy expended through flight). Some insect

herbivores have been shown to extend their normal dietary range during a population outbreak after depletion of their usual food sources (Kovalev et al. 2004). Facultative host switching may be detrimental to the fitness of the herbivore if the new host is a suboptimal species that would normally be avoided (Häggström et al. 1995). However, switching hosts remains beneficial if the alternative is starvation or inability to reproduce to due lack of sufficient nutrients, especially among Hemiptera (Panizzi et al. 1997). Dietary flexibility is also often regarded as a trait facilitating both the establishment and spread of the exotic herbivore into new environments (Kenis et al. 2009). We are then presented with the following question: does the annual host switch of kudzu bug represent a response to a suboptimal environment (i.e. overcrowding on kudzu), or is soybean simply a better host?

Several previous studies (Srinivasaperumal et al. 1992, Eger et al. 2010, Zhang et al. 2012) quantified aspects of the life history of *M. cribraria*, but there is little insight in the literature as to definitive traits of the annual overwintering emergent, first, and second generations and any differences that exist between them. Herein the life history of each generation is examined in a controlled greenhouse setting simulating the natural bivoltine cycle of *M. cribraria.* Objectives were to assess the effects of maternal and developmental host on fecundity, host preference, length and width of scutellum and notum, and distance flown on a flight mill. We hope that subsequent analysis of these parameters will provide much needed insight into the annual phenology and ecology of *M. cribraria* in order to bolster existing knowledge of its life history and better target means of control. In particular, we hope to elucidate key differences in host quality between kudzu and soybean so we can better understand the mechanism driving the midsummer host shift and the differences in imparted by the two substrates.

Methods:

Colony Establishment and Plant Cultivation

Adult *M. cribraria* and egg masses were collected in late march from a kudzu patch in Athens, Georgia (33°55'43.9"N, 83°22'06.0"W) and then established in colonies at the UGA greenhouse complex on Riverbend Road. We examined differences in life history traits among wild caught individuals (WC), individuals that develop from eggs laid by the wild caught females that were reared on either soybean or kudzu ($LabGen_1$), and individuals from eggs laid by the $LabGen_1$ females that were reared on either soybean or kudzu ($LabGen_2$). The kudzu used in rearing was cultivated from locally gathered stolon cuttings originally collected from the roadside of Riverbend Road in Athens, Georgia (33°55'43.9"N, 83°22'06.0"W). Cuttings were planted in potting soil in standard 8 inch diameter, 3.8 L volume plastic pots and allowed to establish roots, so that further cuttings could be taken as needed. The soybean used for rearing individuals was cultivar NK S52-Y2 (Syngenta AG, Basel, Switzerland) and was grown in identical pots. Both soybean and kudzu cultures were maintained under standard greenhouse conditions in a greenhouse at the University of Georgia Riverbend Greenhouse Complex (111 Riverbend Rd, Athens, GA 30602) using BugDorm™ tent cages (60x60x60 cm, 150x150 mm mesh, item #2120) with no artificial light or humidity modification (Figure 3.2). Both the soybean and kudzu were planted in identical potting soil mixed with 14:14:14 N:P:K nutrient formulation and watered daily at the same time.

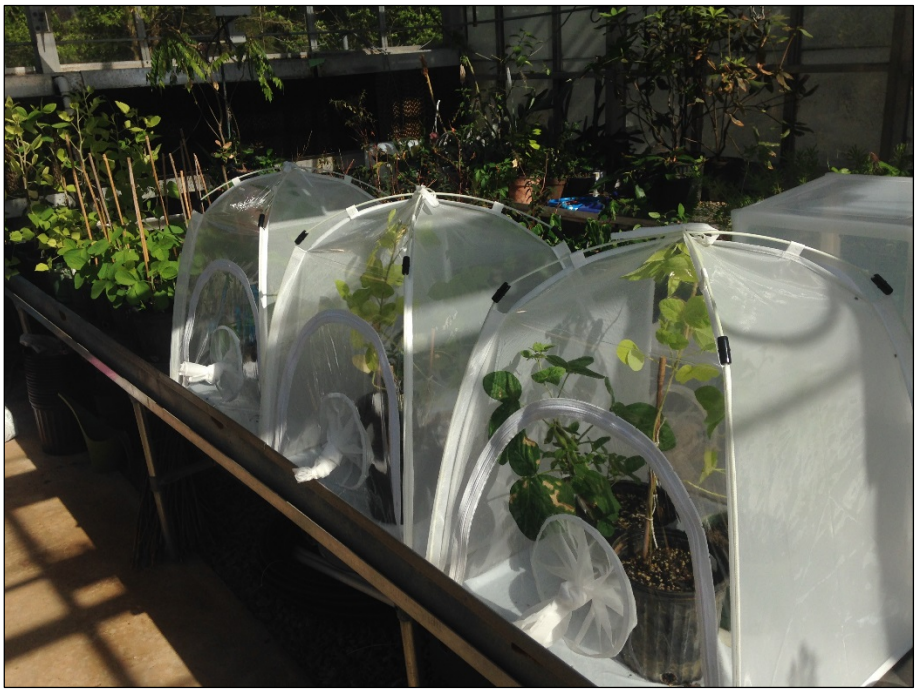

Figure 3.2 Tables and cages in greenhouse used for colony establishment and plant cultivation.

Effect of generation and larval host plant on total fecundity and host preference

From each generation (WC, $LabGen_1$, and $LabGen_2$) a single adult female and two adult male *M. cribraria* of similar age and raised on the same developmental host were randomly selected and enclosed in fine mesh ventilated plastic Petri dishes in growth chamber on a 14:10 day/night cycle at 32° C and 68% humidity (Figure 3.3). Females were given a sequential unique ID number to allow us to follow the offspring of individual females across experiments. The adults were provisioned with damp absorbent cotton for moisture and one each fresh soybean and kudzu leaves from plants of similar growth stage (R1 or R2) and size (≈ 10 cm^2) each day between 9:00 and 10:00 AM. Each trio was monitored twice daily for one week between 8:00-10:00 AM and 4:00-6:00 PM. To assess host preference the following response variables were recorded: number of eggs laid on each of the two available host plants, total number of eggs laid over the period of observation and female mortality. Data were collected on 150 individual females in total. The total number of eggs laid by each female were analyzed using an analysis

of variance (ANOVA), while host preference was analyzed using a Chi-square test. The effect of generation, maternal substrate choice, and developmental host on host preference was analyzed as the percent of eggs laid on soybean using a GLM with a Poisson error function. All analyses were performed in JMP Pro version 11.0.0 (SAS Institute 2014).

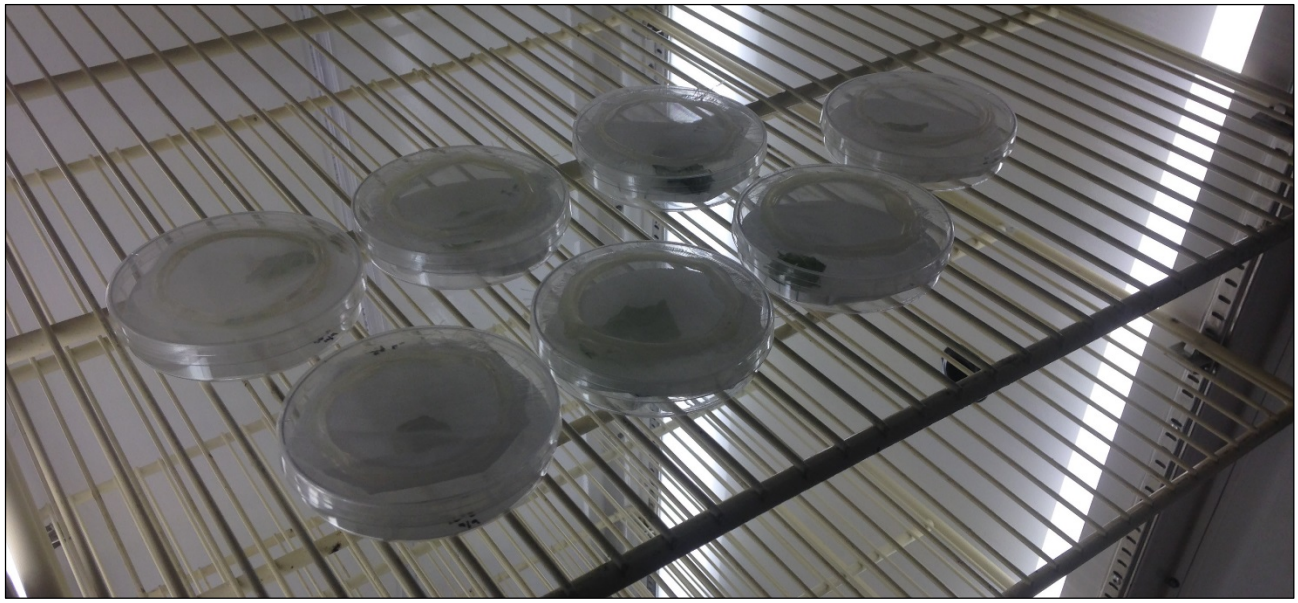

Figure 3.3 Ventilated Petri dishes containing egg-bearing leaf material inside rearing chamber.

Effect of generation and larval host plant on larval development and survival

Eggs laid during the host preference trials were taken on the same day they were oviposited and randomly assigned using a coin flip to determine the treatment the clutch would receive; either kudzu or soybean. These clutches were individually and sequentially numbered, placed on host plants of approximately identical age, and allowed to emerge in BioQuip™ clip cages for the first three instars to avoid losses of individuals due to their small size. Once the majority (>90%) of clutch cohorts had reached 4th instar they were then transferred using soft forceps or a fine bristled paintbrush to caged soybean (growth stage V1-V2) or kudzu (of similar age) to develop until eclosion. Clutch instars were estimated by observing individuals twice daily during and recording when 90% of the cohort had molted indicating an instar change. Total development

days and percent mortality were recorded as response variables and analyzed using ANOVA in JMP Pro 11.0 (SAS Institute 2014).

Effect of generation and larval host plant on adult size

25 andomly selected females from each generation were separated into 1.5ml plastic vials and frozen at -80°C. Individuals were imaged at 1.25X magnification with Leica© M60 Scope and digital imaging software. Length and width of both scutellum and notum measured in millimeters, with each measurement to be used as a proxy for overall body size relative to other individuals in this study. Size data were normally distributed and analyzed using ANOVA in JMP Pro 11.0 (SAS institute 2014).

Effect of generation and larval host plant on adult flight behavior

We measured several flight characters as a proxy for dispersal potential using the flight mill apparatus and accompanying protocol as described in Chapter 2 of this thesis. For each trial four random adult females (3-4 weeks post eclosion) were selected using a random number generator (WC=1-50, F_1=51-100, F_2=101-150). Individuals were attached to flight mill arm using hot glue applied to the anterior thorax with a size 3 insect pin, allowed to fly for one hour under observation. Data were recorded with a DataQ™ DI-149 data logger, interpreted and visualized using a proprietary Python script (Attisano et al. 2015, Appendix A). Response variables recorded included: longest single flight bout (time and distance), total number of bouts, total flight time, and total flight distance.

Results:

Host preference and fecundity

Female fecundity was dependent on generation (ANOVA; $F_{2, 147} = 46.187$, $p < 0.001$) In pairwise comparisons, wild caught females and the first generation of females born from eggs laid in the lab laid similar total numbers of eggs (ANOVA; $F_{2, 147} = 46.187$, $p = 0.229$), while the mean number of total eggs laid by the second generation of laboratory females was less than wild caught and $LabGen_1$ females (ANOVA; $F_{2, 147} = 46.187$, $p < 0.001$). The fecundity of the $LabGen_2$ females was too low to allow for further analysis of host preference for this generation (Figure 3.3A).

Both WC and $LabGen_1$ females laid more eggs on soybean than on kudzu (WC $\chi^2 = 40.5$, d.f. = 1, $p < 0.001$; $LabGen_1$ $\chi^2 = 20.8$, d.f. = 1, $p < 0.001$). Wild caught females laid an average of 65.1% of their eggs on soybean and $LabGen_1$ females laid 59.6% of their eggs on soybean. There was no significant difference in the proportion of eggs laid on soybean between the WC and $LabGen_1$ females (ANOVA, $F_{1,88} = 0.377$, $p = 0.541$)(Figure 3.3B). Within the $LabGen_1$ females, both developmental host and the oviposition choice of their mothers effected their own oviposition choice (Figure 3.4A: GLM; maternal substrate choice $\chi^2 = 186.5$, d.f. = 1, $p < 0.001$, developmental host $\chi^2 = 25.4$, d.f. = 1, $p < 0.001$, maternal substrate*developmental host $\chi^2 = 21.3$, d.f. = 1, $p < 0.001$).

Females that developed from eggs oviposited onto soybean laid significantly more eggs on soybean compared to females that developed from eggs oviposited onto kudzu, despite the fact that these females were moved to their developmental host within 24 hours following hatching. Among the females that hatched from eggs laid on kudzu, those that were transferred to soybean for nymphal development were more likely to lay their eggs on soybean than females

that hatched from eggs laid on kudzu that developed on kudzu(Figure 3.4A) Larger bodied individuals across all generations correlated with higher fecundity (Figure 3.6A).

Development and Morphometrics

There was no significant difference between the number of days from egg to adult among nymphs developing on soybean and kudzu for any generation. There was a significant difference in development time among eggs laid by females in the different generations (ANOVA; $F_{5,89}$=18.1464, $p < 0.0001$), but no interaction between development substrate and generation. When development time is analyzed separately from nymphal substrate, the differences in development time are less clear. The fastest developing offspring are those collected from the field, originally laid by the wild caught females (mean=38.6 days, SE=1.209), while the eggs laid by the WC females (mean=50.35 days ± SE=0.557) take significantly longer to develop than the eggs laid by the $LabGen_1$ females (mean=47.831 days ± SE=0.557) (ANOVA; $F_{2,89}$=46.1697, $p < 0.0001$). $LabGen_2$ females did not have sufficient oviposition or nymphal survivability for analysis (Figure 3.4B).

There was a significant effect of both developmental host and generation on adult size and a significant interaction between diet and generation (ANOVA; $F_{3,49}$=12.7058, $p < 0.0001$). Nymphs that develop on soybean are significantly larger ($LabGen_1$=3.367 mm, $LabGen_2$=2.854 mm) than nymphs that develop on kudzu ($LabGen_1$=2.851 mm ± SE=0.054, $LabGen_2$=2.744 mm ± SE=0.048) in both generations (ANOVA; $F_{1,21}$=11.6933, p=0.0027) and the $LabGen_1$ adults are significantly larger (mean notum width =2.85 mm ± SE=0.107 kudzu, mean notum width =3.367 mm ± SE=0.107 on soybean) than the $LabGen_2$ adults on both diets (mean notum width=2.744 mm ± SE=0.047 on kudzu, mean notum width=2.854 mm ± SE=0.044 on soybean)(Figure 3.5A,B).

Flight behavior

Generation (p=0.0087), but not developmental host (p=0.3144), had significant effects on total distance flown in the two laboratory generations, and there was no significant interaction between generation and (Generation*Diet: t=-0.23, p=0.8159). Wild caught females flew significantly farther (mean=394.3 m ± SE=17.325) in flight trials than either $LabGen_1$ (mean=165.9 m ± SE=14.306) or LabGen2 (mean=128.9 m ± SE 12.681) females (ANOVA; $F_{2,64}$= 85.6570, p<0.001). $LabGen_1$ females flew significantly longer distances than $LabGen_2$ females (Figure 3.6B). In all generations there were positive correlations between body size and distance flown (Figure 3.7).

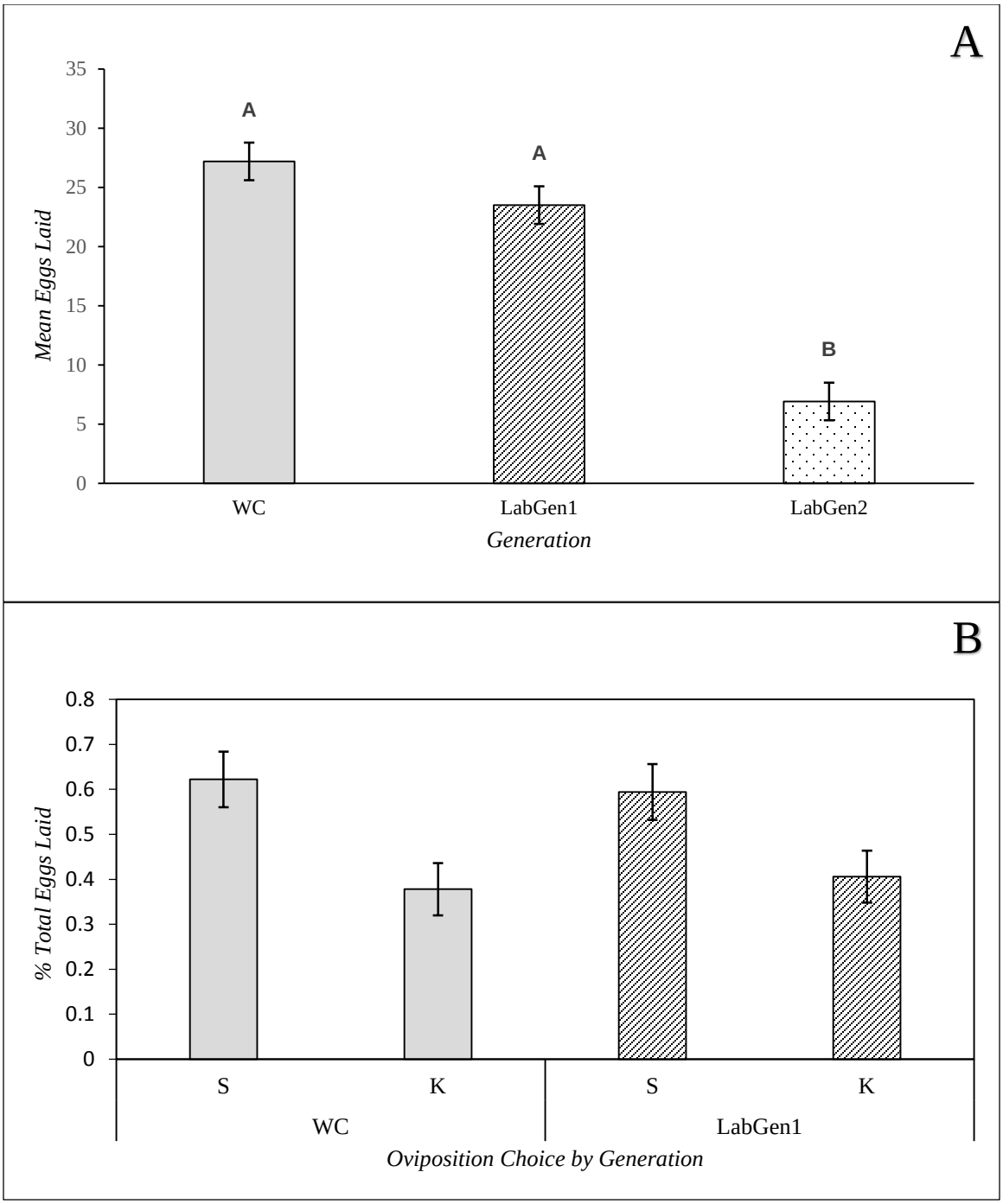

Figure 3.3 Fecundity and host preference of *Megacopta cribraria* A: Mean (± SE) number of eggs laid by females of both rearing substrates of all three generations. Letters above bars indicate significantly different means as determined by post hoc Tukey's HSD test. **B:** Percentage (± SE) of eggs laid on soybean (S) or kudzu (K) in WC and $LabGen_1$

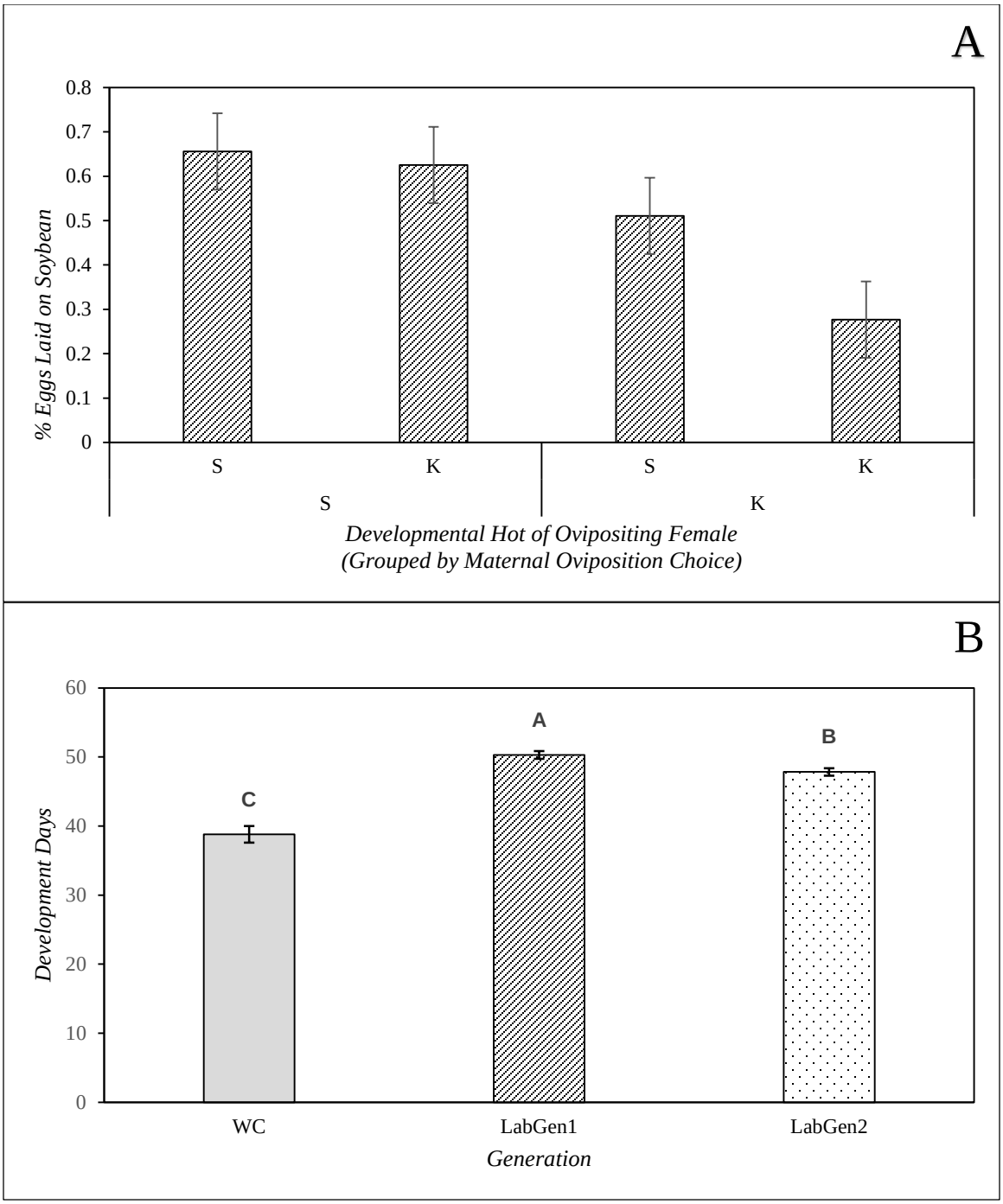

Figure 3.4 Within-generation host preference and development time of *Megacopta cribraria*
A: Mean (± SE) percentage of eggs laid on soybean by $LabGen_1$ females of both diets. Females are grouped into two categories based on the host plant they were originally oviposited on. **B:** Mean (± SE) development time from instar I to adult eclosion of eggs laid by each generation. Letters above bars indicate significantly different means as determined by post hoc Tukey's HSD test.

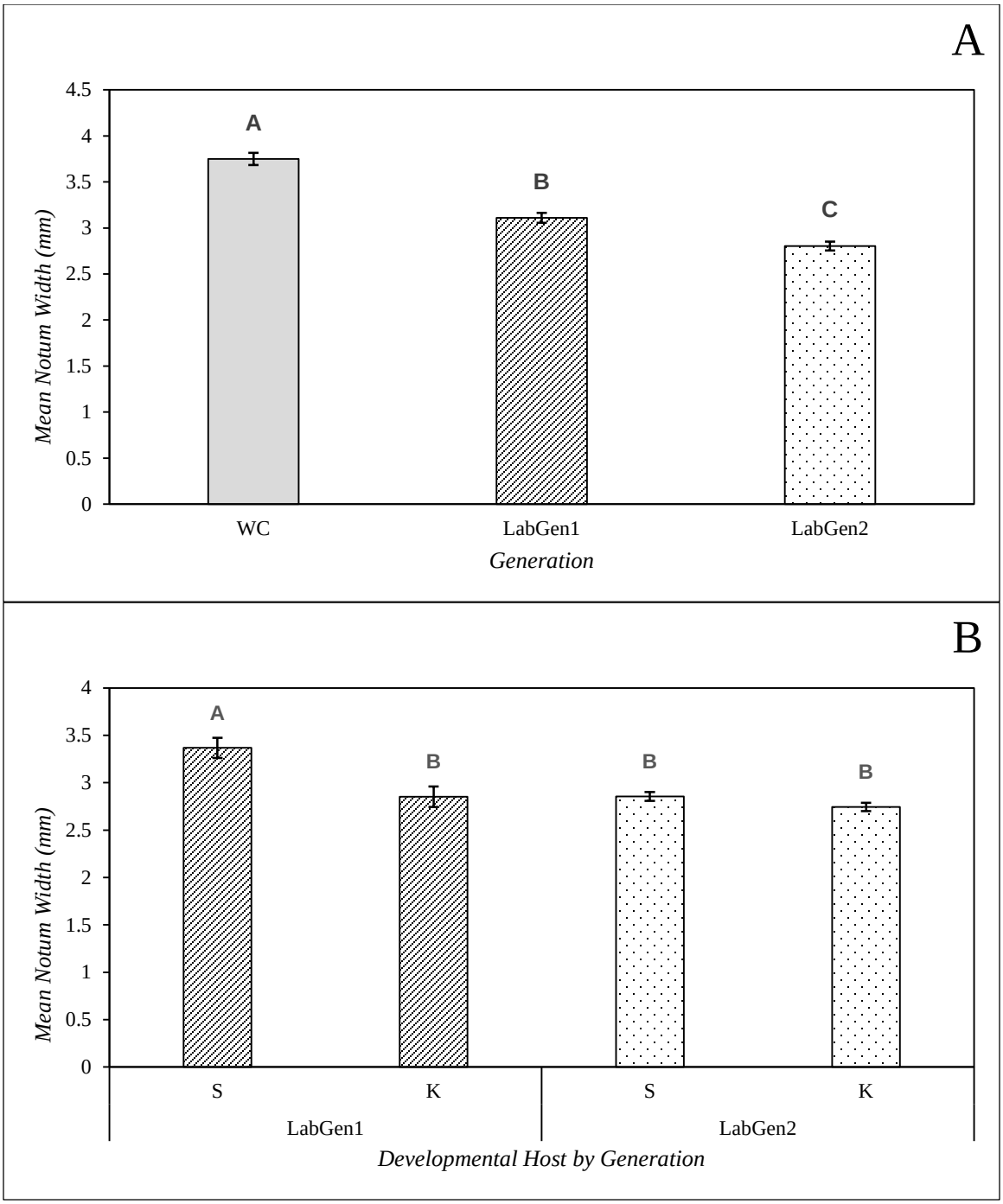

Figure 3.5 Body size of *Megacopta cribraria* relative to annual generation and developmental host A: Mean (± SE) notum width of all three generations. Letters above bars indicate significantly different means as determined by post hoc Tukey's HSD test. **B:** Mean (± SE) notum width of $LabGen_1$ and $LabGen_2$ females. Females are subdivided by developmental host of soybean (S) or kudzu (K). Letters above bars indicate significantly different means as determined by post hoc Tukey's HSD test.

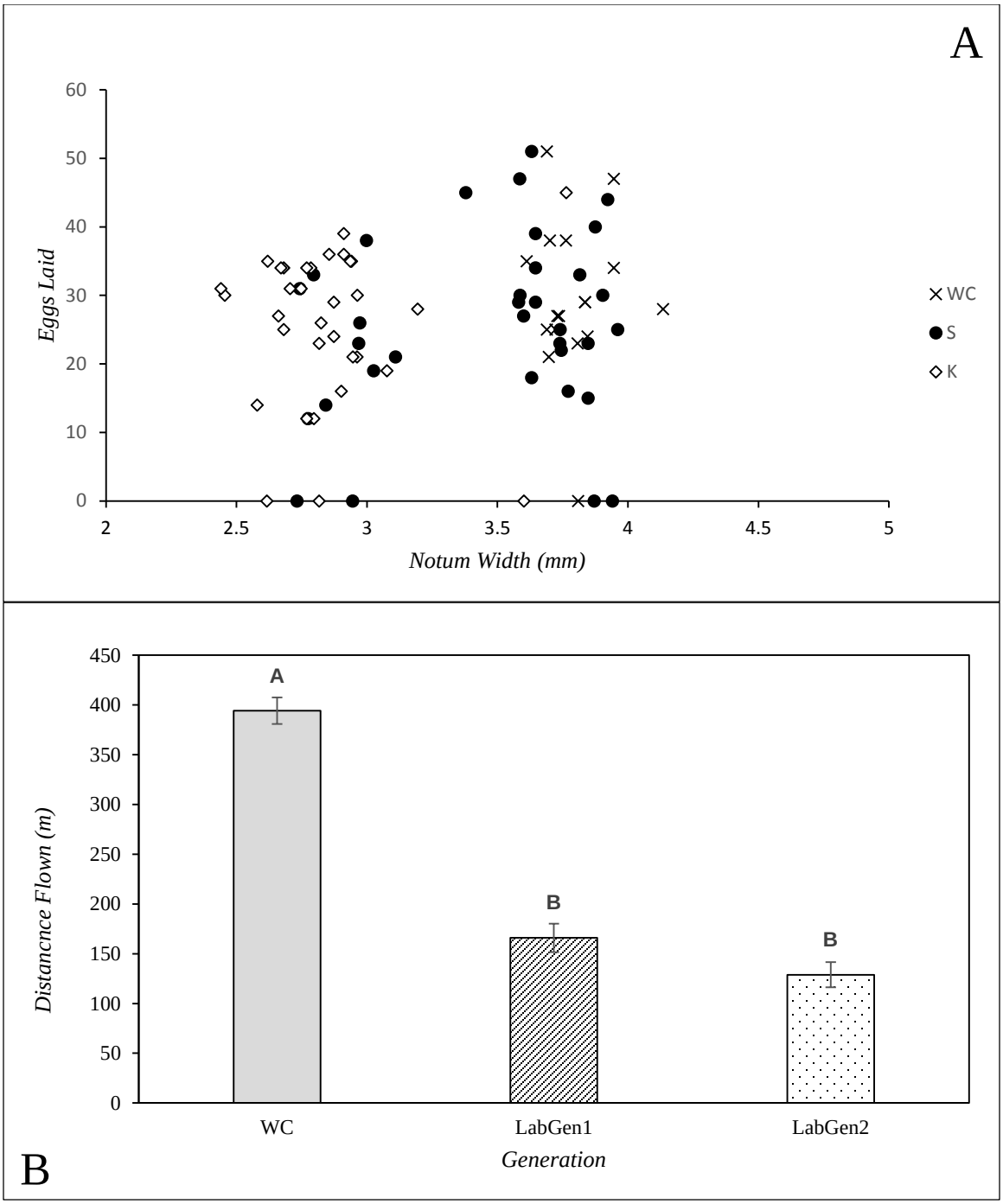

Figure 3.6 Relationship between body size, fecundity, and distance flown of each annual generation of *Megacopta cribraria* A: Potential fecundity of randomly selected females of all three generations and both rearing substrates plotted against their respective notum width. **B:** Mean (± SE) total flight distance of all three generations. Letters above bars indicate significantly different means as determined by post hoc Tukey's HSD test.

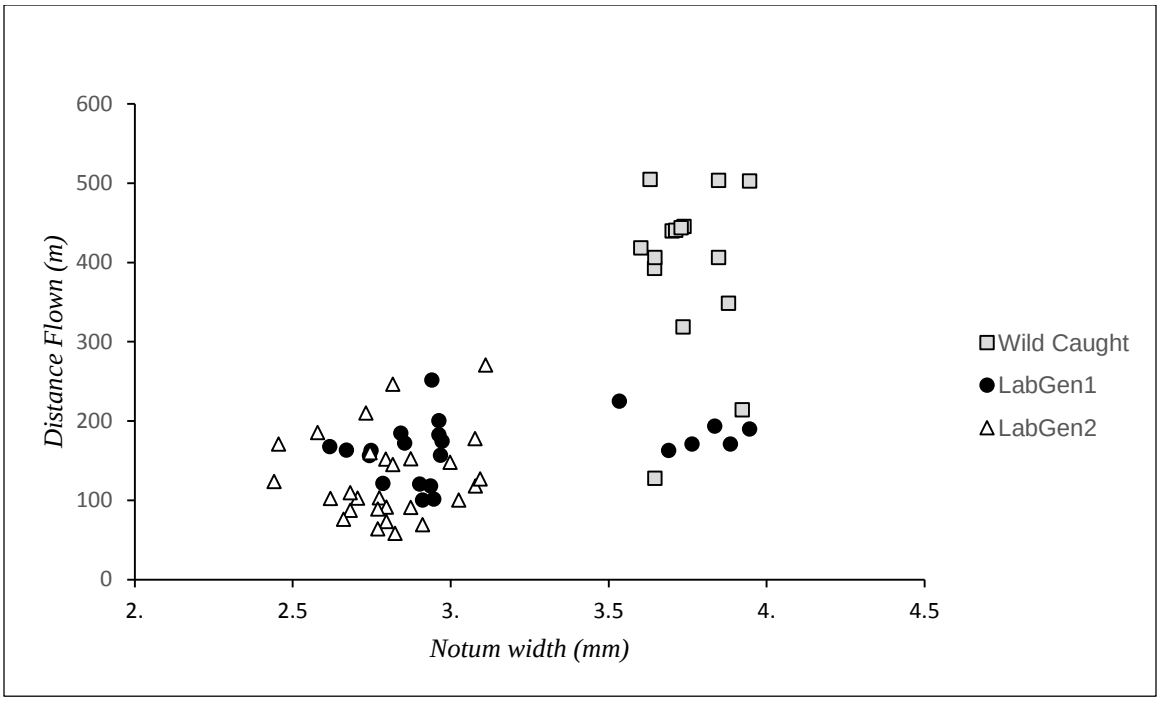

Figure 3.7 Relationship between body size and flight ability of annual generations of *Megacopta cribraria* Flight distance of randomly selected females from all three generations plotted against their respective notum width.

Discussion:

Host switching can have significant costs to the fitness of phytophagous insects, especially if the insect has developed an acquired preference for its initial diet (Bernays et al. 1996, Silva et al. 2014). Understanding the impact of switching hosts is an important aspect of understanding the basic biology of a pest insect, so that effective control methods can be designed and implemented. Our results show that switching between rearing nymphs on the two major hosts of kudzu bug (soybean and kudzu) does not have a significant impact on nymphal development or adult fitness. However, our results do demonstrate significant differences between the annual generations of kudzu bug, indicating that while current chemical and cultural control strategies for kudzu bug may remain unchanged in intensity of application, their temporal arrangement may need adjustment to account for this new information.

Host Preference and Fecundity

Reproduction is an essential component of fitness, and female fecundity as measured by either eggs produced or eggs laid is often chosen as a proxy measurement for fitness (Jaenike 1990). Previous investigations of the host preference of *M. cribraria* are both few in number and inconclusive in nature. Some only investigate adult and nymphal presence/absence on various host plants (Blount et al. 2013) and others reported greater oviposition on cage walls than on a particular host plant when given a choice of several (Seiter 2014). A no-choice study by Medal et al. (2013) found that kudzu bugs given kudzu as a feeding and oviposition substrate laid more eggs, had more eggs hatch, and had a larger number of nymphs eclose to adulthood compared to adults given soybean. However, the results were not significantly different between the two substrates. Similarly, our results indicate that rearing on soybean or kudzu does not produce a significant difference in the fecundity of adult females reared on either substrate.

Fecundity of $LabGen_2$ was significantly, if not catastrophically lower than either wild caught (WC) or $FieldGen_1$ females. It was so low in fact, that we did not have sufficient data to analyze for host preference. Raw percentage data show that only 38% of $LabGen_2$ females oviposited at all, with 26% ovipositing on kudzu and 12% ovipositing on soybean. This is the first and only instance of kudzu preference in the entire study, but due to the low amount of females ovipositing (n=19) and high number of zeros reported for oviposition at the end of the week, we did not analyze for preference further.

Both remaining generations (WC and $LabGen_1$) showed similar potential fecundity across both substrates, with WC females ovipositing slightly more eggs than $LabGen_1$ females. Investigations of host plant preference indicated significant differences in oviposition frequency for with WC females choosing to oviposit on soybean 62% of the time and $LabGen_1$ females

choosing it 59% of the time. We decided to use $LabGen_1$ females to investigate the broader impacts of upstream maternal choices on downstream nymphal fitness. Oviposition behavior is considered extremely important as reproductive decisions made by mothers can define the environment of their offspring and exaggerate or censor expression of their phenotype, thus having a pivotal role in their overall fitness (Abrahamson and Weis 1997). Analysis of host preference within $LabGen_1$ displayed interesting additive effects of developmental host and maternal oviposition choice. Females of this generation preferentially oviposited on soybean (59% of all eggs laid). However, an examination of the developmental hosts of the mothers indicated that soybean reared mothers also oviposited significantly greater proportions of their eggs on soybean. Compounding this, we found an impact of maternal oviposition choice shown by mothers originally oviposited on soybean demonstrated even greater preference for soybean oviposition. Other factors of nymphal development (e.g. rearing conditions and host plant quality) remained constant between nymphal host assignment. We therefore hypothesize this relationship indicates the presence of a maternal effect, influencing females to preferentially oviposit on the substrate chosen by their mother.

Maternal effects are considered ubiquitous in the animal kingdom (Roitberg 1998) and are defined as the modification of or influence on offspring's genotype and resultant phenotype as a direct result of the mother's phenotype and environmental conditions before oviposition (Kempthorne 1969, Wright 1969, Falconer 1989). In the case of our study we show that the environment of a nymph is somehow secondary in influence to that of the substrate her mother chose for oviposition. Thus, female *M. cribraria* have some mechanism of signaling their eggs to prefer certain substrates over others. It is possible that this signaling takes place through the deposition of symbiont capsules during oviposition. These capsules, contain two essential

obligate bacterial endosymbionts: the primary gamma-proteobacterium, *Candidatus Ishikawaella capulata*, and the secondary alpha-proteobacterium, *Wolbachia* (Fukatsu and Hosokawa 2002 , Zhang et al. 2012). The former is essential for proper development as demonstrated by Fukatsu and Hosokawa et al. (2007) and is therefore a likely target for modification or genetic crosstalk leading to downstream maternal effect. *Wolbachia* is also well known to modify the behavior of insects (Stouthammer et al. 1999, Werren 1997, Werren et al. 2008), and so may also be of interest for future studies to elucidate the source of this link between maternal choice and offspring preference.

Development and Morphometrics

Results of our study indicate that both soybean and kudzu are equally good hosts for development, with no significant difference in development time from egg emergence to adult eclosion demonstrated between soybean and kudzu. This result is somewhat unsurprising as previous studies have reported kudzu and soybean as subequal hosts for the development of *M. cribraria* (Eger et al. 2010, Medal et al. 2012, Del Pozo-Validivia et al. 2013). There were differences in development time found in the three generations investigated over the course of our study. Eggs collected from the field developed faster (mean=38.6 days ± SE=1.209) than any eggs laid by kudzu bugs reared in the greenhouse. It is possible that this effect is due at least in part to differences in developmental temperatures following oviposition. Shi et al. (2014) that found that rearing *M. cribraria* at different constant temperatures demonstrated faster development through all nymphal stadia at higher temperatures (mean=34.23 days at 29°C) than at low temperatures (mean=96.79 days at 17°C). Greenhouse temperatures were not kept constant and likely resulted in the variability seen among the generations.

Using body size as a measure of fitness in correlation with fecundity allows for predictions at both the individual and population level (Guha et al. 2012). In contrast to the development data gathered, we found significant differences in body size as a result of both generation and most importantly developmental host. Larger bodies are often correlated with improved fecundity (Briegel 1990). In our study larger bodied $LabGen_1$ and WC females correlated with higher fecundity than kudzu reared females, though there was no significant difference demonstrated between the two host plants indicating that neither diet results in a difference in reproductive potential of *M. cribraria*.

Flight Trials

Results of flight trials exhibited similar trends of morphometric and fecundity experiments. WC females flew farther than either greenhouse reared generation regardless of host plant. This information is troubling, because one of the main goals of our study was to examine the midsummer host shift of kudzu bug. Without a clear difference between host plants it is difficult to understand the reasons for kudzu bug to expend energy to disperse from readily available kudzu to potentially distant soybean that appears to confer few benefits. Studies of Lepidoptera have shown a link between flight capability and fecundity (Watt 1992). Our study similarly indicates that large bodied and more fecund females are also possess greater flight ability. Thus, larger females may represent a novel target of control if planting schedules or chemical application can be modified to select against the rearing of large females.

Conclusions:

The spread of the kudzu bug since its discovery in the United States in 2009 has been both rapid and prolific. A purported single maternal lineage (Jenkins et al. 2010) gave rise to the North American population that is both an agricultural and urban nuisance across the American

southeast each year. This study shows that while the particular nymphal or maternal feeding substrate may not have a strong impact on fitness of *M. cribraria,* there are interesting and important differences amongst the annual generations that may lend themselves to informing control decisions and agricultural practices. Regardless of rearing substrate, larger body size seems to be most highly correlated with the fitness parameters we have investigated. Soybean rearing produced the largest females in our studies, however they were still significantly smaller than the WC females that presumably were reared on soybean in the wild before collection.

It seems that the dynamics of host plant impacts on the fitness of *M. cribraria* are more complex than we have been able to ascertain with our study. We still suffer from a lack of understanding on the basic principles of culturing *M. cribraria* as a side effect of the current paucity of literature on the insect and lack of an established or published rearing protocol yielding success in development past two subsequent generations in a lab or greenhouse setting. Confounding variables including ambient conditions in the greenhouse differing significantly from the rearing chamber and flight room, presence of competitors and natural enemies (e.g. whitefly and ants) on host plants during rearing, and cage effects restricting natural movement of nymphs and adults may have contributed to the lack of clarity of our results. It is also possible, though unlikely due to the low number of generations reared that inbreeding within the colony led to some degree of lab adaptation or concentration of deleterious traits.

We believe that this study provides an informative primer into the life history and behavior of *M. cribraria* in its newly invaded range. By establishing patterns of oviposition preference and the impacts of developmental host on the fitness of *M. cribraria* we have elucidated previously understudied aspects of its life history. However, due to the aforementioned limitations brought on unintentionally by isolating this experiment to the

greenhouse we also believe that further investigation of host plant impacts on fitness should be undertaken in a field context in order to mitigate or control for as many of these issues as possible. It is the authors' hope that by continuing to investigate the biology, ecology, and phenology of kudzu bugs, we may better understand them and in turn understand how they may be controlled.

CHAPTER 4

EFFECTS OF HOST PLANT AND GENERATION ON FITNESS OF *MEGACOPTA CRIBRARIA*: A FIELD STUDY

Introduction:

The kudzu bug, *Megacopta cribraria* (F.) (Hemiptera: Plataspidae) is a new invasive pest of the southeastern United States known to feed on a variety of legume (Fabales: Fabaceae) and non-legume plant hosts (Eger et al. 2010) in its native Asian distribution. *M. cribraria* was first discovered in North Georgia in 2009 and has quickly spread through the southeastern United States (Suiter et al. 2010). Following its introduction and subsequent invasion, it has been collected on 33 species of plant, 17 of which were legumes (Gardner et al. 2013). While adults of *M. cribraria* are commonly encountered on a variety of plants during their annual period of seasonal activity from spring to fall, only legumes have been confirmed to support complete development of kudzu bugs from egg to adult. (Zhang et al. 2012, Del Pozo-Valdivia and Reisig 2013, Medal et al. 2013). Kudzu, *Puereria montana* (Loureiro) Merrill variety *lobata* (Montandon), and soybean, *Glycine max* (L.) (Zhang et al. 2012), both legumes in subtribe Glycininae are found in the kudzu bug's introduced range and are important due to their own invasive status in the case of the former, or economic significance in the case of the latter.

It is well known that in insects, parental host diets influence offspring phenotype and fitness parameters such as egg mass and offspring growth and development (Rossiter 1991, Fox & Mousseau 1998, Kyneb & Toft 2006). In order to effectively control a new invasive it is best

to have as complete an understanding of its biology as possible so that prudent management strategies can be designed and implemented. With both of these things in mind, we seek to explore the host plant interactions between kudzu bug and its two main host plants in the United States (kudzu and soybean) to understand the fitness costs and benefits associated with rearing on each. To this end we will examine the fitness impacts of rearing *M. cribraria* on both host plants in a field setting by measuring several parameters as proxy measures of total fitness. Objectives were to measure potential fecundity (as measured by the mean number of eggs per egg mass laid), development time (as measured by the time in days from egg emergence to adult eclosion), dispersal potential (as correlated with flight ability measured in meters flown per hour), body size (using notum width as a proxy measure for total body size), and lipid energy stores (as measured by total triglyceride titer of abdominal fat body tissue).

Methods:

Collection of Overwintering Females

Adult female *M. cribraria* were collected by hand in mid-February from beneath loose pine bark (Figure 4.1) adjacent to a kudzu patch on Riverbend Road in Athens, GA (33°55'43.9"N 83°22'06.0"W). Individuals randomly selected for flight analysis were submitted to trials within an hour of collection and not allowed any feeding substrate to ensure that all flight activity was resultant of energy stores remaining during overwintering. Individuals designated for morphometric analysis and tissue assay were separated into labelled 1.5ml plastic vials and frozen at -80°C until needed.

Figure 4.1 Overwintering *Megacopta cribraria* adults found under pine bark in Feb. 2015

Effect of Host Plant on Nymphal Development

Beginning 20 April 2015, *M. cribraria* egg masses were isolated in a kudzu patch in Athens, Georgia (33°55'43.9"N 83°22'06.0"W) using BioQuip foam and mesh clip cages (www. bioquip.com, item #: 1458). Caged egg masses were then monitored daily for emergence. After emergence, five randomly selected nymphs were taken from the cohort and re-caged within 1 meter to monitor development. Caged nymphs was monitored daily until eclosion to adulthood with transitions through stadia recorded and analyzed using JMP/SAS. An identical protocol was utilized to monitor nymphal development on soybean at the University of Georgia Plant Science Farm (33.865460, -83.544784) with caging beginning on 13 July 2015. Soybean (NK S52-Y2, Syngenta AG, Basel, Switzerland) was planted on 22 May 2015 in single rows on 36 inch centers. Data were subjected to analysis by ANOVA and post hoc student's t-Tests or Tukey's HSD tests for separation of means as needed in JMP Pro 11.0. (SAS Institute 2014)

Effect of Host Plant on Flight Ability and Dispersal

To compare differences in flight ability and dispersal potential, fifth instar *M. cribraria* nymphs were caged *in situ* on both kudzu and soybean, allowed to eclose to adulthood, and then remained caged for 1 week to continue feeding (Figure 4.2). 185 adult females were collected throughout the summer on a biweekly schedule beginning in June for the first annual generation of kudzu reared individuals ($FieldGen_1$) and again in early August for both soybean and kudzu reared individuals ($FieldGen_2$). Data were also collected from the overwintering individuals (OW) collected 16 February 2015 as well as 'late season' individuals (FieldGenLate) to gather comprehensive data on the flight characteristics across an entire active season. All individuals used for flight trials were taken on the same day of collection to the University of Georgia biological sciences building (120 Cedar St. Athens, GA 30602) for flight trials. Flight trial protocol was otherwise identical to the one described in chapter 2 and utilized in chapter 3. All data were subjected to analysis by ANOVA and post hoc Tukey's HSD tests for separation of means in JMP Pro 11.0. (SAS Institute 2014)

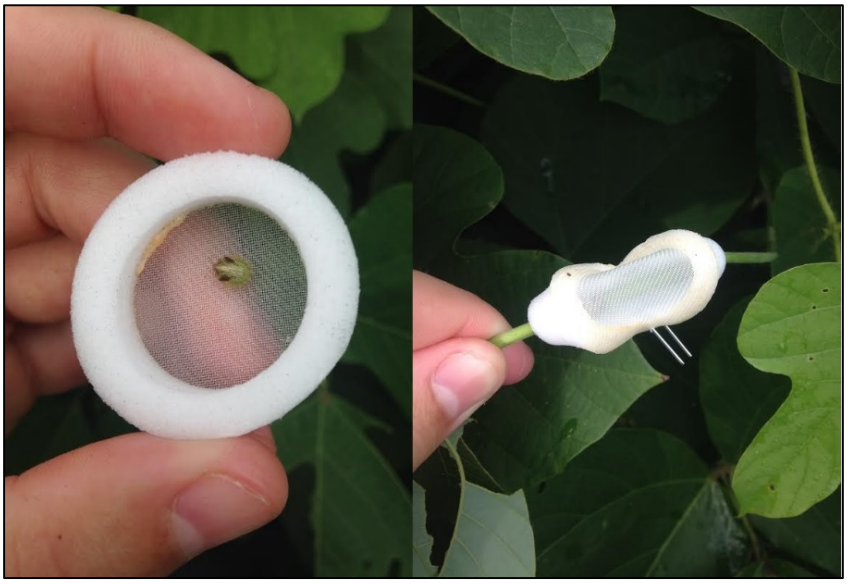

Figure 4.2 Caged instar V *M. cribraria* nymph on kudzu in Athens, Georgia.

Effect of Host Plant on Body Size

208 female *M. cribraria* in total were collected and separated into 1.5ml plastic vials and frozen at -80°C. Individuals were imaged at 1.25X magnification with Leica© M60 Scope and digital imaging software Length and width of both scutellum and notum measured in millimeters, with each measurement to be used as a proxy for overall body size relative to other individuals in this study. Size data was normally distributed and analyzed using ANOVA in JMP Pro 11.0 (SAS Institute 2014)

Effect of Host Plant on Lipid Energy Stores

A standard commercial assay kit (Infinity Triglycerides Liquid Stable Reagent Assay, Thermo Scientific®) was used to assess adult energy stores by dissecting and analyzing fat body tissue of 208 female *M. cribraria*. The protocol for triglyceride (TAG) assay is as follows: First, place the dissected abdominal fat body tissue of one female in 0.5 ml PBS/TX in a 1.5ml vial. Warm to 37°C if using frozen material. Next, homogenize with electronic pestle for 1-2 minutes or until major tissue is crushed. Warm homogenate at 37°C for a few minutes. Next, spin in centrifuge at 2800 RPM for 10 minutes. While tissue samples spin down warm the triglycerides reagent provided in the kit to 37°C. After removing sample from the centrifuge, pipette 5 µl of each tissue sample in triplicate and 5 µl of each standard in triplicate into a 96-well plate. Then add 150 µl Triglycerides Reagent to each sample and standard and tap gently to mix. Wait 10-30 minutes for the assay to develop at room temperature. Finally, place in a plate reader and read at a range of 500nm-550nm. Save and export results to Excel as a .csv file for ANOVA analysis in JMP Pro 11.0. (SAS Institute 2014)

Stats. Would like to see a brief discussion of experimental unit, experimental design, blocking factors, transformations, and replication.

Results:

Development Time

Average fecundity of females remained constant across the entire experimental period with no significant differences shown among generations or between hosts (Figure 4.3A). There was also no significant difference in development time from egg hatch to adult eclosion among generations (t Test DF=148, t=1.976. However, across both generations, soybean reared individuals developed significantly faster (mean=43.81days ± SE= 0.376) on average (t Test: prob < t=0.001, α=0.05). Development time differed slightly among generations, $FieldGen_1$ eggs caged on 20 April developed slower than eggs collected on all other dates (mean=45.91 days ± SE= 0.554). $FieldGen_2$ nymphs caged on soybean on 13 July developed the fastest overall (mean=42.36 days ± SE= 0.338) (Figure 4.3B)

Flight Trials

Flight distance was significantly impacted by both generation and rearing substrate. Overwintering (OW) individuals flew lesswhen compared to all other generations sampled (mean=281.38 m ± SE=139.88). Statistical analysis shows that flight ability of $FieldGen_1$ and $FieldGen_2$ are not significantly different (ANOVA, $F_{1,148} = 0.209$, $p = 0.8955$, means=3239.09 m ± SE= 97.94 and 3404.6 m ± SE= 70.29 respectively). However, FieldGenLate females flew less distance than both FieldGen1 and $FieldGen_2$ (ANOVA, $F_{2,183} = 17.531$, $p < 0.001$, mean=2551.04 m ± SE=116.57), though still significantly better than OW females. Soybean reared females flew the furthest across all generations (mean=3449.29 m ± SE=91.11) compared to kudzu reared females (mean=3053.61 m ± SE=67.57). Independent of generation and rearing substrate, collection date did not show a significant effect on flight distance, except in

FieldGenLate females that flew less than females from all other collection dates besides overwintering females(ANOVA, $F_{4,185} = 9.1575$, $p < 0.001$, mean=2551.04 m). Kudzu reared females ($FieldGen_1$) flew an average distance 3239.09 m with no significant difference in flight ability shown between both collecting dates. $FieldGen_2$ females showed significant differences in flight ability between rearing substrates with soybean reared females flying significantly farther (3776.92 m ± SE= 90.422) than kudzu reared females (3039.74 m ± SE=91.340) (ANOVA, $F_{1,98} = 32.897$, $p < 0.001$). There was again no significant difference in flight ability between collecting dates. FieldGenLate individuals performed similarly on both substrates with no significant difference demonstrated (t Test: Prob < t=0.349, α=0.05)(Figure 4.4A)

Triglyceride Content

Triglyceride titer did not vary significantly between rearing substrates (p=0.6014), but OW females had significantly less triglyceride stores (mean=9.767 μg/L ± SE=4.13) compared to kudzu and soybean reared females (means = 24.34 μg/L and 21.4927 μg/L), respectively (ANOVA, $F_{2,207} = 5.316$, p=0.0056). A Tukey's HSD test found that triglyceride titer did not vary significantly with collection date, except for OW females (mean = 9.766 μg/L ± SE=4.13) and FieldGenLate females collected on 15 September who were significantly different (mean=21.81 μg/L ± SE= 3.24, p=0.0456) (ANOVA, $F_{5,207} = 2.0296$, p = 0.0760). Analysis of triglyceride titer compared against generation mirrored the result of collection data analysis, with $FieldGen_1$ and $FieldGen_2$ females (means= 22.825 μg/L ± SE= 2.71 and 24.14 ± SE=1.95 μg/L respectively) not differing significantly from FieldGenLate females, but significantly greater than overwintering females (mean = 9.766 μg/L ± SE=4.13) (Figure 4.4B).

Morphometrics

A Tukey's HSD test showed that OW females' notum width were significantly larger than all females except for $FieldGen_1$ kudzu reared females (means= 3.72 mm ± SE=0.1 and 3.33 mm ± SE=0.09 respectively). However $FieldGen_1$ females were not significantly larger than other groups. (ANOVA, $F_{5,202} = 3.1489$, $p = 0.0093$). Tukey's HSD test showed that neither rearing substrate produced significantly larger females independent of collection date across the entire summer (Figure 4.5A). An interesting bimodal distribution appears when analyzing body size. All females regardless of generation, rearing substrate, or collection date appear to fall into one of two discrete categories of body size. However, all overwintering females sampled were found to belong to the larger of these two categories (Figure 4.5B).

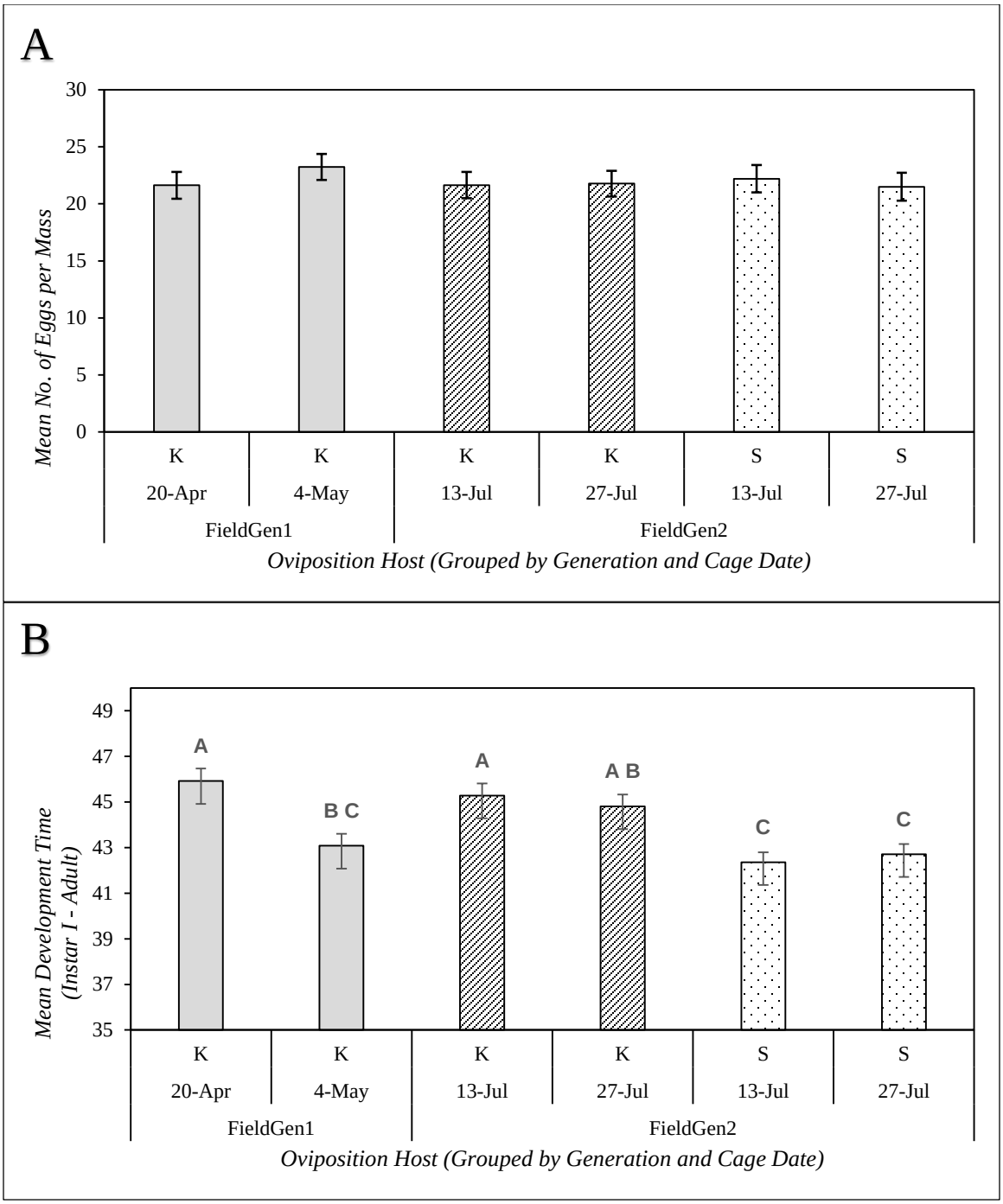

Figure 4.3 Development time and fecundity of *Megacopta cribraria* A: Mean (± SE) number of eggs per egg mass recorded on soybean (S) and kudzu (K). Host plant bars are grouped by egg mass cage date and further grouped by generational category (FieldGen1 or FieldGen2) **B:** Mean (± SE) development time from instar I to adult eclosion of *M. cribraria* on soybean (S) and kudzu (K). Host plant bars are grouped by egg mass cage date and further grouped by generational category ($FieldGen_1$ or $FieldGen_2$). Letters above bars indicate significantly different means as determined by post hoc Tukey's HSD test.

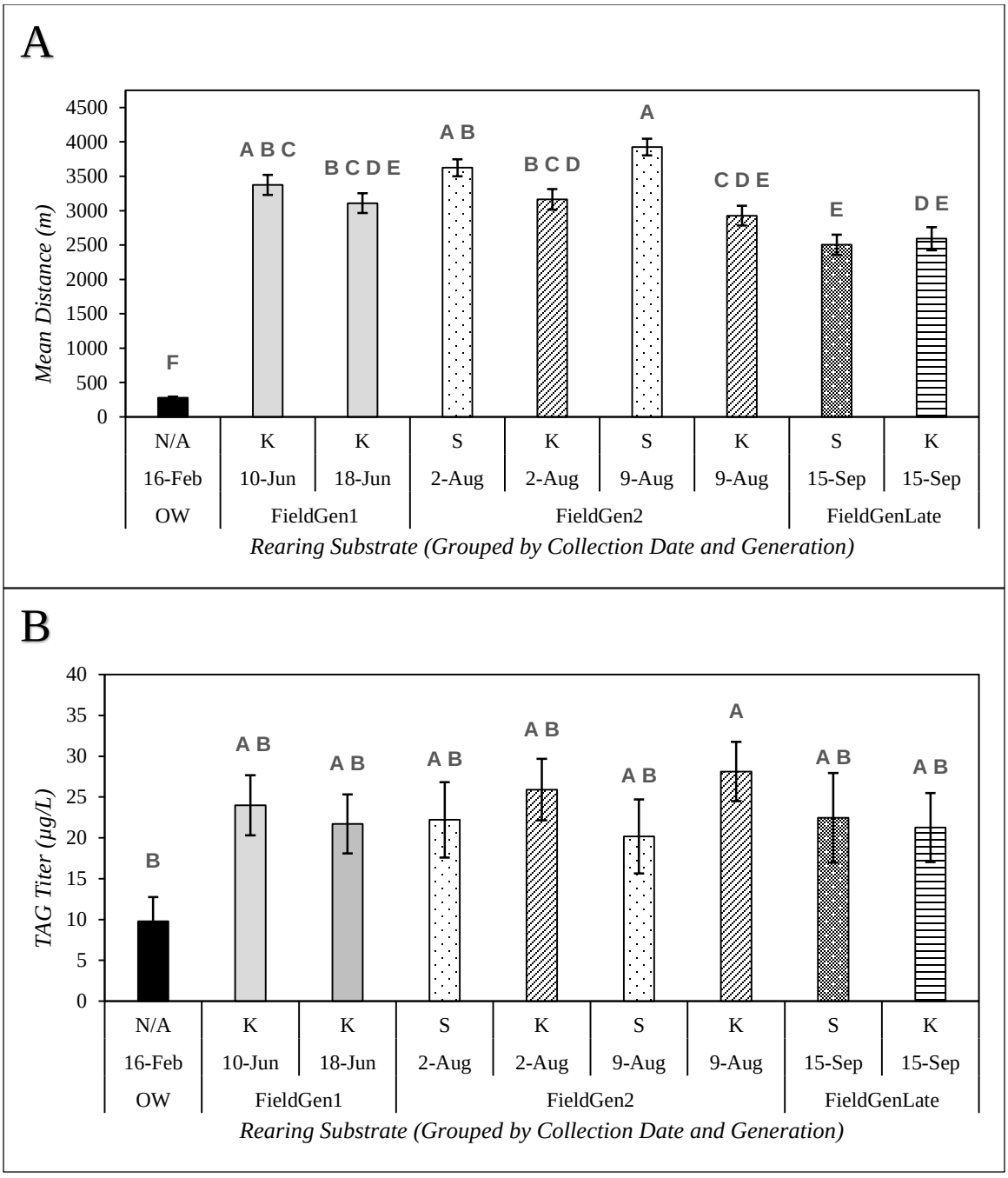

Figure 4.4 Flight ability and lipid energy stores of *Megacopta cribraria* A: Mean (± SE) flight distance of *M. cribraria* reared on soybean (S), kudzu (K), or overwintering. Host plant bars are grouped by collection date and further grouped by generational category (OW, $FieldGen_1$, or $FieldGen_2$). Letters above bars indicate significantly different means as determined by post hoc Tukey's HSD test. **B:** Mean (± SE) TAG titer of *M. cribraria* reared on soybean (S) kudzu (K), or overwintering (N/A). Host plant bars are grouped by collection date and further grouped by generational category (OW, $FieldGen_1$, or $FieldGen_2$). Letters above bars indicate significantly different means as determined by post hoc Tukey's HSD test

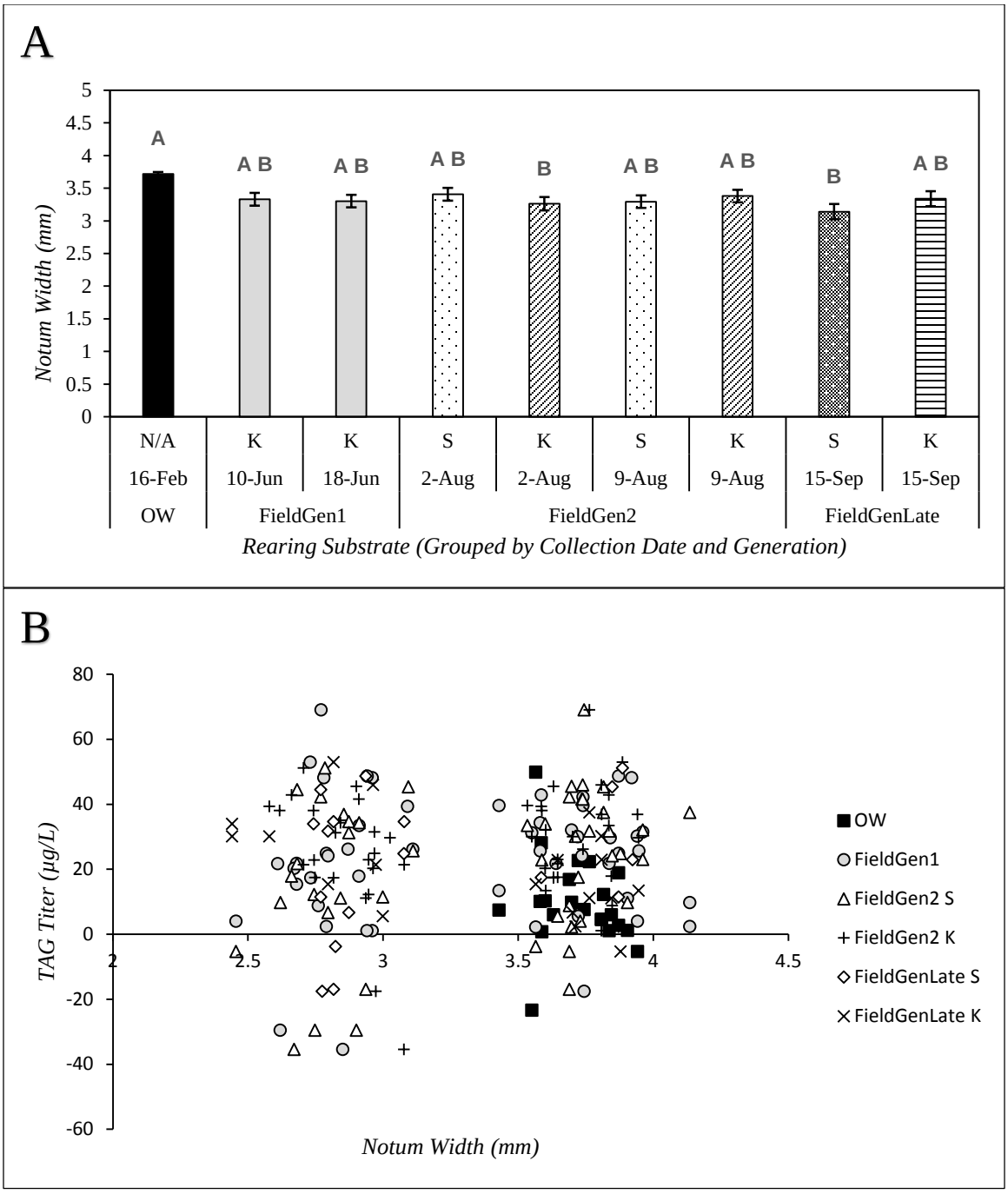

Figure 4.5 Body size and the relationship between body size and lipid energy stores of *Megacopta cribraria* A: Mean (± SE) notum width of *M. cribraria* reared on soybean (S), kudzu (K), or overwintering. Host plant bars are grouped by collection date and further grouped by generational category (OW, $FieldGen_1$, or $FieldGen_2$). Letters above bars indicate significantly different means as determined by post hoc Tukey's HSD test **B:** Notum width plotted against TAG titer of randomly selected females from all three generations (OW, $FieldGen_1$, and $FieldGen_2$). Females are subdivided by rearing substrate as indicated by figure legend.

Discussion:

The primary goal of this study was to maintain the central philosophy of experimental design used in chapter 3 while conducting trials in a natural setting in order to reduce artifacts of cage rearing and greenhouse plant cultivation on the behavior and development of *M. cribraria*. We hoped to better measure our chosen fitness paramters (development time, body size, lipid energy stores, and flight ability) to understand the relative fitness impacts of both soybean and kudzu. To that end, it appears we have achieved a degree of success in some areas, where performance of individuals was markedly different as a result of field rearing (e.g. flight ability and development time). However, other life history parameters measured (e.g. body size and triglyceride titer) demonstrated less clear or insignificant differences between generations or substrates that were measured during our greenhouse trials.

Development Time

Our results indicate that *M. cribraria* develop from first instar nymphs to adulthood in roughly the same amount of time across their annual period of activity, from emergence in spring to the beginning overwintering in fall. Eggs laid mid-spring to early summer on kudzu by females emerging from overwintering developed faster than eggs laid in the same field only two weeks prior. This is strange, because eggs laid by females of presumably the same overwintering generation laid eggs in the same field only two weeks earlier that developed the slowest of all kudzu oviposted cohorts measured, and in fact were took the longest to develop of all cohorts studied regardless of oviposition substrate. It is possible that this effect is due at least in part to differences in developmental temperatures following oviposition. This is corroborated by a recent study by Shi et al. (2014) that found that rearing *M. cribraria* at different constant temperatures demonstrated faster development through all nymphal stadia at higher temperatures

(mean=34.23 days at 29°C) than at low temperatures (mean=96.79 days at 17°C). Though recorded temperatures in the field regularly exceeded 29°C, our data did not reflect a similarly rapid development. We hypothesize that daily fluctutation of temperaure in the field rather than the constant conditions in the lab as well as other environmental factors (e.g. host quality and stress induced by crowding) may have led to this discrepancy.

Soybean reared individuals developed significantly faster than kudzu reared individuals in field trials. This result may be of some concern to growers as our findings indicate that soybean fields left untreated may produce more flight-ready adults that can disperse to adjacent soybean or other available plant resources. However, because the development time of *M. cribraria* appears to differ by approximately two days between host plants the realized developmental difference among cohorts is likely neglible given that progression from instar to instar generally takes seven to ten days on average.

It is also possible that this result is due to the some maternal effect similar to the one detected in our 2014 greenhouse trials. Our results indicate that egg masses laid by females that have emerged from overwintering are signaled by upstream mechanisms of their mother's phenotype and environment to develop more rapidly. This may result in several adaptive and evolutionary advantages. By signaling their eggs to develop faster, this may allow females to ensure that their offspring can more rapidly colonize available host plants and subsequently reproduce, increasing the overall fitness of their genotype through reinforcement of the phenotype. As an invasive pest, this could allow *M. cribraria* to have an advantage over other herbivores that feed on the same plants in the early spring and provide the foundation for its annual population explosion during the summer months, triggered by the process of overwintering as this effect is not seen in the development of kudzu bugs throughout the rest of

the season. This hypothesized maternal effect may be result of overwintering, but could also be mediated by environmental effects as well. It is reasonable to infer that eggs laid later on the same substrate and in the same patch of host plant are the result of females that have had a greater amount of time to feed and to mate. This could mean that the eggs that develop faster may do so as a result of having better fed mothers or mothers that have mated more frequently or have been more selective about choozing mates with a higher potential fitness benefit. These questions are still outside of the scope of our study, but in the future they could be addressed by paying much greater attention to this first generation. In particular, the overwintering females represent an area ripe for investigation. Collecting overwintering females throughout the winter and in the weeks leading to the natural spring emergence and analyzing genes linked to egg production and maturation as well as development could reveal important impacts of overwintering on the maternal genomics of *M. cribraria* and downstream impacts on the fitness of their offspring. Avenues for investigation include observation of mating behavior after overwintering and examination of male physiology and reproductive genetics. Genetic and bacterial assessment of first generation egg masses may provide similarly fruitful lines of inquiry for elucidating the relationship between the maternal phenotype and her environment on her offspring's fitness.

Flight Trials

Results of flight trials indicate that overwintering individuals appear to lack the ability to disperse as far as other individuals, at least for our chosen collection date. This is important in the context of management, because it suggests that indiciduals that overwinter will not be able to travel far from their refuge. Unfortunately this may not be indicative of the dispersal potential of adults naturally emerging from overwintering, as diapause is a complex and often lengthy

process. Its induction, intensity and termination are regulated by a variety of factors like photoperiod, temperature and humidity (e.g., Tanaka et al. 1987; Hodek and Hodková 1988; Nunes and Saunders 1999) meaning that our sample of individuals from February may not be represent the true ability of females emerging from overwintering in the spring. To remedy this, we recommend sampling with greater frequency in the weeks leading up to female emergence to better understand the flight ability of the overwintering cohort.

Both midsummer generations ($FieldGen_1$ and $FieldGen_2$) flew similar distances on the flight mill, however within each generation there were significant differences in flight ability depending on the host plant that females were raised on. Our data show that soybean reared individuals are capable of greater flight ability than kudzu reared individuals during the same time period. A study by Seiter et al. (2013) found that *M. cribraria* oviposition typically peaks in early August, while peak levels of adults occur in mid-late September. Thus it appears that the dispersal ability and reproductive potential of kudzu bug appear to coincide. This is valuable information for growers, as it may help to better delineate periods of peak activity and therefore better inform pesticide application schedules. Current IPM literature recommends a sampling threshold of only a single nymph per sweep to spray insecticide (Greene et al. 2012). With this low threshold and the propensity of kudzu bug to invade in high numbers with significant potential for damage, it is critical to provide relevant biological and phenological information to growers to avoid economically inefficient control practices. We therefore recommend that growers concentrate spray efforts after mid-Julyin order to eliminate both adults at the peak of their dispersal ability, and early nymphs recently emerged from egg masses in order to effect the greatest and most efficient control.

Triglyceride Lipid Stores and Body Size

In insects, lipids are stored in the fat body. Besides sequestering valuable energetic stores it is also the site of synthesis of hemolymph proteins, as well as facilitating the metabolism and storage of and glycogen (Beenakers et al 1985, Candy 1985). More than 90% of the lipid stored in the fat body is triacylglyceride (TAG) (Arrese and Wells 1997, Beenakers et al. 1985, Canavoso et al. 1998, Downer 1985). Insect flight muscle is the most metabolically active tissue known, but it is also incapable of storing significant amounts of lipid to sustain flight activity (Canavaso et al. 2001). To engage in flight insects have a complex hormone and enyzme mediated cascade is responsible for the mobilization of TAG from the fat body and converting it into usable fatty acids. TAG storage in the fat body is also a direct result of transfer of dietary fat from the midgut. Therefore, examining the link between available energy stores in the form of TAG in the fat body is an excellent means of assessing direct effects of impacts of host quality on the physiology and phenotype of *M. cribraria*, as well as getting at broader questions about downstream impacts on offspring fitness.

Our results show little difference between mean TAG titer between substrates, indicating that fat stores as a result of nutrition are not influenced differentially between the two hosts. However, an interesting inverse relationship between flight ability and TAG titer emerges when comparing $FieldGen_2$ females. Soybean reared $FieldGen_2$ females have a lower mean TAG titer than their kudzu raised counterparts, while simultaneously exhibiting greater flight ability. This seems counterintuitive as logic would indicate that having greater available TAG stores would correlate with greater flight ability. One potential explanation for this is that flight muscle has the capacity to use several fuel sources other than TAG. Trehalose, proline, and ketone bodies have been described as sources of energy for flight muscle (Candy et al. 1997). It is also possible that because the soybean reared individuals demonstrate greater flight ability that that

some portion of the hormone or enzyme pathway responsible for mobilizing TAG to diacylglyceride (DAG) for use in flight muscles is upregulated as a result of feeding on soybean. This in turn would result in measurably lower TAG titers in assay, but because of additional mobilized DAG in the hemolymph the insects would in fact have greater energy stores available already primed for use in flight actvity. To clarify this this we recommend that future investigations analyze not only TAG titer, but also DAG and perhaps any of the other aforementioned molecular sources of metabolic energy as well. Assay of Adipokinetic hormone (AKH), Octopamine, or TAG-lipase titers between different diets may also provide a clearer picture of what impact the diet has on the physiology and ultimately the fitness of *M. cribraria*.

Adult body size has significant consequences for fitness (Blanckenhorn 2000). In perhaps the most curious result of the our study is the emergence of a bimodal distribution in the body size of all females sampled, illustrared above (Figure 4.5B). Our data show that overwintering females invariably fell into the larger of the two tiers of body size recorded. This indicates that in order to successfully undergo overwintering kudzu bugs may have to attain a certain minimum body size. The adaptive significance of this is unclear. All kudzu bugs besides the overwintering females were of similar age and had been reared in otherwise identical conditions, eliminating our treatment or sampling regimen as a potential cause of this trend. Even more curious is the presence of this large bodied phenotype in early summer when there would be no impending threat of cold weather and the need to overwinter. It is possible that the large body phenotype within the popultion is conserved to serve as a 'safety net' phenotype in case of the onset of adverse conditions so that at least some portion of the population may enter dormancy to emerge once conditions are more favorable; this adaptation would be particularly helpful in temperature zones where late spring freezes are possible. This discovery could have

important implications on the annual phenology of kudzu bug and is worthy of further work with more care given to the life history and fitness traits of this purported distinct phenotype.

Conclusions:

Kudzu bug, like any invasive species is best managed by first understanding the basics of its biology and life history. In service of this goal, we investigated the impacts of feeding on soybean and kudzu on the realized fitness of *M. cribraria* across a typical annual active period. The findings of our 2015 study indicate that soybean and kudzu are relatively equal hosts for development and do not appear to significantly impact the development, fecundity, or dispersal ability of *M. cribraria*. This result is perhaps unsurprising as previous studies have indicated that kudzu bugs can survive and indeed thrive on both hosts. However, our study has brought about the discovery of important aspects of the life history of *M. cribraria*. Of principle importance is the difference in development time between soybean and kudzu. Though this difference is subtle, by our measures it is still significant indicating that feeding on soybean may provide some benefit to fitness over feeding on kudzu. Our findings also give insight into the dispersal ability of *M. cribraria* across its seasonal voltinism. In order for growers to make informed choices for control it is imperative to understand when and how quickly fields may be colonzied. Our study indicates that individuals reared on both plants show a greater ability to disperse as the summer goes on ($LabGen_2$), thus control efforts may need to be timed either effectively to eliminate early season bugs before they have a chance to disperse, or long enough after initial arrival in fields to avoid subsequent reinfestation from similarly dispersing populations. Moreover, because our results coincide with evidence from the literature (Seiter et al. 2013) indicating that kudzu bugs show greater reproductive potential during this same time

period it appears that the second annual generation exhibits the highest relative fitness making them also potentially the most destructive and invasive.

Further studies should investigate the underlying mechanisms of the fitness tradeoffs we have described. Our study has laid the foundation for continued work on kudzu bug, but there are still numerous avenues of investigation that could clarify the results we have presented. The work of Hosokawa et al. (2007) and Panizzi (1997) regarding gut symbionts of *M. cribraria* in the case of the former and the impacts of host switching in Hemiptera in the case of the latter provide an excellent basis future study in the context of the data we present here. We suggest targeting research on the genetic interactions between the gut biota of kudzu bugs and their environment, with specific attention given to their diet and annual generation. We are also curious about the adaptive significance of the appearance of two distinct body sizes for females and whether if as indiciated by our study attaining a sufficient body size is needed for *M. cribraria* to successfully complete overwintering.

Since its intital discovery in 2009, *M. cribraria* spread through the American southeast at a breakneck pace and left researchers and growers alike racing to understand its biology to curb the effects of its intrusion. It has presented itself first as a nuisance, but in the years since it has grown exponentially in population and now represents a significant threat as an economically injurious pest. We therefore present this new information in the hope that it may aid any person or entity impacted by the kudzu bug and ultimately become part of the literature leading to its successful management in the United States.

CHAPTER 5

CONCLUSIONS AND FUTURE DIRECTIONS

Our results indicate that soybean and kudzu are nearly equal hosts for the development and dispersal of *Megacopta cribraria* as measured by the fitness parameters measured in both the greenhouse and field components of this study. However, we have also demonstrated a number of interesting aspects of the insect's life history including a more nuanced understanding of its biology, ecology, and phenology across a typical annual active period than has been previously reported in the literature.

In 2014 greenhouse trials we demonstrated that maternal oviposition choice has a significant downstream impact on their offspring's choice of host plant regardless of the host plant used for rearing from nymph to adult stage. Specifically, we documented that soybean seems to be the preferred host under our experimental conditions, and this choice is somehow reinforced through a hypothesized maternal effect to signal successive generations to preferentially choose this host as well. This effect could possibly be a result of the documented benefits of host switching, but could also be an artifact of our experimental design only using a single soybean cultivar. Current work being undertaken by researchers at North Carolina State University indicates that certain cultivars of soybean are preferred by *M. cribraria* over others for oviposition (Del Pozo-Valdivia *unpublished data*). We therefore recommend future studies undertaken with a similar methodology to provide multiple soybean cultivars to clarify this effect. Our study has also neglected to quantify host quality of the plants used or to investigate

epigenetic effects associated with different diets. Future studies should investigate quantitative metrics of host plant quality (e.g. sugar and amino acid content) as well as target the gut biota of *M. cribraria* as a site of potential genetic crosstalk.

Other results seen in greenhouse trials are less clear. With near uniformity, wild caught individuals showed greater fitness than the generations reared in culture. Wild caught individuals were all collected from the same kudzu patch and so it is possible that the population sampled for our study was just particularly fit. However, we also believe that our greenhouse trials suffered from a lack of a defined protocol for rearing and maintain an experimental colony of *M. cribraria.* In personal communications with several researchers from institutions across the southeast, it is clear that kudzu bugs are tricky to maintain in colony and to our knowledge no other study has ever successfully maintained a colony past two generations. The underperformance of our experimentally reared populations in comparison to the wild caught cohort leads us to believe that there is some essential component of the insect's biology that has not yet been reproduced in a lab setting. A likely avenue for investigation is facilitated diapause to signal second generation females to pause reproductive activity before resuming again and continuing with reproduction. This would likely require an entire study of its own to optimize temperatures, photoperiod cues, and timing to properly induce overwintering without negatively impacting reproductive potential.

Our 2015 field trials similarly resulted in new and interesting data concerning the life history of *M. cribraria*. Again soybean and kudzu reared individuals performed similarly across the fitness metrics we measured, however soybean reared individuals had a greater dispersal ability than their kudzu reared counterparts. This result may be concerning to growers as it means insects that successfully develop on soybean may be more able to disperse and in the

context of our greenhouse trials may in turn prefer soybean as an oviposition site. In order to clarify this result we recommend future studies submit field reared soybean individuals to lab host preference choice trials to see if this effect is conserved. Periods of greatest dispersal ability during early August in our studies coincide with periods of greatest reproductive potential in other published studies (Seiter et al. 2013), so we conclude that this population peak may represent the period of greatest risk to growers and therefore should be investigated more closely when drafting recommendations for mid- and late-summer management of kudzu bug.

Overwintering individuals performed worse in almost all fitness metrics measured except for body size, where they were significantly larger than all other individuals. Our results reveal that body size has a large impact on the fitness of *M. cribraria*. In both 2014 greenhouse trials and 2015 field trials larger individuals correlated with greater potential fecundity and increased flight ability. Most curious is that in both cases the first generation chosen for each study (WC in 2014 and OW in 2015) exhibited similarly large body size that was also significantly different from all other individuals sampled. Presumably both populations were of the same annual generation, as we have established that the overwintering generation comes from the previous year's second generation and emerges in the spring during the same time period that our wild caught individuals were collected. We therefore hypothesize that large body size is a requirement for successful overwintering and may have important implications for the phenology and biology of *M. cribraria*. Future studies should investigate the role that body size has on other fitness parameters in order to better understand the relationship between these characteristics.

In conclusion, the findings of our study demonstrate important aspects of the life history and biology of *Megacopta cribraria* in its newly established range throughout the United States southeast. Since 2009, this insect has transformed in significance from a minor nuisance to a

serious threat. By devoting time to understanding as many aspects of its biology as possible we are able to make more informed decisions leading to greater efficiency in its management and a greater quality of life for those impacted by its invasion. We hope that the knowledge we have assembled here may contribute to the growing body of literature on the insect, and that it may aid in continued successful management in both agricultural and urban contexts.

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APPENDIX– PYTHON CODE FOR FLIGHT DATA ACQUISITION AND ANALYSIS

Standardize Peaks – Script for standardizing raw data input from DATAQ data loggers

standardize_peaks.py

```
def peak_standardization(colum):
    format_colum = []
    new_list=[]
    peaks=[]

    for i in range(0, len(colum)):
        format_colum.append(round(colum[i], 2))

    min_val=round((sum(format_colum)/len(format_colum)) - 0.01, 2)
    max_val=round((sum(format_colum)/len(format_colum)) + 0.02, 2)
    for ii in range(0, len(format_colum)):
        x=(format_colum[ii]-min_val)/(max_val-min_val)
        if x > 1:
            new_list.append(1)
        else:
            new_list.append(0)
```

```
    for iii in range(0, len(new_list)-1):
        if new_list[iii] > new_list[iii-1] and new_list[iii] >= new_list[iii+1]:
            peaks.append(1)
        else:
            peaks.append(0)
    peaks.append(0)


    return peaks
filename = input("File path or file name -> ")
InputFile = open("C:\\Users\\Moorelab\\Desktop\\Flight\\" + filename, "r")
Lines = InputFile.readlines()
time_colum = []
first_colum = []
second_colum = []
third_colum = []
fourth_colum = []
fifth_colum = []
sixth_colum = []
seventh_colum = []
eighth_colum = []
for i in range(0, len(Lines)):
    raw = Lines[i]
```

```
    a,b,c,d,e,f,g,h,j = raw.split(",") # if > 5 channels then a,b,c,d,e,f,g,h,j
    time_colum.append(float(a))
    first_colum.append(float(b))
    second_colum.append(float(c))
    third_colum.append(float(d))
    fourth_colum.append(float(e))
    fifth_colum.append(float(f))
    sixth_colum.append(float(g))
    seventh_colum.append(float(h))
    eighth_colum.append(float(j))

InputFile.close()

first_colum = peak_standardization(first_colum)
second_colum = peak_standardization(second_colum)
third_colum = peak_standardization(third_colum)
fourth_colum = peak_standardization(fourth_colum)
fifth_colum = peak_standardization(fifth_colum)
sixth_colum = peak_standardization(sixth_colum)
seventh_colum = peak_standardization(seventh_colum)
eighth_colum = peak_standardization(eighth_colum)
OutputFile = open("C:\\Users\\MooreLab\\Desktop\\Flight\\peaks_standardized.txt","w")
for i in range(0, len(Lines)):
```

```
    OutputFile.write('%.1f' % time_colum[i] + ", " + '%.2f' % first_colum[i] + ", " + '%.2f' %
second_colum[i]
                + ", " + '%.2f' % third_colum[i] + ", " +  '%.2f' % fourth_colum[i] + ", "
                + '%.2f' % fifth_colum[i] + ", " + '%.2f' % sixth_colum[i] + ", "
                + '%.2f' % seventh_colum[i] + ", " + '%.2f' % eighth_colum[i] +"\n")
OutputFile.close()
```

Flight Analysis – Script for converting standardized data into interpretable flight characters

flight_analysis.py

```
def time_list(time, channel):
    time_channel=[]
    for i in range(0, len(channel)):
        if float(channel[i]) == 1.00:
            time_channel.append(float(time[i]))

    return time_channel
def speed_list(time, ch_number):
    ch = str(ch_number)
    speed_t=0
    speed_channel=[]
    speed_channel.append(0)
    if len(time) > 0:
        if len(time) > 2:
            for i in range(1, len(time)):
```

```
            if float(time[i]) != float(time[i-1]):
                speed_t = 1.0996/(float(time[i]) - float(time[i-1]))
                speed_channel.append(float(speed_t))
            else:
                speed_channel.append(10)
    for x in range(0, len(speed_channel)):
            if float(speed_channel[x]) < 0.15:
              speed_channel[x] = 0


        else:
            print ("Channel ",ch, "has only one peak - impossible to calculate motion stats")
    else:
        print ("Channel ",ch, "is empty")


    return speed_channel
def distance(time, speed):
    distance=0
    average_speed=0
    time_new=[]
    speed_new=[]
    time_new_new=[]
    speed_new_new=[]
    if len(time) > 2:
```

```
        for i in range(1, len(speed)):
            if float(speed[i]) > 0 and float(speed[i]) < 1.8:
                time_new.append(float(time[i]))
                speed_new.append(float(speed[i]))
                distance += 1.0996
        if len(time_new) > 2:
            time_new_new.append(time_new[0])
            speed_new_new.append(speed_new[0])
            for ii in range(0, len(time_new)-1):
                if float(time_new[ii+1]) <= float(time_new[ii]) + 7:
                    time_new_new.append(time_new[ii+1])
                    speed_new_new.append(speed_new[ii+1])
            average_speed = sum(speed_new_new)/len(speed_new_new)
        else:
            print('Cannot calculate distance and average speed')
    else:
        print('Cannot calculate distance and average speed')
    return (time_new_new, speed_new_new, distance, average_speed)
def flying_bouts(time, speed, ch):
    t_odd = []
    t_even = []
    tot_t = []
    last_time = 0
```

```
diff = 0

flight_time = 0

longest_bout = 0

shortest_bout = 0

to_remove=[]

bout_time = []

fly_time = 0

flight_60_300 = []

sum_60_300 = 0

flight_300_900 = []

sum_300_900 = 0

flight_900_3600 = []

sum_900_3600 = 0

flight_3600_4500 = []

sum_3600_4500 = 0

flight_4500 = []

sum_4500 = 0

events_300 = 0

events_900 = 0

events_3600 = 0

events_4500 = 0

events_more_4500 = 0

if len(time) > 2:
```

```
        if float(time[1]) < float(time[0]) + 20:
            bout_time.append(time[0])
for i in range(0, len(time)-1):
            if float(time[i+1]) >= float(time[i]) + 20:
                bout_time.append(time[i])
                bout_time.append(time[i+1])
        if bout_time[-1] != time[-1]:
            bout_time.append(time[-1])
  for iii in range(1, len(bout_time)):
            if float(bout_time[iii]) == float(bout_time[iii-1]):
                to_remove.append(bout_time[iii])


        for iiii in range(0, len(to_remove)):
            while to_remove[iiii] in bout_time:
                bout_time.remove(to_remove[iiii])
if len(bout_time)%2 != 0:
            last_time = float(bout_time[-1])
            del bout_time[-1]
        t_odd = bout_time[0::2]
        t_even = bout_time[1::2]
        for ii in range(0, len(t_odd)):
            diff = float(t_even[ii]) - float(t_odd[ii])
            tot_t.append(diff)
```

```
if float(last_time) != 0:
    diff = float(last_time) - float(t_even[-1])
    tot_t.append(diff)
flight_time = sum(float(i) for i in tot_t)
for index in range(0, len(tot_t)):
    if float(tot_t[index])>60 and float(tot_t[index])<=300:
        flight_60_300.append(float(tot_t[index])/flight_time)
    elif float(tot_t[index])>300 and float(tot_t[index])<=900:
        flight_300_900.append(float(tot_t[index])/flight_time)
    elif float(tot_t[index])>900 and float(tot_t[index])<=3600:
        flight_900_3600.append(float(tot_t[index])/flight_time)
    elif float(tot_t[index])>3600 and float(tot_t[index])<=4500:
        flight_3600_4500.append(float(tot_t[index])/flight_time)
    elif float(tot_t[index])>4500:
        flight_4500.append(float(tot_t[index])/flight_time)
if len(flight_60_300) > 0:
    sum_60_300=sum(float(a) for a in flight_60_300)
    shortest_bout = float(min(flight_60_300))*flight_time
if len(flight_300_900) > 0:
    sum_300_900=sum(float(b) for b in flight_300_900)
if len(flight_900_3600) > 0:
    sum_900_3600=sum(float(c) for c in flight_900_3600)
if len(flight_3600_4500) > 0:
```

```
            sum_3600_4500=sum(float(d) for d in flight_3600_4500)
        if len(flight_4500) > 0:
            sum_4500=sum(float(e) for e in flight_4500)
        longest_bout = max(tot_t)
        fly_time=flight_time/5062
        events_300=len(flight_60_300)
        events_900=len(flight_300_900)
        events_3600=len(flight_900_3600)
        events_4500=len(flight_3600_4500)
        events_more_4500=len(flight_4500)
    else:
        print('Channel', ch, 'has only one peak - cannot perform calculation')

    return (flight_time, shortest_bout, longest_bout, fly_time, sum_60_300, sum_300_900,
sum_900_3600, sum_3600_4500, sum_4500, events_300, events_900, events_3600,
events_4500, events_more_4500)
def graph(time, speed):
    time_new=[]
    speed_new=[]
    x=0
    y=0
    for i in range(0, len(time)-1):
        if float(time[i+1]) > float(time[i]) + 20:
```

```
            time_new.append(time[i])
            speed_new.append(speed[i])
            x=float(time[i]) + 1
            time_new.append(x)
            speed_new.append(0)
            y=float(time[i+1]) -1
            time_new.append(y)
            speed_new.append(0)
        else:
            time_new.append(time[i])
            speed_new.append(speed[i])
    time_new.append(0)
    speed_new.append(0)
    return time_new, speed_new


def cls(): print ("\n" * 100)


cls()
filename = input('File path or file name -> ')
input_file = open("C:\\Users\\Moorelab\\Desktop\\Flight\\" + filename, "r")
data_list = list(input_file)
time_column = []
list_dict=dict()
```

```
peaks1 = []

peaks2 = []

peaks3 = []

peaks4 = []

peaks5 = []

peaks6 = []

peaks7 = []

peaks8 = []


for i in range(0, len(data_list)):

    raw = data_list[i]

    a,b,c,d,e,f,g,h,j = raw.split(",")

    time_column.append(a)

    peaks1.append(b)

    peaks2.append(c)

    peaks3.append(d)

    peaks4.append(e)

    peaks5.append(f)

    peaks6.append(g)

    peaks7.append(h)

    peaks8.append(j)


list_dict[1]=peaks1
```

```
list_dict[2]=peaks2

list_dict[3]=peaks3

list_dict[4]=peaks4

list_dict[5]=peaks5

list_dict[6]=peaks6

list_dict[7]=peaks7

list_dict[8]=peaks8


input_file.close()


for i in range(1, len(list_dict)+1):

    print('CHANNEL ' + str(i) + ' ----------------------------------------')

    time_channel = time_list(time_column, list_dict[i])

    speed_channel = speed_list(time_channel, i)

    time_n, speed_n, dist, av_speed = distance(time_channel, speed_channel)

    fly_time, short_bout, long_bout, flight, fly_to_300, fly_to_900, fly_to_3600, fly_to_4500,
fly_more_4500, event_300, event_900, event_3600, event_4500, event_more_4500 =
flying_bouts(time_n, speed_n, i)

    print('Average speed channel ' + str(i) + ' -> ' + '%.2f' % av_speed)

    print('Total flight time channel ' + str(i) + ' -> ' + '%.2f' % fly_time)

    print('Distance channel ' + str(i) + ' -> ' + '%.2f' % dist)

    print('Shortest flying bout channel ' + str(i) + ' -> ' + '%.2f' % short_bout)

    print('Longest flying bout channel ' + str(i) + ' -> ' + '%.2f' %long_bout)
```

```
print('This individual spent ' + '%.3f' %flight + ' of its time flying with this composition: ')
print('  60s-300s = ' + '%.3f' %fly_to_300 + ' with ',event_300, 'events')
print('  300s-900s = ' + '%.3f' %fly_to_900 + ' with ',event_900, 'events')
print('  900s-3600s = ' + '%.3f' %fly_to_3600 + ' with ',event_3600, 'events')
print('  3600s-4500s = ' + '%.3f' %fly_to_4500 + ' with ',event_4500, 'events')
print('  4500s = ' + '%.3f' %fly_more_4500 + ' with ',event_more_4500, 'events')
print('\n')
time_graph, speed_graph = graph(time_n, speed_n)
OutputFile=open('C:\\Users\\Moorelab\\Desktop\\Flight\\'+str(i)+'.DAT', "w")
for index in range(0, len(time_graph)):
    OutputFile.write('%.1f' % time_graph[index] + ',' + '%.2f' %speed_graph[index] + '\n')
OutputFile.close()
```