Nutritional Ecology of *Pseudacteon* species (Diptera: Phoridae) and Its Impact on Their Fitness

by

Olufemi Seyi Ajayi

A thesis submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Auburn, Alabama
August 01, 2015

Keywords: *Pseudacteon* spp., *Solenopsis* spp., sugars, lipid, longevity, ovigeny index

Approved by

Henry Fadamiro, Chair, Professor and Assistant Dean & Director of Global Programs
Nannan Liu, Department Chair and Endowed Professor
Lawrence Graham, Entomology Research Fellow IV

Abstract

Natural enemies, such as *Pseudacteon* parasitic flies, have the potential to provide

sustainable suppression of imported fire ants, *Solenopsis* species. Multiple releases of various

species of these flies have been made across the Southeastern United States for biological control

of imported fire ants. Five species (*P. cultellatus*, *P. curvatus*, *P. litoralis*, *P. obtusus*, and *P.

tricuspis*) have been released in Alabama, and *P. curvatus* is one of the established species in the

state. Much research is being conducted on these flies, but very little is known about some aspects

of their nutritional ecology and life-history strategies, which are essential for conservation

biological control. The goal of the study was to address some important questions related to the

nutritional ecology and life-history strategies of *Pseudacteon* phorid flies.

In chapter II, a series of biochemical tests (i.e. anthrone tests) were used to test if *P.

curvatus* utilizes sugar and lipid sources in the field, by comparing the nutrient levels in flies from

three treatments: i) field-collected, ii) sugar-fed laboratory-reared (positive control), and iii) sugar-

starved laboratory-reared (negative control) flies. Field-collected flies showed similar levels of gut

sugars, body sugars and glycogen as sugar-fed laboratory-reared flies. When compared with sugar-

starved laboratory-reared flies, field-collected flies showed marginal (but insignificant) increase in

levels of gut sugars, body sugars and glycogen. Field-collected flies showed significantly higher

lipid levels than both sugar-starved and sugar-fed laboratory-reared flies. The ecological

significance and practical implications of these results are discussed.

In chapter III, the effects of sugar feeding on the longevity of three phorid fly species, *P.

cultellatus, P. curvatus*, and *P. obtusus*, were investigated. The timing of egg maturation and

ovigeny of *P. obtusus* was also determined. The results showed significant and positive effect of sugar feeding on the longevity of all three species when compared with sugar-starved individuals. Comparing species, the largest-sized species, *P. obtusus*, lived the longest, with almost twice the lifespan of the smallest species, *P. cultellatus*. The ovaries of all specimens of newly emerged *P. obtusus* analyzed by light microscopy showed only post-vitellogenic oocytes, suggestive of a pro-ovigenic life history strategy. The ecological significance and practical implications of these results are discussed.

Acknowledgements

I want to thank my major professor and committee chair, Dr. Henry Fadamiro, for giving me the opportunity to work on this interesting project. I continue to feel blessed by the multitude of opportunities he has provided me throughout the years. His patience, understanding, support and invaluable advisory role kept me going through the challenging phases of this study. He took a step further from being an advisor to becoming a mentor. Special thanks are also offered to my committee members: Nannan Liu and Fudd Graham. I feel blessed to have had the opportunity to get to work with you, and for your readiness in offering support any time I needed it.

When I came to Auburn, it was my first time of coming to the United States but I give much thanks to my major professor and Tolulope Morawo who made me settle down pretty quickly. I thank Amy Bass at the USDA-APHIS-PPQ-CPHST Laboratory/Florida DPI, Gainesville, FL, USA who provided the *Pseudacteon* flies utilized for this work. Thank you to Dr. Esther Ngumbi for working with me in the rearing and separation of the flies. Dr. Rammohan Balusu was very helpful in getting me familiarized with the use of different materials in the laboratory. This helped me to quickly start the research work in my first semester. Dr. Simon Zebelo was very helpful when I was conducting the body nutrient analysis experiments, my sincere thanks to him. Many thanks to Kelly Palmer for her invaluable assistance with field collection of phorid flies. Special thanks to Drs. Michael Miller and Roland Dute for their assistance with light microscopy part of this work. I also thank Dee Fowler, Tolulope Morawo and Dr. Joseph Kloepper for editorial assistance with this thesis. Sincere thanks go out to Dr. Rammohan Balusu, Dr. Simon Zebelo, Joseph Disi and Tolulope Morawo for their technical assistance in Microsoft Office and statistics. Thanks to other

Table of Contents

List of tables

Chapter 3

List of figures

Chapter 2.

Chapter 3.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1   Fire ants

Imported fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae), are invasive pests that

are native to the flood plains of Argentina and Brazil in South America (Vander Meer & Lofgren

1990). Both the black imported fire ant, *Solenopsis richteri* Forel, and the red imported fire ant,

Solenopsis invicta Buren, were accidentally introduced into the United States. The black

imported fire ant was first identified in the United States in 1929 (Löding 1929) and is thought to

have been introduced through the port of Mobile, Alabama, around 1918 (Creighton 1930). The

red imported fire ant was first discovered in Mobile, Alabama, in the 1930s (Vinson 1997). The

two fire ant species do not readily hybridize in their native South America, likely due to limited

geographical overlap between the species (Tshinkel 2006). However, the species are in continual

contact with one another in the U.S., which allows for frequent hybridization (Palmer 2014).

Like many other non-native insect species that are liberated from the regulatory effects of natural

enemies in their native range, imported fire ants pose a significant ecological, economic and

medical threat to invaded areas (Lofgren 1986, Porter et al. 1992).

Since their introductions, the populations of the two species have undergone an explosive

dispersal and range expansion across the gulf-coast and eastern seaboard states of the U.S.

Imported fire ants continue to spread approximately 2.4 million ha per year (Callcott 2002).

Today, approximately 172 million ha of the southeastern U.S. have been infested by these

species, including much of Texas, and parts of Oklahoma, New Mexico and California (Imported Fire Ant eXtension Community of Practice 2014).

Populations of imported fire ants pose serious health and economic risks in areas where they are established. Fire ants cause approximately $7 billion in loss in the United States each year (Lard et al. 2001). These costs are associated with damage, repair and control of the invasive pests. Damage is known to occur to electrical equipment (Drees 1998), agricultural equipment such as hay cutters (Lard et al. 2006), and to turf with the general upheaval of dirt caused by the creation of the fire ant's mound (Lard et al. 2006). In addition, approximately 1% of human population in the United States is highly allergic to fire ant venom and could potentially die when stung (Adams 1968). Fire ants are known to be highly aggressive and will defend their colony by biting and stinging intruders when their mound is disturbed (Tshinkel 2006). This is a successful way for the ants to defend their colony against predators; however, this defensive mechanism can be quite painful and even deadly when disturbed by humans. This makes fire ant control especially important in sensitive environments such as hospitals and schoolyards (Palmer 2014).

1.2   *Pseudacteon* **phorid flies**

Many species of parasitic phorid flies (*Pseudacteon* spp.; Diptera: Phoridae) parasitize workers of *Solenopsis saevissima* complex fire ants (including *S. invicta* and *S. richteri*) throughout their native South American range (Folgarait et al. 2000, Calceterra et al. 2005). Twenty three species of *Pseudacteon* are known as parasitoids of fire ants (LeBrun et al. 2012b). These parasitic flies can be distinguished by the morphology of their ovipositors (Porter and Pesquero 2001). As a part of an integrated pest management strategy to control imported fire ants

in the southeastern U.S., a biological control program has been implemented that includes *Pseudacteon* decapitating flies.

Phorid flies respond to pheromones that imported fire ants emit during foraging, mound disturbance, or mating flights (van der Meer and Porter 2002, Morrison and King 2004, Chen and Fadamiro 2007). Thus, the flies become attracted to active imported fire ant workers. Female phorids then inject a single egg into the thorax of imported fire ant workers (Morrison et al. 1997). Neonate larvae travel into the head capsule of the ant where they develop for a period of 2-3 weeks (Consoli et al. 2001, Porter and Pesquero 2001). Before pupation occurs, the intersegmental membranes between the head and thorax of the host are chemically dissolved and the ant head capsule becomes separated from the body. The decapitated head serves as a puparium for the developing fly (Puckett 2008).

Host specificity is of major concern, as in any natural enemy used in classical biological control (Simberloff and Stiling 1996). A number of native species of fire ants exist in North America such as *S. geminata* Forel and *S. xyloni* McCook, as well as native ants of other genera. Several studies have investigated the host specificity of *Pseudacteon* phorid flies that parasitize *Solenopsis saevissima* complex of fire ants (Porter et al. 1995b, Porter 1998b, Gilbert and Morrison 1997, Morrison and Gilbert 1999, Estrada et al. 2006, Porter and Alonso 1999). These tests all conclude that *Pseudacteon* phorid flies are very host specific. Although there may be some concern about non-target effects, such effects are rare and would be unlikely to occur in a field setting. These reports show that there is little reason to expect any host shift. In addition to host specificity, Porter and Gilbert (2004) investigated the possibility of the flies becoming a nuisance. They found no significant attraction to any food or moist tissues and noted that the flies were never attracted to human faces or hands, thereby showing no indication of becoming

nuisances to people (Barrow 2014).

These flies are thus considered potential candidates for classical biological control of imported fire ants and are being released in the United States (Porter 1998a, Graham et al. 2003, Vogt and Street 2003, Porter et al. 2004). Thus far, five species have been released in Alabama: *P. tricuspis*, *P. curvatus*, *P. litoralis*, *P. obtusus* and *P. cultellatus* (Imported Fire Ant eXtension Community of Practice 2015). These parasitoids vary in size: *P. cultellatus*, a small species; *P. curvatus*, also a small species and *P. obtusus*, a medium-large species (Porter and Calcaterra 2012). Recent surveys for the establishment of these introduced species show establishment of *P. curvatus*, *P. litoralis* and *P. tricuspis* in Alabama (Imported Fire Ant eXtension Community of Practice 2015).

Mortality of imported fire ants due to parasitization is low, only 1-3% in the field (Morrison et al.1997, Morrison and Porter 2005a). However, phorid flies have a greater indirect effect on imported fire ants rather than a direct effect of population reduction. Imported fire ants have developed multiple defensive responses to the attack of phorid flies. Ants exhibit reduced foraging (Feener 1981, Folgarait and Gilbert 1999) and mound rebuilding and often freeze in place to avoid parasitization (Porter and Gilbert 2004), all of which result in colonies being less efficient at bringing in resources. This weakens the colonies and allows native ants to compete for food more effectively (Mehdiabadi and Gilbert 2002). These indirect effects together with direct impacts may result in population-wide suppression of imported fire ants in the U. S. (Morrison et al. 1997).

1.3   Parasitoid nutrition and its benefits

Many adult insect parasitoids are obligate consumers of plant-derived foods, including sugar-rich solutions such as nectar and homopteran honeydew (Wäckers 2005). Parasitoids derive multiple benefits from sugar sources. Parasitoids use sugar sources to cover energetic needs (Jervis et al 1993, Steppuhn and Wäckers 2004). Sugar feeding may enhance the longevity and fecundity of parasitoids (e.g., Heimpel et al. 1997, Lewis et al. 1998, Wäckers 2001, Costamagna and Landis 2004). In laboratory studies, sugar feeding significantly increases the lifespan of *P. tricuspis* (Fadamiro et al. 2005, Chen et al. 2005, Fadamiro and Chen 2005, Chen and Fadamiro 2006). However, the ability to utilize sugar does vary among species of the same genus (Ferreira et al. 1998, Wäckers 2001), and this may be the case in *Pseudacteon* species. Carbohydrate consumption also affects other aspects of parasitoid reproductive success such as egg maturation (Azzouz et al 2004). These effects show that availability of sugar sources can potentially be important in the fitness of parasitoids in host suppression.

The extent to which parasitoids benefit from dietary sources such as sugars partly depends upon the degree of provisioning of oocytes in teneral adults (O'Brien et al. 2004) which is correlated with the ovigeny index, defined as the proportion of the potential lifetime egg complement that is mature upon adult emergence (Jervis et al. 2005a, Bernstein and Jervis 2008). Species that have all of this complement mature upon emergence are termed 'pro-ovigenic', while those that do not are termed 'synovigenic' (Jervis et al. 2001). A synovigenic female parasitoid stands to gain future oviposition opportunities from dietary sources, due to the potentially positive effects the dietary nutrients will have on her life expectancy and on her rate of egg manufacture (Bernstein and Jervis 2008). On the contrary, a pro-ovigenic parasitoid rarely manufactures more eggs after eclosion, therefore benefiting from dietary nutrients only through

the potential increment in her life span. The state of egg maturation of a number of *Pseudacteon* species have been reported on (Wasmann 1918, Zacaro and Porter 1997, Cônsoli et al. 2001, Zacaro and Porter 2003). However, there is no report about the egg maturation state in newly emerged *Pseudacteon* parasitic flies.

Floral nectar, extra-floral nectar, and honeydew are widely available in the field and regularly exploited by parasitoids (Elliott et al. 1987, Idoine and Ferro 1988, Gilbert and Jervis 1998, Jervis 1998, Singh et al. 2000, Heimpel et al. 2004, Lee et al. 2006). The suitability of food depends on species-specific degrees of gustatory acceptance and metabolization of consumed compounds (Ferreira et al. 1998, Baggen et al. 1999, Olson et al. 2000, Hausmann et al. 2005, Wäckers 2005, Winkler et al. 2005, Chen and Fadamiro 2006, Wyckhuys et al. 2008).

Sugar feeding substantially affects the levels of body sugar, glycogen, and lipid parasitoids (Olson et al. 2000, Lee et al. 2004, Fadamiro and Chen 2005, Chen and Fadamiro 2006). In contrast to lipids, which apparently cannot be synthesized by Hymenoptera (Giron and Casas 2003), carbohydrate (sugar and glycogen) levels consistently increase upon consumption of sugar-rich foods and are considered indicative of parasitoid nutritional status (Fadamiro and Heimpel 2001, Lee et al. 2004). As fructose levels are typically very low in unfed insects, this sugar has clear potential value as a marker for sugar feeding (van Handel 1984, Olson et al. 2000). Certain biochemical assays, e.g., anthrone tests, have been developed to characterize carbohydrate metabolism in insects (Jervis et al. 1992, Heimpel et al. 2004a, Fadamiro et al. 2005, Wyckhuys et al. 2008).

1.4   Justification of the study

In the past few decades, studies on natural history of *Pseudacteon* parasitic flies have received significant attention. *Pseudacteon* parasitic flies are promising biocontrol agents of imported fire ants that can be incorporated into Integrated Pest Management (IPM) programs. Similar to parasitoid wasps, parasitoid flies rely largely on non-host foods such as sugar-rich foods to meet their energetic needs, increase female life expectancy, and increase dispersal capacity (Bernstein and Jervis 2008). However, very little is known about some aspects of the nutritional ecology and life-history strategies of *Pseudacteon* parasitic flies, which are essential for conservation biological control. In particular, the following questions still remain unanswered: i) do *Pseudacteon* parasitic flies utilize sugar and lipid sources in the field? ii) what role does sugar feeding play in enhancing their fitness? To address these questions, the present study investigated the utilization of sugar and lipid sources in the field and the effects of sugar feeding on the fitness, timing of egg maturation and ovigeny of phorid flies.

With relatively few studies on the nutritional ecology and life-history strategies of *Pseudacteon* parasitic flies, further studies are required to confirm the hypothesis that these parasitic flies rely on sugar sources in the field to enhance their fitness to suppress the imported fire ant pests. Studies comparing the relative suitability and utilization of sugar sources by parasitoids have yielded different results for different species, even species within the same genus. This may be the case in *Pseudacteon* species. These studies may provide insights regarding the ecological and practical implications of sugar sources on the life-history strategies of *Pseudacteon* parasitic flies. The present study is expected to be applied in biological control programs in many agro-ecosystems in the form of habitat management.

Within the context of classical biological control, an in-depth assessment is needed to

determine the effects of non-host foods on *Pseudacteon* parasitic flies' fitness, efficacy and ultimately their conservation as biological control agents. Such studies can potentially address long-standing questions in *Pseudacteon* parasitic fly field ecology. These include the role of sugar feeding in the suppression of imported fire ant pests by the flies and the effects on realized lifetime reproductive success of these flies in the field. Basic information about the timing of egg maturation and ovigeny index in *Pseudacteon* flies is also important for biocontrol efforts because it provides answers to important questions about their fecundity.

1.5   Thesis Goal and Outline

The goal of this research was to address some important questions related to the nutritional ecology and life-history strategies of *Pseudacteon* phorid flies, natural enemies of imported fire ants. Behavioral, biochemical and analytical techniques were used to address the following key questions: i) do *Pseudacteon* phorid flies utilize sugar and lipid sources in the field? ii) does feeding on such nutrient sources enhance their fitness in terms of longevity and egg maturation? This study followed a stepwise order in the variation of nutrient concentrations available to the flies in the field. In chapter II, it was tested if *P. curvatus* utilizes sugar and lipid sources in the field. In the next set of experiments in chapter III, the effects of sugar feeding on the longevity of *P. cultellatus, P. curvatus*, and *P. obtusus*, were investigated and compared. Also, the timing of egg maturation and ovigeny in *P. obtusus* was determined. The results of these experiments are discussed.

CHAPTER 2

NUTRITIONAL ECOLOGY OF *PSEUDACTEON CURVATUS*: EVIDENCE

SUPPORTING UTILIZATION OF SUGAR AND LIPID SOURCES IN THE FIELD

2.1   Introduction

Imported fire ants (Hymenoptera: Formicidae) are invasive pests that pose serious threats to public health, the economy, and the environment. Multiple releases of various species of *Pseudacteon* parasitic flies have been made across the southeastern United States for biological control of these pests. *Pseudacteon curvatus* is one of the released species, and recent surveys show its establishment in several parts of the southeastern United States such as in Florida (Porter et al. 2004, Vazquez et al. 2006), Alabama, Mississippi and Tennessee (Thead et al. 2005), Georgia (Gardner et al. 2008), Texas (Plowes et al. 2011, Puckett et al. 2013), and Arkansas (Farnum and Loftin 2011).

Most parasitoids in the field depend on plant-derived sugar sources such as extrafloral and floral nectar or honeydew for energy (Jervis et al. 1993, Steppuhn and Wäckers 2004). Variation in sugar type and quantity exists among sugar sources, however, the predominant sugars in nectar and honeydew are sucrose, glucose and fructose (Baker and Baker 1983, Kloft et al. 1985, Koptur 1992, Wäckers 2001). Parasitoids derive several benefits from feeding on sugar sources. One benefit is increased longevity (Fadamiro et al. 2005, Fadamiro and Chen 2005, Chen and Fadamiro 2006), which allows more time for host location and parasitization. Parasitoids also benefit from sugar feeding through maintenance of high glycogen levels (Fadamiro and Heimpel 2001, Fadamiro et al. 2005). Lee and Heimpel 2008 suggested that

ingesting sugars/ nectar may reduce the rate of lipid decline in parasitoids. These mechanisms are expected to eventually enhance egg production, female fecundity, and foraging ability in parasitoids. Carbohydrate levels are considered a good indicator of a parasitoid's nutritional state (Casas et al. 2003, Steppuhn and Wäckers 2004, Rusch et al. 2013).

Despite the many research projects being conducted on *Pseudacteon* parasitic flies, very little is known about some aspects of their nutritional ecology (Fadamiro and Chen 2005), which is essential for conservation biological control. Sugar feeding was documented for *P. tricuspis* in the laboratory (Fadamiro and Chen 2005). While such laboratory study is informative about the nutritional ecology of *Pseudacteon* parasitic flies, it does not fully address questions related to their food use under field conditions. The overall objective of this study is to better understand the nutritional ecology of phorid flies by characterizing their food utilization in the field. It is hypothesized that *P. curvatus* feeds on sugar and lipid sources in the field.

2.2   Materials and Methods

2.2.1   Parasitoids. Females of *P. curvatus* were caught at mounds of red imported fire ants, *Solenopsis invicta*, in Auburn, AL, USA, during September 2014. The flies were caught with aspirators between 12:00 and 16:00 h. Females were then identified by the presence and shape of their ovipositor under a stereomicroscope. Other *P. curvatus* flies (laboratory-reared) tested in this study were reared on workers of *S. invicta* at the phorid fly rearing facility of the USDA-APHIS-PPQ-CPHST Laboratory/Florida DPI, Gainesville, FL, USA, as described by Porter et al. (1995). Parasitized fire ant worker heads were received in batches and kept in a plastic jar (25 cm × 13 cm) covered with a mesh lid until emergence in an incubator held at 25 ± 1^0C, $70 \pm 5\%$ RH and LD 14: 10 h photoperiod. Newly emerged female *P. curvatus* were removed daily with an aspirator and placed in groups of 2 individuals in a 6-cm diameter plastic

Petri dish. Water was provided by filling a 0.5 mL microcentrifuge tube with distilled water and threading a cotton string through a hole in the cap of the tube and refilled as needed. For the sucrose-fed treatment, 25 % sucrose solution was presented in a second tube and also smeared on the inside of the Petri dish cover (Fadamiro et al. 2005). All flies were frozen prior to use for assays.

2.2.2   Experimental design. The following three treatments were tested for this experiment: (1) field-collected, (2) sucrose-fed laboratory-reared (positive control), and (3) sucrose-starved laboratory-reared (negative control) *P. curvatus*. There were 11 replicates per treatment.

2.2.3   Body nutrient analyses. The amounts of fructose, body sugars, glycogen and lipid in individual flies were quantified using a series of biochemical tests originally developed by van Handel (1965, 1967, 1985a, b, van Handel and Day 1988) for mosquitoes and recently adapted for phorid flies by our laboratory (Fadamiro and Chen 2005, Fadamiro et al. 2005, Chen and Fadamiro 2006). Briefly, an individual fly was crushed with a plastic pestle in a 1.5mL microcentrifuge tube containing 50 µL of 2% sodium sulphate solution and placed on ice. The dissolved nutrients were then extracted with 450 µL of chloroformmethanol (1: 2), after which the tube was vortexed. The tube was then centrifuged at 14,000 g for 2 min, and 200 µL of the resulting supernatant was transferred to a glass tube (12-mm diameter $\times$ 75-mm long) for the sugar assays. Another 200 µL was transferred to a similar glass tube for the lipid assay. The precipitate was left in the microcentrifuge tube for the glycogen assay. All tubes were heated at 90°C until approximately 50 µL of solution was left in the sugar tube and all solution was evaporated from the lipid and glycogen tubes.

Fructose: To estimate the amount of fructose, 950 µL of anthrone reagent was added to the sugar tube, mixed thoroughly. It was then left to react at room temperature for 1.5 h (cold anthrone reading). After the reaction time elapsed, the solution was then poured into a 1.5 mL methacrylate cuvette, and the optical density (absorbance) was measured at 625 nm wavelength using a spectrophotometer. To convert absorbance readings to absolute fructose amounts (µg), standard curves were generated by determining the cold anthrone absorbance (at 625 nm) of different amounts (1–50 µg; three replicates per dose) of pure fructose (Fisher, Fairlawn, New Jersey). The total amount of gut sugars (amount of sugars present in the insect crop) in each fly was estimated by multiplying the fructose amount by five. [This was done because sucrose consists of equal parts of fructose and glucose, and the glucose does not react at room temperature (van Handel 1967, Fadamiro and Heimpel 2001). Therefore, the cold anthrone reading was then multiplied by two to give an estimate of total gut sugars. Furthermore, because only 200 µL of the original 500 µL was used for the fructose (cold anthrone) assay, it was necessary to multiply this amount further by 2.5 to estimate the total amount of gut sugars].

Body sugars. The amount of total sugars in each fly was estimated by using the hot anthrone test. Total sugars refer to the total amount of sugars present in an insect and it consists of two components: first is the body or blood sugars present in the hemolymph and body tissues, and second is the gut sugars contained in the crop (van Handel and Day 1988). The same solution used for the cold anthrone test was poured back into a glass tube, heated at 90 °C for 10 min and cooled on ice. The absorbance was again read at 625 nm to give an estimate of total sugars. To convert absorbance readings into absolute amounts (µg), standard curves were generated by determining the hot anthrone absorbance (at 625 nm) of different amounts (1–50 µg, three replicates per dose) of pure sucrose (Fisher). The total amount of body sugars present in each fly

12

was estimated by multiplying the amount of sugars from the hot anthrone test by 2.5 because only 200 µL of the original 500 µL was used for the hot anthrone assay. To estimate the amount of body sugars (nonglycogen sugars) present within the hemolymph and tissues of the flies, the estimated total amount of gut sugars was subtracted from the estimated amount of total sugars. Presumably, body sugars of *Pseudacteon* phorids consist mainly of trehalose, the dominant sugar in insect haemolymph and other tissues (Wyatt 1967, van Handel 1969, Fadamiro and Chen 2005).

Glycogen: One mL of anthrone reagent was added to the microcentrifuge tube containing the precipitate. After centrifugation, the tube was heated at 90 °C for 10 min and then cooled on ice, and the absorbance read at 625 nm. Glycogen standard (calibration) curves were generated by determining the absorbance of oyster glycogen (ICN Biomedicals, Aurora, Ohio) at a range of 1–50 µg (three replicates per dose). Regression analyses were carried out, and the equation was thus used to convert absorbance readings to absolute glycogen amount. The amount of glycogen estimated above was considered to be representative of the whole fly because all glycogen in the sample was presumed to precipitate to the bottom of the tube.

Lipids: The amount of lipids in each fly was determined by adding 40 µL of sulphuric acid to the tube containing the lipid precipitate. The tube was then heated at 90 °C for 2 min and cooled on ice, and 960 µL of a vanillin phosphoric acid reagent was added. The solution in the tube was mixed and left to react at room temperature for 30 min, and the absorbance was read at 525 nm. To convert absorbance values to absolute lipid amounts (mg), lipid standard curves were generated by determining the absorbance of pure vegetable oil at a range of 1–50 µL (three replicates per dose). The resulting quadratic equation was used to convert absorbance readings to absolute lipid amount. To estimate the total amount of lipids present in each fly, the lipid amount

was multiplied by 2.5 because 200 µL of the original 500 µL was used for the assay. All reagents were prepared as described by van Handel (1985a).

2.2.4 Statistical analyses. Nutrient data were analyzed using analysis of variance (ANOVA) followed by Tukey-Kramer HSD comparison test ($P < 0.05$; SigmaPlot 13.0, Systat Software Inc., 2014) to establish significant differences among the treatments.

2.3 Results

2.3.1 Body nutrient analyses

Gut sugars: The field-collected treatment group had an average gut sugar level of 11.81µg ± 0.25; the sucrose-fed laboratory-reared group had an average gut sugar level of 12.03 µg ± 0.25; while the sucrose-starved laboratory-reared group had an average gut sugar level of 11.61µg ± 0.20. The effect of diet was significant, $F (2, 30) = 8.37$, $P = 0.001$. The gut sugar level in sucrose-fed flies was significantly higher than that in sucrose-starved individuals, while those of field-collected flies and sucrose-fed flies were similar. There was only a marginal (approximately 0.2µg) but insignificant increase in gut sugar level of field-collected flies when compared with that of sucrose-starved flies. Gut sugars were detected in sucrose-starved female *P. curvatus* (Fig. 1).

Body sugars: The field-collected treatment group had an average body sugar level of 2.64 µg ± 0.29; the sucrose-fed laboratory-reared group had an average body sugar level of 2.64 µg ± 0.53, and the sucrose-starved laboratory-reared group had an average body sugar level of 2.51µg ± 0.27. The effect of diet was not significant, $F (2, 30) = 0.378$, $P > 0.05$. Although there was no significant effect of diet on level of body sugars, there was marginal (approximately 0.1µg) but insignificant increase in body sugar level of field-collected flies when compared with those of

sucrose-starved flies. The body sugar levels of both field-collected and sugar-fed flies were the same (Fig. 2).

Glycogen: The field-collected treatment group had an average glycogen level of 2.53 µg ± 0.07; the sucrose-fed laboratory-reared group had an average glycogen level of 2.54µg ± 0.08; and the sucrose-starved laboratory-reared group had an average glycogen level of 2.49µg ± 0.08. The effect of diet was not significant, F (2, 30) = 0.786, $P > 0.05$. Although there was no significant effect of diet on level of glycogen, there was marginal (approximately 0.04µg) but insignificant increase in body sugar levels of field-collected flies when compared with those of sucrose-starved flies, while the glycogen levels of both field-collected and sugar-fed flies were the same (Fig. 3).

Lipid: The field-collected treatment group had an average lipid level of 13.09 µg ± 2.64; the sucrose-fed laboratory-reared group had an average lipid level of 7.87µg ± 4.47; and the sucrose-starved laboratory-reared group had an average lipid level of 8.09µg ± 3.10. The effect of diet was significant, F (2, 30) = 7.840, P= 0.002. The means of lipid levels were significantly higher in field-collected flies than those in both sugar-fed and sugar-starved individuals (Fig. 4). Among the nutrients, lipid was the most abundant, followed by gut sugars, body sugars, and then glycogen.

2.4   Discussion

The overall objective of this study was to demonstrate sugar and lipid feeding in field-collected *P. curvatus.* Although the gut sugar levels in sucrose-fed flies were significantly higher than that in sucrose-starved individuals, while those of field-collected flies and sucrose-fed flies were similar, there was only a marginal (approximately 0.2µg) but insignificant increase in gut sugar levels of field-collected flies when compared with that of sucrose-starved flies. Although

there was no significant effect of diet on levels of body sugars and glycogen, these nutrient levels showed marginal but insignificant increases in field-collected flies when compared with those of sucrose-starved flies. This suggests that *P. curvatus* utilizes sugar sources, though moderately, in the field. Such sugar sources could be floral nectar, extra floral nectar and honeydew. Similar results have been reported for several Hymenopteran parasitoids (Jervis et al. 1993, Steppuhn and Wäckers 2004).

The results show significantly higher levels of lipid in field-collected female *P. curvatus* relative to those of both sucrose-fed and sucrose-starved laboratory-reared individuals, indicating the potential roles lipid plays in field-collected female *P. curvatus*. Lipid is crucial in female insects, because lipid reserves are critical for egg production (Ellers and van Alphen 1997). This high lipid level in field-collected female *P. curvatus* may also suggest that the phorid flies feed on lipid-rich food sources in the field, as several insects are known to utilize lipids for egg production, including hymenopteran parasitoids (Ellers and van Alphen 1997, Giron and Casas 2003b).

Gut sugars were detected in sucrose-starved female *P. curvatus*. This finding is not supported by the studies of Fadamiro et al (2005), which did not detect any gut sugar in females and males of both newly emerged and older sugar-starved *P. tricuspis*. The presence of gut sugar in these sucrose-starved female *P. curvatus* is notable as similar studies did not detect any gut sugar in the crop of their tested insects (Olson et al 2000, Fadamiro et al. 2005). The detection of gut sugars in the crop of these sucrose-starved female *P. curvatus* raises questions about how these insects came about these sugars in their crop and if they have it at eclosion. The results indicate that our knowledge about insects' gut sugars may be inadequate, or possible contamination during the experiment

There are several assumptions that shortages of nectar and other sugar sources in modern agro-ecosystems can limit the efficacy of biological control agents (e.g. Wolcott 1942, Hocking 1966). This has sparked a growing interest in the concept of augmenting beneficial insects by providing food supplements such as flowering plants (Gurr and Wratten 1999) or sugar sprays (Hagen 1986, Evans and Swallow 1993). Results from the present study show higher but marginal levels of gut sugars in field-collected flies than sucrose-starved flies, thereby suggesting that the provision of supplemental food sources will ensure that these flies have abundant essential nutrients.

In summary, this study establishes that field-collected female *P. curvatus* are capable of utilizing sugar sources, though moderately, in the field, thereby giving basis for provisioning of supplemental sugar sources in the field in southeastern U.S. for conservation of *P. curvatus*, parasitoids of imported fire ants. The potential sugar sources will be identified in future studies.

Figure legends

Figure 1. Mean amount of gut sugars per female *P. curvatus* (µg ± SE) fed three diet treatments [Means with different letters are significant ($P < 0.05$, Tukey HSD test)]

Figure 2. Mean amount of body sugars per female *P. curvatus* (µg ± SE) fed three diet treatments ($P < 0.05$, Tukey HSD test)

Figure 3. Mean amount of glycogen per female *P. curvatus* (µg ± SE) fed three diet treatments [Means with different letters are significant ($P < 0.05$, Tukey HSD test)]

Figure 4. Mean amount of lipid per female *P. curvatus* (µg ± SE) fed three diet treatments [Means with different letters are significant ($P < 0.05$, Tukey HSD test)]

Figure 1.

Gut sugars

Figure 2.

Body sugars

Figure 3.

Glycogen

Mean amount of glycogen per female fly (µg ± SE)

Sucrose-fed | Sucrose-starved | Field-collected

Figure 4.

Lipid

Mean amount of lipid per female fly (µg ± SE)
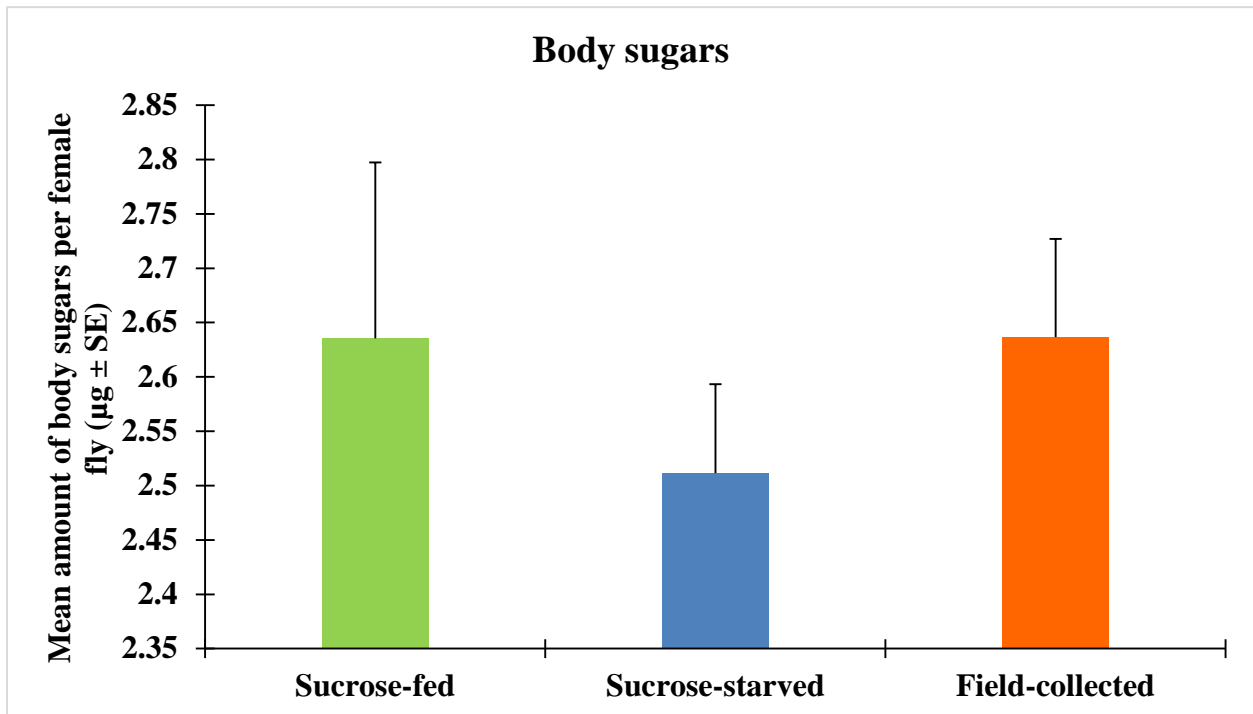
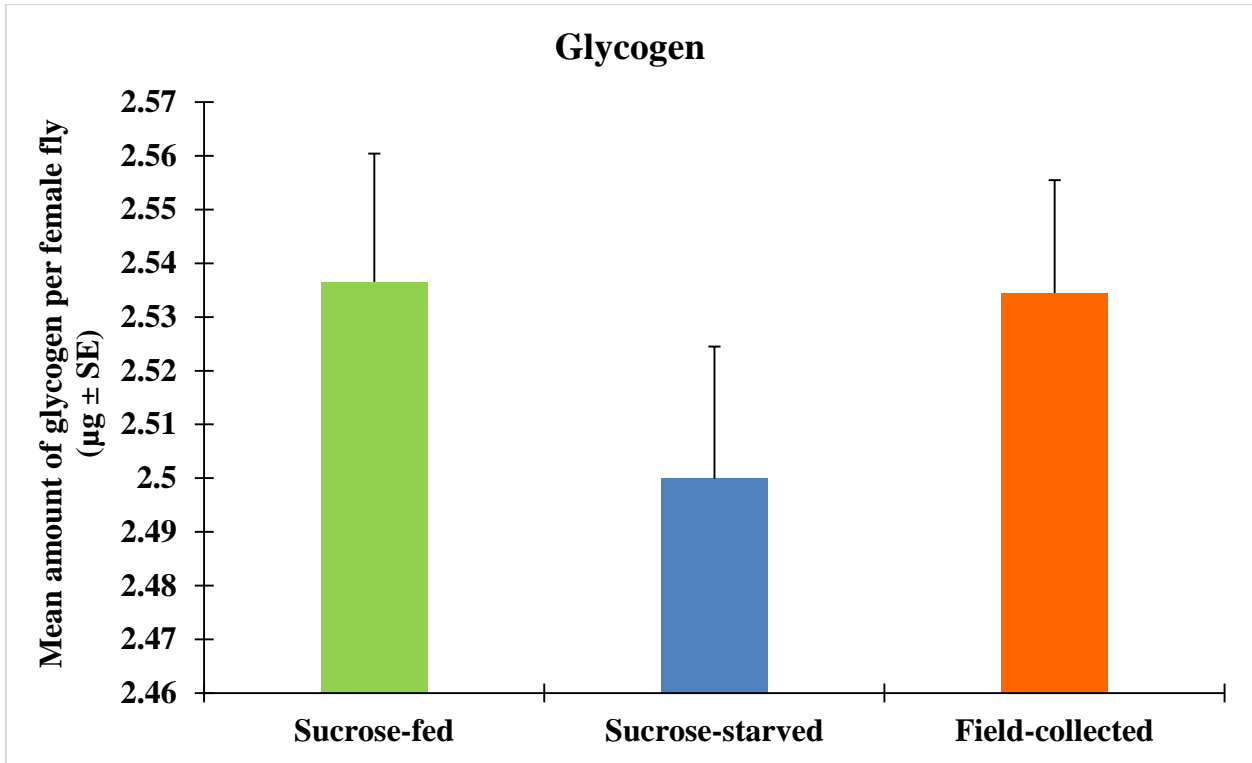Field-collected | Sucrose-fed | Sucrose-starved
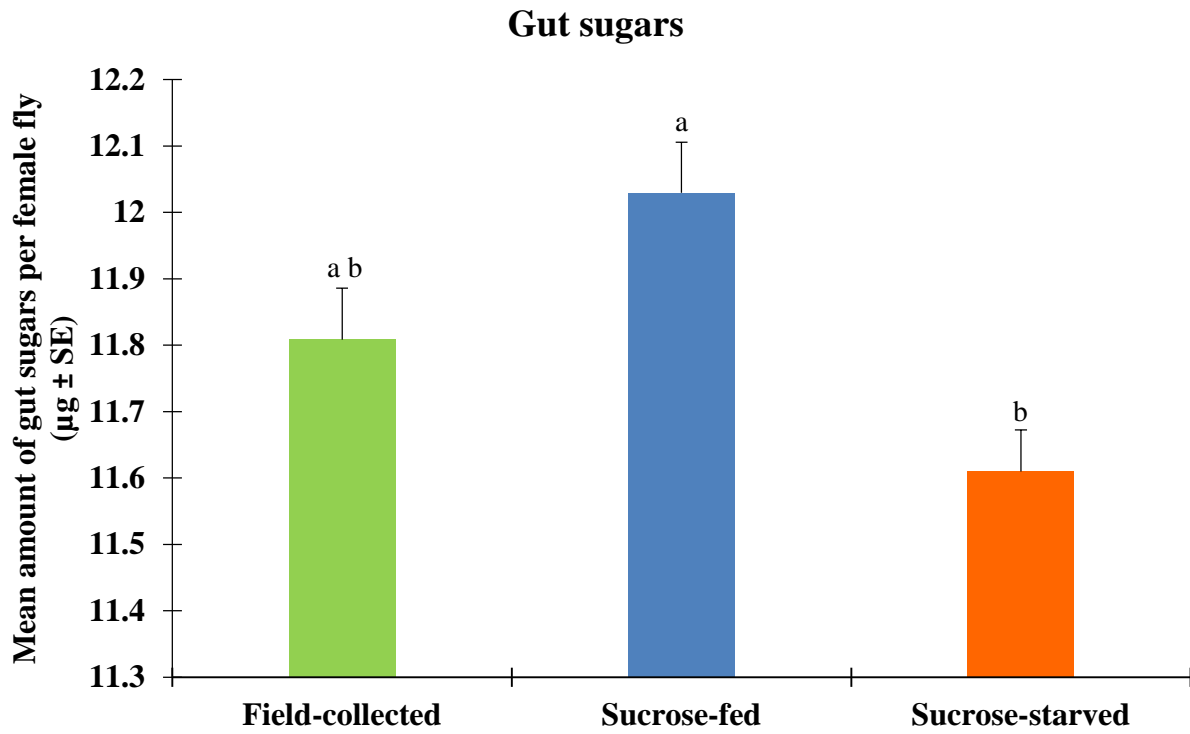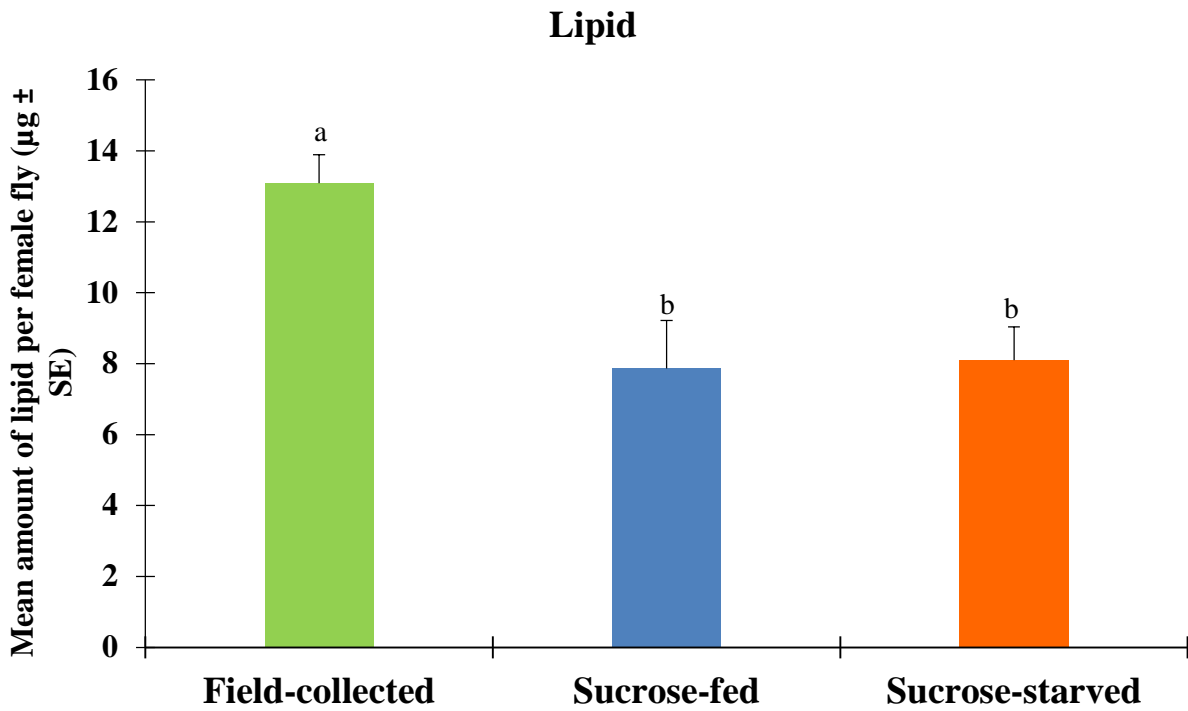
CHAPTER 3

SUCROSE FEEDING ENHANCES THE FITNESS OF *PSEUDACTEON* SPP. (DIPTERA: PHORIDAE), NATURAL ENEMIES OF IMPORTED FIRE ANTS

3.1 Introduction

The red imported fire ant, *Solenopsis invicta*, is an invasive pest accidentally introduced into the United States (U.S.) from South America in the 1930s and is now common in the southern U.S. (Morrison et al. 2004). The various negative economic and ecological impacts of these ants have been documented (Allen et al. 2004, LeBrun et al. 2012a). Their aggressiveness, painful and often allergenic sting, extremely high densities, and large unsightly mounds constitute them as pests (Adams 1986, Vinson 1997, Eubanks 2001). The treatment of an individual mound can cost as high as $6.00, and bait treatments cost as high as $133.00 per acre (Imported Fire Ant eXtension Fire Ant Control Materials 2015). Controlling the pests cost the U.S. $6 billion a year, including cost of insecticides (Imported Fire Ant eXtension Fire Ant Control 2014). Chemical control alone is however, not sustainable, thus the need for other control methods, such as biological control.

Twenty-three *Pseudacteon* species are known to attack imported fire ants in their native South America (LeBrun et al. 2012b). In the last two decades, multiple releases of various *Pseudacteon* species, natural enemies of fire ants, have been made in the southeastern region of the U.S. for classical biological control of imported fire ants (Henne et al. 2007a). Recent surveys in Alabama show a reduction in the population of *P. tricuspis* (Palmer 2014). Factors responsible for this decline may be complex (Morrison et al. 1999). Nutrition, an environmental factor, plays an important role in the conservation of biocontrol agents (Linker et al. 2009).

Although much research is being conducted on *Pseudacteon* spp, very little is known about their nutritional ecology (Fadamiro and Chen 2006).

Most adult parasitoid species depend on sugar-rich food, such as nectar, for their survival and metabolic needs. Some can also feed on honeydew (Jamont et al. 2014). Lee and Heimpel (2008) highlight the ways that feeding on common sugars or floral nectars enhance parasitoid longevity and fecundity. These include direct ways, such as significantly reducing starvation risk, reducing egg resorption or enhancing egg maturation, and indirect ways, such as giving parasitoids more time and energy for host-location, thereby increasing their oviposition rate.

Several studies have highlighted the importance of sucrose feeding, among other sugars. Comparing several naturally occurring sugars for their benefits on *P. tricuspis*, Chen and Fadamiro (2006) reported that sucrose appeared to be one of the most beneficial in terms of increased fly longevity, glycogen level, and total body sugar level. Sucrose had similar benefits in terms of longevity on the parasitoid *Cotesia glomerata*, when compared with other naturally occurring sugars (Wäckers 2001). Binns (1980) reported that among various sugars, sucrose is more preferred by the phorid fly, *Megaselia halterata*.

Sucrose feeding has been reported to significantly increase the longevity of *P. tricuspis*, thus suggesting that *Pseudacteon* phorid flies have the ability to feed on sucrose solution (Fadamiro et al. 2005, Chen et al. 2005, Chen and Fadamiro 2006). However, longevity studies indicate that variation exists among insect species in their ability to utilize specific sugars (Ferreira et al. 1998, Wäckers 2001). This may be the case among species of *Pseudacteon* phorid flies. A parasitoid's egg maturation strategy may lead to differences in sensitivity to carbohydrates by parasitoids (Williams III and Roane 2007).

Many studies have documented the positive impact of sugar feeding on the fecundity of

21

some parasitoids (e.g. Lee and Heimpel 2008, Idris and Grafius 1995, Dyer and Landis 1996, Berndt et al. 2002). Fadamiro and Chen (2005) suggested that the fecundity of *P. tricuspis* may be increased with feeding on honeydew or other sugar sources. Studies have indicated that two *Pseudacteon* species, *P. tricuspis* and *P. wasmanni* are pro-ovigenic in nature. (Cônsoli et al. 2001, Zacaro and Porter 2003), thereby suggesting that no post-emergence treatment, such as sugar feeding, enhances fecundity in these species with pro-ovigenic life history since they contain only mature eggs. In contrast, sugar feeding may enhance fecundity in synovigenic parasitoid species by maturing additional eggs (Heimpel and Jervis 2005, Jervis et al. 2001, Fadamiro and Chen 2005). A pair of ovaries is usually part of the insect female reproductive system. Each ovary is formed by ovarioles whose number and physiological states are closely related to egg production. However, relatively little is known about the egg maturation state (pro-ovigenic or synovigenic) of *Pseudacteon* species (Cônsoli et al. 2001, Zacaro and Porter 2003). Such studies are needed to better understand the timing of egg maturation in *Pseudacteon* species to aid development of field-based strategies for improving biological control of *S. invicta* in the Southeast U. S.

The objectives of this study are to investigate the effect of sucrose-feeding on the longevity of the parasitoids, *P. cultellatus*, *P. curvatus* and *P. obtusus*, and todetermine the ovigeny index of newly emerged *P. obtusus*. The overall hypothesis is that sucrose-feeding in these will significantly increase their longevity and thus improve their fitness to control their hosts, imported fire ants. It is also hypothesized that at emergence *P. obtusus* will have all its lifetime egg complement mature, thus indicating a pro-ovigenic life history strategy.

3.2 Materials and Methods

3.2.1 Parasitoids. The three phorid fly species tested (*P. cultellatus, P. curvatus* and *P. obtusus*) were reared on workers of red imported fire ants, *S. invicta*, at the fire ant rearing facility of the USDA-APHIS-PPQ-CPHST Laboratory/Florida DPI, Gainesville, FL, USA as described by Porter et al. (1995). Because larvae of *Pseudacteon* phorid flies have the habit of decapitating fire ant workers and pupating inside the empty head capsule (Porter et al. 1995), heads of parasitized fire ant workers were received in batches and maintained in a plastic jar with a lid (25cm diameter, 13cm high) at 26 ± 1^0C, under an LD 14: 10 h photoperiod and $65 \pm 5\%$ RH, until emergence. The jar was checked at least five times daily for fly emergence. Emerging flies were not given access to food or water in the jar and were removed with an aspirator five times daily. The sex was determined by the presence or absence, and shape of the distinct female ovipositor upon examination under a stereomicroscope.

3.2.2 Longevity

The methods described below were adapted from Chen et al. (2005).

3.2.2.1 <u>Experimental design</u>: There were four diet treatments: (i) sugar-starved, but provided water only, hereafter referred to as water only (the control); (ii) 50% (w/v – i.e. weight to volume) sugar water, fed only for 24 hours during the first day of emergence and starved thereafter until death, hereafter referred to as 24 h fed 50%; (iii) 25% (w/v) sugar water, fed continuously from emergence throughout life, hereafter referred to as 25% sucrose; and (iv) 50% (w/v) sugar water, fed continuously from emergence throughout life, hereafter referred to as 50% sucrose. The number of replicates was 35 flies of each sex and each species for each treatment.

3.2.2.2 <u>Experimental diet</u>: Adult females and males of *P. cultellatus, P. curvatus* and *P. obtusus*

emerging on the same day from the same batch of decapitated imported fire ants' heads were

distributed evenly across the four diet treatments. Flies were fed *ad libitum* for 24 h during the

day of feeding. Water was provided in all treatments by filling a 0.5 mL microcentrifuge tube

with distilled water and threading a cotton string through a hole in the cap of the tube. Water

tubes were refilled as needed. Each newly-emerged fly was placed in a 6 cm diameter plastic

Petri dish. For the treatments involving sugar feeding, the sucrose solution was applied to the

inside of each Petri dish cover with a cotton-tipped applicator. Flies that were fed only during the

first day of emergence and then starved until death were transferred to new dishes without

sucrose (but with water tubes) after exposure in sucrose-smeared dishes for 24 h.

Petri dishes were checked once daily for dead flies, which were promptly removed from

the dishes. The longevity (in days) of each fly was recorded. All experiments were conducted at

28 ± 1^{0}C, under an LD 14 : 10 h photoperiod and $65 \pm 5\%$ RH.

3.2.2.3 <u>Statistical analyses</u>: The means from each treatment group were analyzed using analysis

of variance (ANOVA) followed by Tukey-Kramer HSD comparison test ($P < 0.05$; SigmaPlot

13.0, Systat Software Inc., 2014) to establish significant differences among the treatments.

3.2.3   Egg maturation type in *P. obtusus*

The method is similar to that of Zacaro and Porter (2003) and is described as follows.

Eight newly emerged female *P. obtusus* had their abdomen severed, and light microscopy (serial-

sectioning stained with Mayer's haematoxylin and aqueous eosin (HE)) was carried out on each

as described below.

For histological preparations, abdomens were severed to facilitate fixing with a modified

paraformaldehyde solution for 4–8 h at room temperature (4 g of paraformaldehyde in 90 ml of

distilled water; after dissolving, add 0.75 g NaCl, 0.23 g Na_2HPO_4 and 0.27 g KH_2PO_4; finally,

bring final volume to 100 ml with 0.1 M sodium phosphate buffer, pH 7.4). After fixation, the

abdomens were transferred to a sodium phosphate buffer (0.1 M, pH 7.4), dehydrated in ethanol

(70 to 95%), and then infiltrated and embedded in JB 4/ Polysciences resin. The specimens were

serially sectioned (4–5 mm thickness) with glass knives in a Sorvall/ DuPont microtome, and the

sections were stained with Mayer's haematoxylin and aqueous eosin (HE).

The sections were examined with a Zeiss photomicroscope. The egg maturation type in

the ovaries of each female was then described. The eggs were determined using the basophilic

and spherical appearance in the ovaries (Zacaro and Porter 2003).

3.3   Results

3.3.1   Longevity

3.3.1.1 Comparison among treatments for each species

Pseudacteon cultellatus: Survivorship curves for both sexes are shown in Figures 1A and 2A.

Table 1 shows the average longevity of *P. cultellatus* under different diet treatments. Females fed

50% sucrose continuously throughout their lifespan had greater longevity (3.6 ± 0.14 days) than

females provided 25% sucrose continuously throughout their lifespan (3.0 ± 0.10 days), females

provided 24 h fed 50% sucrose (2.8 ± 0.11 days), and females provided with water only ($2.6 \pm$

0.15 days) (Table 1). Similar results were obtained for male *P. cultellatus*. Males fed 50%

sucrose throughout their lifespan lived longer than males in the remaining three treatments

(Table 1). Feeding on 50% sucrose increased average longevity by 1 day for females and by

approximately 2 days for males when compared with individuals provided with only water. The

effect of sex was not significant, as males and females under each diet treatment had similar

lifespan.

Pseudacteon curvatus: Survivorship curves for both sexes are shown in Figures 1B and 2B.

Table 1 shows the average longevity of *P. curvatus* under different diet treatments. Females fed

50% sucrose continuously throughout their lifespan (4.6 ± 0.29 days), 25% sucrose continuously

throughout their lifespan (4.8 ± 0.28 days), and 24 h fed 50% sucrose (4.4 ± 0.24 days) all had

greater longevity than females provided with water only (3.3 ± 0.10 days) (Table 1). Males fed

25% sucrose continuously during their lifespan (4.3 ± 0.24 days) had greater longevity than

males provided with water only (3.4 ± 0.11 days). Feeding on 25% sucrose increased average

longevity by approximately 2 days for both females and males when compared with individuals

provided with only water. The effect of sex was not significant as males and females under each

diet treatment had similar longevity.

Pseudacteon obtusus: Survivorship curves for both sexes are shown in Figures 1C and 2C. Table

1 shows the average longevity of *P. obtusus* under different diet treatments. Females fed 50%

sucrose continuously throughout their lifespan had greater longevity (7.3 ± 0.40 days) than

females provided 25% sucrose continuously throughout their lifespan (6.3 ± 0.41 days), females

provided 24 h fed 50% sucrose (5.1 ± 0.23 days), and females provided with water only (3.9 ±

0.19 days) (Table 1). Males fed 50% sucrose continuously throughout their lifespan had greater

longevity (8.7 ± 0.42 days) than males provided 24 h fed 50% sucrose (5.2 ± 0.19 days), and

males provided with water only (3.5 ± 0.12 days). There was no difference between males fed

50% and 25% sucrose continuously. Also, there was no difference between males fed 25%

sucrose continuously and 50% sucrose for 24 h. Feeding on 50% sucrose increased average

longevity by 3 days for females when compared with females provided with only water and

increased average longevity by 5 days for males when compared with males provided with only water. The effect of sex was not significant as males and females under each diet treatment had similar longevity.

3.3.1.2 Comparison among the species

50% sucrose: Female *P. obtusus* had greater longevity (7.3 ± 0.40 days) than female *P. curvatus* (4.6 ± 0.29 days) and *P. cultellatus* (3.6 ± 0.14 days). Similar results were obtained among males. Male *P. obtusus* had greater longevity (8.7 ± 0.42 days) than male *P. curvatus* (4.0 ± 0.22 days) and *P. cultellatus* (4.2 ± 0.18 days) (Table 1). Longevity of female *P. obtusus* was more than double that of female *P. cultellatus*. Similar results were obtained among males. Longevity of male *P. obtusus* was more than double that of male *P. cultellatus*. The effect of sex was not significant.

25% sucrose: Female *P. obtusus* had greater longevity (6.3 ± 0.41 days) than female *P. curvatus* (4.8 ± 0.28 days) and *P. cultellatus* (3.0 ± 0.10 days). Similar results were obtained among males. Male *P. obtusus* had greater longevity (6.6 ± 0.34 days) than male *P. curvatus* (4.3 ± 0.24 days) and *P. cultellatus* (3.2 ± 0.11 days) (Table 1). The longevity of female *P. obtusus* was more than double that of female *P. cultellatus*. Similar results were obtained among males. Longevity of male *P. obtusus* was more than double that of male *P. cultellatus*.

24 h fed 50% sucrose: Average longevity of female *P. obtusus* (5.1 ± 0.23 days) and female *P. curvatus* (4.4 ± 0.24 days) were both greater than that of female *P. cultellatus* (2.8 ± 0.11 days). Male *P. obtusus* had greater longevity (5.2 ± 0.19 days) than male *P. curvatus* (3.6 ± 0.13 days) and *P. cultellatus* (2.8 ± 0.11 days) (Table 1).

Water only: Female *P. obtusus* had greater longevity (3.9 ± 0.19 days) than female *P. curvatus*

(3.3 ± 0.10 days) and female *P. cultellatus* (2.6 ± 0.15 days). Longevity of both male *P. obtusus* (3.5 ± 0.12 days) and male *P. curvatus* (3.4 ± 0.11 days) were greater than that of male *P. cultellatus* (2.7 ± 0.09 days).

3.3.2 Egg maturation type in *P. obtusus*

The histology analysis showed that the eggs of *P. obtusus* appeared as slugform or torpedo shape and were extremely basophilic. Inside the ovarioles, no nurse cell was observed and the eggs appeared to be post-vitellogenic (Fig. 3). The ovaries of *P. obtusus* were spherical and enclosed by a thick muscular sheath (Fig. 3).

3.4 Discussion

3.4.1 Longevity

The overall hypothesis of this study was that sugar feeding will increase the longevity of *P. cultellatus*, *P. curvatus*, and *P. obtusus*. The results support this hypothesis, as continuous sugar provisioning increased the longevity of both males and females of these species. This result is similar to the positive impact of sugar feeding on the longevity of *P. tricuspis* (Fadamiro et al. 2005). Further, the results indicate that these *Pseudacteon* species are capable of feeding on sucrose solution and utilizing sucrose solution for their increased longevity, which may be generalized to other *Pseudacteon* species.

More effective management of *Solenopsis* species with *Pseudacteon* species in the southern U.S. will require an increased reliance on conservation efforts within the framework of a classical biological control program. Knowledge that sugar increases the longevity of these parasitic *Pseudacteon* phorid flies could assist in the identification of factors that can be manipulated in the field (e.g., sugar sources). With regard to *Pseudacteon* spp., these findings

28

could promote their full parasitization potential, enhance their biological control of *Solenopsis* species, and reduce the constraints of their short lifespan. Maximizing the fitness of *Pseudacteon* species could be achieved by providing flowering cover crops as food and shelter, a strategy that has been demonstrated to improve pest control. (Nicholls et al. 2000, English-Loeb et al. 2003, Gurr et al. 2004).

Comparison among the three *Pseudacteon* species shows that in each diet treatment, longevity of *P. obtusus* is significantly higher than in *P. cultellatus* and *P. curvatus*. *Pseudacteon obtusus* is larger in size than *P. curvatus*, and *P. curvatus* is larger in size than *P. cultellatus* (Folgarait et al. 2002). The results suggest that longevity in *Pseudacteon* species is directly dependent on their body size.

When compared with individuals provided water only, continuous provision of 50% sucrose solution significantly increased longevity of both males and females of all *Pseudacteon* species, except for male *P. curvatus*. Provisioning of 25%, and 50% sucrose solution for 24 h also increased the longevity of the three species. Most plants that are sugar sources in the field in the southern U.S. have sucrose concentrations in the range of 20 - 50% (van Handel et al. 1972). This range suggests that the sucrose concentrations in the nectars of several flowering plants in the area where *Pseudacteon* phorid flies are being released for biological control of imported fire ants are within the suitability span for these *Pseudacteon* species.

3.4.2 Egg maturation type in *P. obtusus*

No pre-vitellogenesis or vitellogenesis features were seen in the histological sections of *P. obtusus*, indicating that oogenesis was completed prior to emergence, probably during the pupal stage. The ovaries of *P. formicarum* and *P. wasmanni* are also filled with vitellogenic eggs

(Wasmann 1918, Zacaro and Porter 2003). In contrast, several saprophytic phorid flies apparently do have follicles (oocyte-nurse cell complex) and developing oocytes (Borgmeier 1930, Benner 1985). The occurrence of only mature or nearly mature eggs inside both ovaries of all *P. obtusus* analyzed characterizes a strict pro-ovigeny state (Jervis et al. 2001), which is probably related to the short life span of the fly and the need to lay large number of eggs rapidly after mating. The pupal stage of most *Pseudacteon* flies provides a long period for egg development (Cônsoli et al. 2001). The presence of only mature or nearly mature eggs in the ovaries of all inspected specimens of *P. obtusus* indicates that, as in other *Pseudacteon* species, *P. obtusus* has the potential for laying a large number of eggs shortly after emergence (Zacaro and Porter 2003). The condition of pro-ovigeny in *P. obtusus* is similar to those of *P. formicarum* and *P. wasmanni* (Wasmann 1918, Zacaro and Porter 2003), which is probably an adaptation for a parasitoid life history in *Pseudacteon* flies.

The presence of only mature or nearly mature eggs in the ovaries of all newly emerged *P. obtusus* inspected indicates no need for further egg maturation. This is in contrast to synovigenic parasitoids, where egg maturation is expected to occur. In the case of synovigenic condition, post emergence treatments, such as sugar feeding, usually enhance egg maturation (Olson and Andow 1998, Tylianakis et al. 2004, Bezemer et al. 2005). Therefore, no post-emergence treatment, such as sugar feeding, is expected to increase egg maturation or fecundity in *P. obtusus*, since they appear to be pro-ovigenic. However, this conclusion should be tested further in future studies.

The eggs of *P. obtusus* appeared as slugform or torpedo shape, were extremely basophilic, and the ovaries of *P. obtusus* appeared as spherical and were surrounded by a thick muscular sheath, a feature common in Diptera. The spherical egg and ovarian shapes are similar to those of *P. wasmanni* (Zacaro and Porter 2003), which may indicate a generalized appearance

30

in *Pseudacteon* species. This finding should be tested in future studies.

These results provide an insight into the nutritional requirements, survival and fitness benefits from sucrose feeding in *Pseudacteon* parasitic flies used in this set of studies, under laboratory conditions. The significant increment in survival in sugar-fed flies compared to those starved indicates that sugar feeding is essential to fitness enhancement in these flies. However, the observation of only post-vitellogenic eggs in the ovaries of newly emerged *P. obtusus* indicates a pro-ovigenic condition, which further indicates that probably, no post-emergence treatment such as sugar feeding will enhance egg maturation in these flies. Accumulation of additional sugars postemergence is nonetheless necessary to increase the longevity of the flies, thus giving them more time for host location and parasitization. Such information can be a basis to improving the longevity of this biocontrol agent by sugar feeding in the field and in greenhouses.

References

Adams CT. 1986. Agricultural and medical impact of the imported fire ant. *In* Lofgren CS and Vander Meer RK [eds], Fire ants and leaf-cutting ants biology and management. Westview Press, Boulder, CO.

Allen, C.R., D.M. Epperson, and A.S. Garmestani. 2004. Red imported fire ant impacts on wildlife: a decade of research. Am. Midl. Nat. 152:88–103

Azzouz, H., Giordanengo, P., Wäckers, F.L., Kaiser, L. 2004. Effects of feeding frequency and sugar concentration on behaviour and longevity of the adult aphid parasitoid: *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae). *Biological Control*. 31:445 – 452.

Barrow, S.M. 2014. Biological control of red imported fire ant (*Solenopsis invicta*) using *Pseudacteon* (Diptera: Phoridae) in Central Arkansas. Thesis submitted to University of Arkansas, Arkansas, U.S.A.

Baggen, L.R., Gurr, G.M., Meats, A., 1999. Flowers in tritrophic systems:mechanisms allowing selective exploitation by insect natural enemies for conservation biological control. Entomologia Experimentalis et Applicata 91, 155–161.

Baker, H. G., and I. Baker. 1983. Floral nectar sugar constituents in relation to pollinator type. In *Handbook of experimental pollination biology*, ed. C. E. Jones and R. J. Little, 117–41. New York: Van Nostrand Reinhold.

Benner, D.B. 1985. Oocyte development and fecundity in *Megaselia scalaris* (Phoridae: Diptera). International Journal of Entomology 27:280–288.

Berndt, L. A., S. D. Wratten, and P. G. Hassan. 2002. Effects of buckwheat flowers on leafroller

parasitoids in a New Zealand vineyard. *Agricultural and Forest Entomology* 4: 39-
45.

Bernstein, C., Jervis, M.A., 2008. Food-searching in parasitoids: the dilemma of choosing
between 'intermediate' or future fitness gains. In: Wajnberg, E. (Ed.), Behavioural
Ecology of Parasitoids. Blackwell Publishing, JJM van Alphen, Oxford, pp. 129–171

Bezemer, T. M., Harvey, J.A., and Mills, N.J. 2005. Influence of adult nutrition on the
relationship between body size and reproductive parameters in a parasitoid wasp.
Ecol. Entomol. 30:571-580.

Binns, E.S. 1980. Mating behaviour, fecundity and feeding in the mushroom phorid, *Megaselia
halterata* (Wood) (Dipt.). Entomol. Monthly Mag. 116, 45–57.

Borgmeier, T. 1930. Zur Morphologie und Biologie von Pseudohypocera nigrofascipes
Borgmeier–Schmitz (Dipt., Phoridae). Zoologischer Anzeiger 90, 92–104.

Bortolotto, O. C., A. De O. Menezes Jr., A. T. Hoshino, M. G. Carvalho, A. Pomari-Fernandes
and G. Salgado-Neto. 2014. Sugar solution treatment to attract natural enemiesand its
impact on fall army worm *Spodoptera frugiperda* in maize fields. Interciercia vol. 39
No. 6

Callcott AMA. 2002. Range expansion of the imported fire ant – 1998-2001. 2002 Annual
Imported Fire Ant Research Conference, Athens, Georgia, USA.

Calceterra, L.A., S.D. Porter, and J.A. Briano. 2005. Distribution and abundance of fire ant
decapitating flies (Diptera: Phoridae: *Pseudacteon*), in three regions of southern
South America. Annals of the Entomological Society of America. 98:85-95.

Casas, J., G. Driessen, N. Mandon, S. Wielaard, E. Desouhant, J. van Alphen, et al. 2003. Energy

dynamics in a parasitoid foraging in the wild. J. Anim. Ecol. 72:691–697.

Chen, L., Onagbola, E.O., Fadamiro, H.Y. 2005. Effects of temperature, sugar availability, sex, mating, and size on the longevity of phorid fly *Pseudacteon tricuspis* (Diptera: Phoridae). Environmental Entomology 34: 246-255.

Chen, L., and Fadamiro, H.Y. 2006. Comparing the effects of five naturally occurring monosaccharide and oligosaccharide sugars on longevity and carbohydrate nutrient levels of a parasitic phorid fly, *Pseudacteon tricuspis*. Physiological Entomology 31: 46-56.

Chen, L., and Fadamiro, H.Y. 2007. Behavioral and electroantennogram responses of phorid fly *Pseudacteon tricuspis* (Diptera: Phoridae) to red imported fire ant *Solenopsis invicta* odor and trail pheromone. Journal of Insect Behavior, Vol. 20, No. 2

Cônsoli, F. L., C. T. Wuellner, S. B. Vinson and L. E. Gilbert. 2001. Immature development of *Pseudacteon tricuspis* (Diptera: Phoridae), an endoparasitoid of the red imported fire ant (Hymenoptera: Formicidae). Annals of the Entomological Society of America 94: 97-109.

Costamagna, A.C., and D. A. Landis. 2004. Effect of food resource provision on adult *Glyptapanteles militaris* (Walsh) and *Meteorus communis* (Cresson) (Hymenoptera: Braconidae), parasitoids of the armyworm, *Pseudaletia unipuncta* (Haworth) (Lepidoptera: Noctuidae). *Environmental Entomology* 33: 128 -137.

Creighton WS. 1930. The New World species of the genus *Solenopsis* (Hymenoptera: Formicidae). Proceedings of American Academy of Arts & Science 66: 39-51.

de Oliveira, C. F., E. Y. Long, and D. L. Finke. 2014. A negative effect of a pathogen on its

       vector? A plant pathogen increases the vulnerability of its vector to attack by natural

       enemies. Oecologia. 174:1169–1177

Disney, R.H.L. 1994. Scuttle Flies: the Phoridae. Chapman and Hall, London.

Drees BM. 1998. Managing red imported fire ant in electrical equipment and utility housings,

       Fact Sheet Fact Sheet 011. Texas Imported Fire Ant Research & Management

       Project, Texas A&M University System, College Station, Texas. 4 pp.

Drees, B.M., Vinson, S.B., Gold, R.E. Merchant, M.E., Brown, E., Engler, K., Keck, M., Nester,

       P., Kostroun, D., Flanders, K., Graham, F., Pollet, D., Hooper-Bui, L., Beckley, P.,

       Davis, T., Horton, O.M., Gardner, W., Loftin, K., Vail, K., Wright, R., Smith, W.,

       Thompson, D.C., Kabashima, J., Layton, B., Koehler, P., Oi, D., and A.M. Callcott.

       2006. Managing Imported Fire Ants in Urban Areas, a Regional Publication

       Developed for: Alabama, Arkansas, California, Florida, Georgia, Louisiana,

       Mississippi, New Mexico, Oklahoma, South Carolina, Tennessee and Texas, B-

       6043. Texas Cooperative Extension, Texas A&M University, College Station, TX,

       22 pp. Available at: http://tcebookstore.org.

Drees, B.M., A.A Calixto, and P. R. Nester. 2013. Integrated pest management concepts for red

       imported fire ants *Solenopsis invicta* (Hymenoptera: Formicidae). *Insect Science*

       (2013) 20, 429–438.

Dyer, L. E. and D. A. Landis. 1996. Effects of habitat, temperature, and sugar availability on

       longevity of *Eriborus terebrans* (Hymenoptera: Ichneumonidae). *Environmental*

       Entomology 25: 1192-1201.

Ellers, J. and van Alphen, J.J.M. 1997. Life history evolution in *Asobara tabida*: plasticity in

allocation of fat reserves to survival and reproduction. Journal of Evolutionary
Biology, 10:771–785.

Ellers, J. and M. A. Jervis. 2004. Why are so few parasitoid wasp species pro-ovigenic?
Evolutionary Ecology Research, 2004, 6: 993–1002

Elliott, N.C., Simmons, G.A., Sapio, F.J., 1987. Honeydew and wildflowers as food for the
parasites *Glypta fumiferanae* (Hymenoptera: Ichneumonidae) and *Apanteles
fumiferanae* (Hymenoptera: Braconidae). J. Kans. Entomol. Soc. 60, 25–29.

English-Loeb, G., Rhainds, M., Martinson, T., Ugine, T. 2003. Influence of flowering cover
crops on *Anagrus* parasitoids (Hymenoptera: Mymaridae) and *Erythroneura*
leafhoppers (Homoptera: Cicadellidae) in New York vineyards. Agric. Forest
Entomol. 5, 173–181.

Estrada, C., R. J. W. Patrock, P. J. Folgarait, and L. E. Gilbert. 2006. Host specificity of four
Pseudacteon spp. (Diptera: Phoridae), parasitoids of fire ants in Argentina
(Hymenoptera: Formicidae). Florida Entomologist 89: 462-468.

Eubanks, M.D. 2001. Estimates of the Direct and Indirect Effects of Red Imported Fire Ants on
Biological Control in Field Crops. Biological Control 21, 35–43

Evans, E. W., and J. G. Swallow. 1993. Numerical responses of natural enemies to artificial
honeydew in Utah alfalfa. Environ. Entomol. 22: 1392-1401.

Fadamiro, H.Y. and Heimpel, G.E. 2001. Effects of partial sugar deprivation on lifespan and
carbohydrate mobilization in the parasitoid *Macrocentrus grandii* (Hymenoptera:
Braconidae). *Annals of the Entomological Society*, 94, 909–916.

Fadamiro, H.Y., Chen, L., Onagbola, E., Graham, L. 2005. Lifespan and patterns of

accumulation and mobilization of nutrients in sugar fed phorid fly *Pseudacteon tricuspis*. Physiological Entomology 30: 212-224.

Fadamiro, H.Y., Chen, L. 2005. Utilization of aphid honeydew and floral nectar by *Pseudacteon tricuspis* (Diptera: Phoridae), a parasitoid of imported fire ants, *Solenopsis* spp. (Hymenoptera: Formicidae). Biological Control, 34: 73-82.

Farnum, J. M., and K. M. Loftin. 2011. Distribution of *Pseudacteon curvatus* and *Pseudacteon tricuspis* (Diptera: Phoridae) in Arkansas. Fla. Entomol. 94: 15-21.

Feener, D. H., Jr. 1981. Competition between ant species: outcome controlled by parasitic flies. Science 214: 815-817.

Ferreira, C., B. B. Torres, and W. R. Terra. 1998. Substrate specificities of midgut β-glycosidases from insects of different orders. *Comp. Biochem. Physiol.* 119B:219-25.

Fischbein, D., C. Bernstein, and J.C. Corley. 2012. Linking reproductive and feeding strategies in the parasitoid *Ibalia leucospoides*: does feeding always imply profit? Evol Ecol. DOI 10.1007/s10682-012-9608-9

Folgarait, P. J., and L. E. Gilbert. 1999. Phorid parasitoids affect foraging activity of *Solenopsis richteri* under different availability of food in Argentina. Ecol. Entomol. 24: 163-173.

Folgarait, P.J., O.A. Bruzzone, R.J. Patrock, and L.E. Gilbert. 2000. Developmental rates and host specificity for *Pseudacteon* parasitoids (Diptera: Phoridae) of fire ants (Hymenoptera: Formicidae) in Argentina. Journal of Economic Entomology. 95(6):1151-1158.

Gardner, W.A., Diffie S, Vander Meer RK, Brinkman MA. 2008. Distribution of the fire ant

      (Hymenoptera: Formicidae) hybrid in Georgia. Journal of Entomological Science

      43: 133-137.

Gilbert, L. E. and L. W. Morrison. 1997. Patterns of host specificity in *Pseudacteon* parasitoid

      flies (Diptera: Phoridae) that attack *Solenopsis* fire ants (Hymenoptera:

      Formicidae). Environmental Entomology 26: 1149-1154.

Gilbert, F.S. and Jervis, M.A. 1998. Functional, evolutionary and ecological aspects of feeding-

      related mouthpart specializations in parasitoid flies. *Biological Journal of the*

      Linnean Society 63: 495–535.

Giron, D. and J. Casas. 2003a. Lipogenesis in an adult parasitic wasp. Journal of Insect

      Physiology 49:141–147

Giron, D. and Casas, J. 2003b. Mothers reduce egg provisioning with age. Ecology Letters,

      6:273–277.

Graham LC, Porter SD, Pereira RM, Dorough HD, Kelley AT. 2003. Field releases of the

      decapitating fly *Pseudacteon curvatus* (Diptera: Phoridae) for control of imported

      fire ants (Hymenoptera: Formicidae) in Alabama, Florida, and Tennessee. Florida

      Entomologist 86: 335-340.

Gurr, G. M. and S. D. Wratten. 1999. "Integrated biological control": a proposal for enhancing

      success in biological control. *International Journal of Pest Management* 45(2): 81-

      84.

Gurr, G.M., Wratten, S.D., Altieri, M. 2004. Ecological Engineering for Pest Management.

      Advances in Habitat Manipulation of Arthropods. CSIRO Publishing, Australia.

Hagen, K. S. 1986. Ecosystem analysis: plant cultivars (HPR), entomophagous species and food

supplements, pp. 151-197. *In* D. J. Boethel and R. D. Eikenbary [eds.], Interactions

of plant resistance and parasitoids and predators of insects. Wiley, New York.

Hausmann, C., Wäckers, F.L., Dorn, S., 2005. Sugar convertibility in the parasitoid *Cotesia*

glomerata (Hymenoptera: Braconidae). Archives of Insect Biochemistry and

Physiology 60, 223–229.

Heimpel, G. E., J. A. Rosenheim, and M. Mangel. 1997. Predation on adult *Aphytis* parasitoids in

the field. *Oecologia* 110: 346-352.

Heimpel, G .E., Lee, J.C., Wu, Z., Weiser, L., Wäckers, F. and Jervis, M.A. 2004. Gut sugar

analysis in field-caught parasitoids: adapting methods originally developed for

biting flies. *International Journal of Pest Management*, 50, 193 – 198.

Heimpel, G.E., and Jervis, M.A. 2005. Does floral nectar improve biological control by

parasitoids? In: Wäckers, F., van Rijn, P., Bruin, J. (Eds.), Plant-provided Food for

Carnivorous Insects: A Protective Mutualism and its Applications. Cambridge

University Press, Cambridge, UK, pp. 267–304.

Henne, D. C., S. J. Johnson, and S. D. Porter. 2007a. Status of *Pseudacteon tricuspis* Borgmeier

(Diptera: Phoridae) releases in Louisiana. Fla. Entomol. 90: 565-569.

Hocking, H. 1966. The influence of food on longevity and oviposition in *Rhyssa persuasoria* (L.)

(Hymenoptera: Ichneumonidae). Journal of the Australian Entomological Society,

6:83–88

Idoine, K., Ferro, D.N., 1988. Aphid honeydew as a carbohydrate source for *Edovum puttleri*

(Hymenoptera: Eulophidae). Environ. Entomol. 17, 941–944.

Idris, A. B. and E. Grafius. 1995. Wildflowers as nectar sources for *Diadegma insulare*

(Hymenoptera: Ichneumonidae), a parasitoid of diamondback moth (Lepidoptera:

Yponomeutidae). *Environmental Entomology* 24: 1726-1735.

Imported Fire Ant eXtension Community of Practice. 2015. Information for Alabama Residents.
http://www.extension.org/pages/16168/information-for-alabama-residents#.VQTa3OTyCm7 (last accessed 15 March 2015)

Imported Fire Ant eXtension fire ant control materials for Alabama home owners. 2015.
http://www.aces.edu/pubs/docs/A/ANR-0175-A/ANR-0175-A.pdf (last accessed 15 June 2015)

Imported Fire Ant eXtension fire ant control: the two-step method and other approaches. 2014.
http://www.extension.org/pages/14345/fire-ant-control:-the-two-step-method-and-other-approaches#.VX9VmrblZ60 (last accessed 15 June 2015)

Jamont, M., C. Dubois-Pot, and B. Jaloux. 2014. Nectar provisioning close to host patches increases parasitoid recruitment, retention and host parasitism. Basic and Applied Ecology 15: 151–160

Jervis, M.A. 1998. Functional and evolutionary aspects of mouthpart structure in parasitoid wasps. *Biological Journal of the Linnean Society* 63: 461–93.

Jervis, M. A., Kidd, N. A. C., Fitton, M. G., Huddleston, T., and Dawah, H. A. 1993. Flower-visiting by hymenopteran parasitoids. *Journal of Natural History*, *27*, 67–105.

Jervis, M.A., Heimpel, G.E., Ferns, P.N., Harvey, J.A. and Kidd, N.A.C. 2001. Life-history strategies in parasitoid wasps: A comparative analysis of 'ovigeny'. *Journal of Animal Ecology* 70: 442–58.

Jervis, M.A., Boggs, C.L. and Ferns, P.N. 2005a. Egg maturation strategy and its associated

tradeoffs in Lepidoptera: A review and synthesis. *Ecological Entomology* 30: 1–17.

Kloft, W. J. J., Maurizio, A. and Kaeser, W. 1985. Waldtracht und Waldhonig in der Imkerei. Ehrenwirth Verlag, München.

Koptur, S. 1992. Extrafloral nectary-mediated interactions between insects and plants. *Insect Plant Interactions*, Vol. IV (ed. E. A. Bernays), pp. 81–129. CRC Press, Boca Raton, Florida.

Lard, C.F., Salin, V., Willis, D.B., Robison, S., Hadley, J. and Schroeder, K. 2001. The statewide economic impact of red imported fire ants in Texas: A part of the Texas Fire Ant Initiative 1999-2001. Department of Agricultural Economics, Texas A&M University, Department of Agricultural and Applied Economics, College Station, TX.

Lard, C.F., Schmidt, J., Morris, B., Estes, L., Ryan, C., and Bergquist, D. 2006. An Economic Impact of Imported Fire Ants in the United States of America. Texas A&M University, College Station, Texas. http://fireantecon.tamu.edu.

LeBrun, E.G., R. M. Plowes, and L.E. Gilbert. 2012a. Imported fire ants near the edge of their range: disturbance and moisture determine prevalence and impact of an invasive social insect. Journal of Animal Ecology 2012, 81, 884–895

LeBrun, E.G., R. M. Plowes, and L.E. Gilbert. 2012b. Death by 1000 cuts: Continuing to assemble communities of phorid fly parasitoids for the biological control of imported fire ants. 2012 Annual Imported Fire Ant Conference Nashville Tennessee.

Lee, J.C. and G.E. Heimpel. 2003. Nectar availability and parasitoid sugar feeding. In *Proceedings, first international symposium on biological control of arthropods* (pp. 220–225).

Lee, J.C. and G.E. Heimpel. 2004. Dynamics of parasitoids and nectar sources. In *Proceedings of California Conference on Biological Control* (pp. 40–44).

Lee, J.C., Heimpel, G.E. and Leibee, G.L. 2004. Comparing floral nectar and aphid honeydew diets on the longevity and nutrient levels of a parasitoid wasp. Entomologia Experimentalis et Applicata, 111, 189–199.

Lee, J., Andow, D.A. and Heimpel, G.E. 2006. Influence of floral resources on sugar feeding and nutrient dynamics of a parasitoid in the field. *Ecological Entomology* 31: 470–80.

Lee, J.C. and G.E. Heimpel. 2008. Floral resources impact longevity and oviposition rate of a parasitoid in the field. *Journal of Animal Ecology* 2008, 77, 565–572

Lewis, W. J., Stapel, J. O., Cortesero, A. M., and Takasu, K. 1998. Understanding how parasitoids balance food and host needs: importance to biological control. *Biological Control* 11, 175-183.

Linker, H. M., D. B. Orr, and M. E. Barbercheck. 2009. Insect Management on Organic Farms. North Carolina Cooperative Extension Service. *Organic Production Series— Insect Management.* Pages 1-37

Löding HP. 1929. An ant (*Solenopsis saevissima richteri* Forel). Insect Pest Survey Bulletin 9: 241. Washington, D. C.: Bureau of Entomology.

Lofgren, C.S. 1986. The economic importance and control of imported fire ants in the

United States, pp. 227-255. *In* Vinson, S.B. (ed.), Economic impact and control of social insects. Praeger, New York.

Mehdiabadi, N. J. and L. E. Gilbert. 2002. Colony-level impacts of parasitoid flies on fire ants. Proceedings: Biological Sciences 269: 1695-1699.

Morrison, L.W., C.G. Dall'Agilo-Holvorcem, L.E. Gilbert. 1997. Oviposition behavior and development of *Pseudacteon* flies (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae). Environmental Entomology. 26:716-724.

Morrison, L. W. and L. E. Gilbert. 1999. Host specificity in two additional *Pseudacteon* spp. (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae). Florida Entomologist 82: 404-409.

Morrison, L. W., E. A. Kawazoe, R. Guerra, and L. E. Gilbert. 1999. Phenology and dispersal in *Pseudacteon* flies (Diptera: Phoridae), parasitoids of *Solenopsis* fire ants (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 92: 198-207.

Morrison, L.W., S. D. Porter, E, Daniels and M.D. Korzukhin. 2004. Potential global range expansion of the invasive fire ant, Solenopsis invicta. *Biological Invasions* 6: 183–191, 2004.

Morrison, L. W. and J. R. King. 2004. Host location behavior in a parasitoid of imported fire ants. Journal of Insect Behavior 17: 367-383.

Morrison, L. W. and S. D. Porter. 2005a. Phenology and parasitism rates in introduced populations of *Pseudacteon tricuspis*, a parasitoid of *Solenopsis*

invicta. BioControl 50: 127-141.

Naksathit, A.T., Edman, J.D. and Scott, T.W. 1999. Amounts of glycogen, lipid, and sugar in
      adult female *Aedes aegypti* (Diptera: Culicidae) fed sucrose. Journal of Medical
      Entomology, 36:8–12.

Nicholls, C.I., Parrella, P., Altieri, A. 2000. Reducing the abundance of leafhoppers and thrips in
      a northern California organic vineyard through maintenance of full season floral
      diversity with summer cover crops. Agri. Forest Entomol. 2:107–113.

Olson, D.M. and Andow, D.A. 1998. Larval crowding and adult nutrition effects on longevity
      and fecundity of female *Trichogramma nubilale* Ertle & Davis (Hymenoptera:
      Trichogrammatidae). Environmental Entomology, 27:508–514.

O'Brien, D. M., Boggs, C. L. and Fogel, M. L. 2004. Making eggs from nectar: the role of life
      history and dietary carbon turnover in butterfly reproductive resourceallocation.
      Oikos 105, 279-291.

Olson, D. M., H. Fadamiro, J. G. Lundgren, and G. E. Heimpel. 2000. Effects of sugar feeding
      on carbohydrate and lipid metabolism in a parasitoid wasp. *Physiological*
      Entomology 24:1-10.

Palmer, K. 2014. Phenology of *Pseudacteon* spp. (Diptera: Phoridae) flies in Alabama.
      Dissertation submitted to Auburn University, Alabama, U.S.A.

Plowes, R. M., E. G. LeBrun, and L. E. Gilbert. 2011. Introduction of the fire ant decapitating fly
      Pseudacteon obtusus in the United States: factors influencing establishment in
      Texas. BioControl 56:295-304.

Porter, S.D., H.G. Fowler and W.P MacKay. 1992. Fire ant mound densities in the

United States and Brazil (Hymenoptera: Formicidae). Journal of Economic Entomology. 85: 1154-1161.

Porter, S.D., Pesquero, M.A., Campiolo, S., Fowler, H.G. 1995. Growth and development of *Pseudacteon* phorid fly maggots (Diptera: Phoridae) in the heads of *Solenopsis* fire ant workers (Hymenoptera: Formicidae). Environ Entomol 24:475–479

Porter, S. D., H. G. Fowler, S. Campiolo, and M. A. Pesquero. 1995b. Host specificity of several *Pseudacteon* (Diptera: Phoridae) parasites of fire ants (Hymenoptera: Formicidae) in South America. Florida Entomologist 78: 70-75

Porter, S.D. 1998a. Biology and behavior of *Pseudacteon* decapitating flies (Diptera: Phoridae) that parasitize *Solenopsis* fire ants (Hymenoptera: Formicidae). Florida Entomologist. 81(3):292-309.

Porter, S. D. 1998b. Host-specific attraction of *Pseudacteon* flies (Diptera:Phoirdae) to fire ant colonies in Brazil. Florida Entomologist 81: 423-429.

Porter, S. D. and L. E. Alonso. 1999. Host specificity of fire ant decapitating flies (Dipera: Phoridae) in laboratory oviposision tests. Journal of Economic Entomology 92: 110-114.

Porter, S.D., and M.A. Pesquero. 2001. Illustrated key to *Pseudacteon* decapitating flies (Diptera: Phoridae) that attack *Solenopsis saevissima* complex fire ants in South America. Florida Entomologist. 84(4):691-699.

Porter, S. D., L. A. Nogueira de Sá, and L. W. Morrison. 2004. Establishment and dispersal of
the fire ant decapitating fly *Pseudacteon tricuspis* in North America. Biological
Control 29: 179-188.

Porter, S. D. and L. E. Gilbert. 2004. Assessing host specificity and field release potential of fire
ant decapitating flies (Phoridae: *Pseudacteon*). pp. 152-176. In R. G. Van Driesche,
Murray, T. and R. Reardon [eds.]. Assessing host ranges for parasitoids and
predators used for classical biological control: a guide to best practice. FHTET-
2004-03, USDA Forest Service, Morgantown, West Virginia.

Porter, S.D., Calcaterra, L.A. 2012. Establishment, dispersal, and competitive impacts of a third
fire ant decapitating fly (Pseudacteon obtusus) in North Central Florida. Biological
Control. 64:66-74.

Puckett, R.T. 2008. Biological and ecological aspects of field-released fire ant decapitation flies
Pseudacteon spp. (Diptera: Phoridae); parasitoids of red imported fire ants
Solenopsis invicta Buren (Hymenoptera: Formicidae). Dissertation submitted to
Texas A&M University, Texas, U.S.A.

Puckett, R. T., A. A. Calixto, J. J. Reed, D. L. McDonald, B. 'Bart' Drees, and R. E. Gold. 2013.
Effectiveness Comparison of Multiple Sticky-Trap Configurations for Sampling
Pseudacteon spp. Phorid Flies (Diptera: Phoridae). Entomological Society of
America 42:4.

Imported Fire Ant eXtension Community of Practice. 2014. Geographic distribution of fire ants.
http://www.extension.org/pages/9725/geographic-distribution-of-fire-

ants#.VCnt3xaZd6U. (last accessed 29 September 2014).

Rusch, A., S. Suchail, M. Valantin-Morison, J.-P. Sarthou, and J. Roger-Estrade. 2013. Nutritional state of the pollen beetle parasitoid *Tersilochus heterocerus* foraging in the field. Biocontrol 58:17–26.

Simberloff, D. and P. Stiling. 1996. How risky is biological control? Ecology 77: 1965-1974.

Sharma, K., and H.Y. Fadamiro. 2013. Fire ant alarm pheromone and venom alkaloids act in concert to attract parasitic phorid flies, *Pseudacteon* spp. Journal of Insect Physiology 59: 1119-1124.

Singh, D.K., Sale, P.W.G., Pallaghy, C.K., and Singh, V. 2000. Role of proline and leaf expansion rate in the recovery of stressed white clover leaves with increased phosphorus concentration. New Phytol 146 (2): 261-269.

Steppuhn, A. and Wäckers, F. L. 2004. HPLC sugar analysis reveals the nutritional state and the feeding history of parasitoids. Volume 18, Issue 6, pages 812–819

Systat Software, 2007. SigmaPlot, Technical Graphing & Statistical Analysis Software, Version 8.0. Systat Software, Inc., Chicago, IL.

Thead, L. G., Vogt, J. T., and Streett, D. A. 2005. Dispersal of the fire ant decapitating fly, *Pseudacteon curvatus* (Diptera: Phoridae) in Northeast Mississippi. Florida Entomol. 88: 214-216.

Tschinkel WR. 2006. The Fire Ants. President and Fellows of Harvard College, Boston, MA.

Tylianakis, J.M., Didham, R. K. and Wratten, S.D. 2004. Improved fitness of aphid parasitoids receiving resource subsidies. *Ecology*, 85, 658–666.

van Handel, E. 1965. Microseparation of glycogen, sugars and lipids. Analytical Biochemistry, 11:266–271.

van Handel, E. 1967. Determination of fructose and fructose yielding carbohydrates with cold anthrone. Analytical Biochemistry, 19:193–194.

van Handel, E. 1969. Metabolism of fructose in the intact mosquito: exclusion of glucose and trehalose as intermediates. Comparative Biochemistry and Physiology, 29:413–421.

van Handel, E., Haeger, J.S. and Hansen, C.W. 1972. The sugars of some Florida nectars. American Journal of Botany, 59:1030–1032.

van Handel, E. 1984. Metabolism of nutrients in the adult mosquito. Mosq. News 44: 573-579.

van Handel, E. 1985a. Rapid determination of glycogen and sugars in mosquitoes. Journal of the American Mosquito Control Association, 1:299–301.

van Handel, E. 1985b. Rapid determination of total lipids in mosquitoes. Journal of the American Mosquito Control Association, 1:302–304.

van Handel, E. and Day, J.F. 1988. Assay of lipids, glycogen and sugars in individual mosquitoes: correlations with wing length in field-collected *Aedes vexans*. Journal of the American Mosquito Control Association, 4:549–550.

van der Meer, R.K. and C.S. Lofgren. 1990. Chemotaxonomy applied to fire ant systematic in the United States and South America. Pp. 75-84 *In* RK Vander Meer, K Jaffe and A Cedeno [eds.], Applied myrmecology: A world perspective. Westview Press, Boulder, CO.

van der Meer, R.K. and S.D. Porter. 2002. Fire ant, *Solenopsis invicta*, worker alarm pheromones attract Pseuacteon phorid flies. Proceedings of the 2002 Imported Fire Ant Conference, Athens, Georgia, pp.77-80.

Vazquez, R. J., S. D. Porter, and J. A. Briano. 2006. Field release and establishment of the decapitating fly *Pseudacteon curvatus* on red imported fire ants in Florida.

BioControl 51: 206-216.

Vinson SB. 1997. Invasion of the red imported fire ant (Hymenoptera: Formicidae): Spread,
     biology, and impact. American Entomologist 43: 23-39.

Vogt, J.T. and Streett, D.A. 2003. *Pseudacteon curvatus* (Diptera: Phoridae) laboratory
     parasitism, release and establishment in Mississippi. *Journal of Entomological
     Science* 38: 317-320.

Wäckers, F. L. 2001. A comparison of nectar and honeydew sugars with respect to their
     utilization by the hymenopteran parasitoid *Cotesia glomerata*. Journal of Insect
     Physiology 47: 1077-1084.

Wäckers, F. L. 2005. Suitability of extrafloral nectar, pollen, and honeydew as insect food
     sources. In F. L. Wäckers, P. C. J. van Rijn, and J. Bruin (Eds.), *Plant-provided
     food for carnivorous insects: A protective mutualism and its applications* (pp. 17–
     74). Cambridge: Cambridge University Press.

Warburg, M.S., and Yuval, B. 1996. Effects of diet and activity on lipid levels of adult
     Mediterranean fruit flies. Physiol Entomol 21: 151-158.

Wasmann, E., 1918. Zur Lebensweise und Fortpflanzung von Pseudacteon formicarum Verr.
     (Diptera, Phoridae). Biologisches Zentralblatt 38, 317–329.

Williams, L. III, and Roane, T.M. 2007. Nutritional ecology of a parasitic wasp: Food source
     affects gustatory response, metabolic utilization, and survivorship. Journal of Insect
     Physiology, 53:1262-1275.

Winkler, K., Wäckers, F.L., Stingli, A., van Lenteren, J.C., 2005. *Plutella xylostella*

(diamondback moth) and its parasitoid *Diadegma semiclausum* show different

gustatory and longevity responses to a range of nectar and honeydew sugars.

Entomologia Experimentalis et Applicata 115, 187–192.

Wolcott, G.N. 1942. The requirements of parasites for more than hosts. Science, 96:317–318.

Wyatt, G.R. 1967. The biochemistry of sugars and polysaccharides in insects. Advances in Insect

Physiology, 4:287–359.

Wyckhuys, K.A.G., Strange-George, J.E., Kulhanek, C.A., Wäckers, F.L., and Heimpel, G.E.

2008. Sugar feeding by the aphid parasitoid *Binodoxys communis*: How does

honeydew compare with other sugar sources? Journal of Insect Physiology 54:481–

491.

Zacaro, A.A., and Porter, S.D. 1997. First report on the ultrastructure (TEM) of a truly parasitic

phorid: the ovary of the scuttle fly *Pseudacteon solenopsidis* Schmitz (Diptera:

Phoridae). Acta Microscopica 6, 622–623. Suppl. B.

Zacaro, A.A., and Porter, S.D. 2003. Female reproductive system of the decapitating fly

Pseudacteon wasmanni Schmitz (Diptera: Phoridae). Arthropod Structure &

Development 31:329–337.

Table 1. Diet-dependent longevity (days ± SE) of female and male *P. cultellatus, P. curvatus*,

and *P. obtusus*

	Female				Male			
Species	Water only	24 h fed 50%	25% sucrose	50% sucrose	Water only	24 h fed 50%	25% sucrose	50% sucrose
Pseudacteon cultellatus	2.6±0.15c(c)	2.8±0.11b(bc)	3.0±0.10c(b)	3.6±0.14b(a)	2.7±0.09b(b)	2.8±0.11c(b)	3.2±0.11c(b)	4.2±0.18b(a)
P. curvatus	3.3±0.10b(b)	4.4±0.24a(a)	4.8±0.28b(a)	4.6±0.29b(a)	3.4±0.11a(b)	3.6±0.13b(ab)	4.3±0.24b(a)	4.0±0.22b(ab)
P. obtusus	3.9±0.19a(d)	5.1±0.23a(c)	6.3±0.41a(b)	7.3±0.40a(a)	3.5±0.12a(c)	5.2±0.19a(b)	6.6±0.34a(ab)	8.7±0.42a(a)

Means within the same column having different letters are significantly different ($P < 0.05$,

Tukey HSD test). Similarly, means across the same row having different letters indicated in

parentheses are significantly different ($P < 0.05$, Tukey HSD test).

Figure legend

Figure 1. Survivorship curves for (A) female *P. cultellatus* (B) female *P. curvatus* and (C)

female *P. obtusus* fed different diet treatments.

Figure 2. Survivorship curves for (A) male *P. cultellatus* (B) male *P. curvatus* and (C) male *P.

obtusus* fed different diet treatments.

Figure 3: Histology features of the ovaries of the parasitic fly *P. obtusus* showing sectioned

ovaries (OV), eggs (g); note the muscular sheath (s) that covers each ovary. Scale: 100µm.
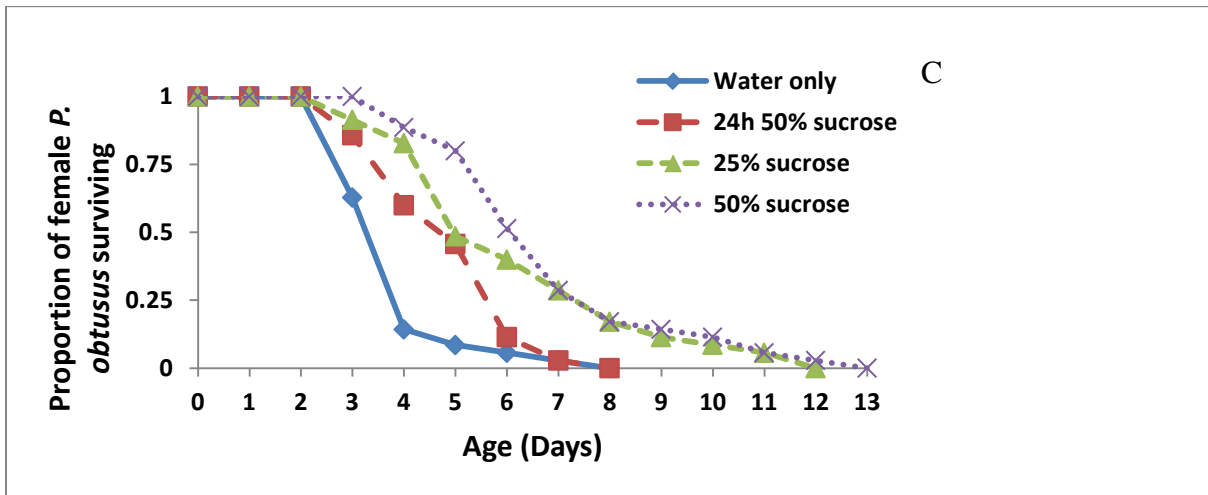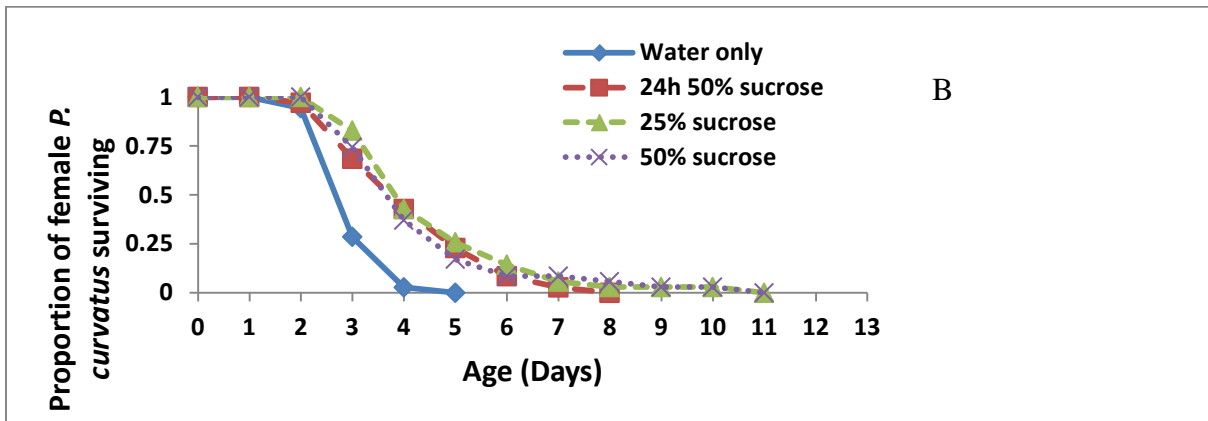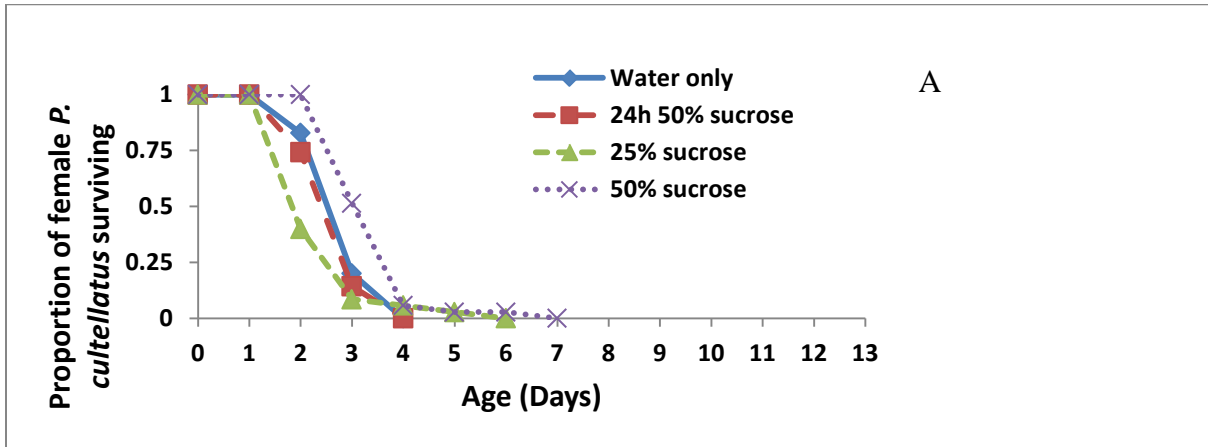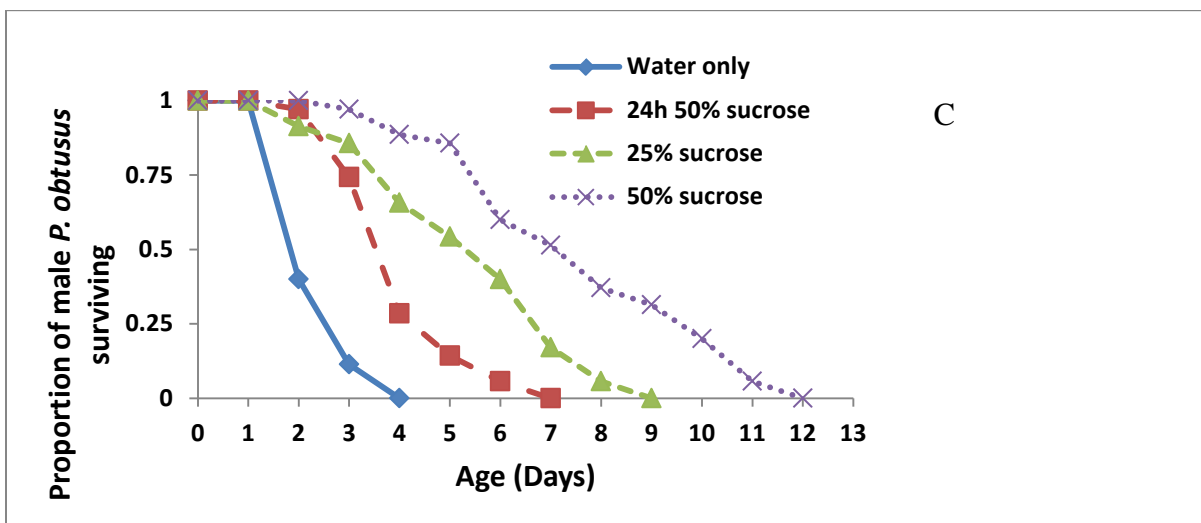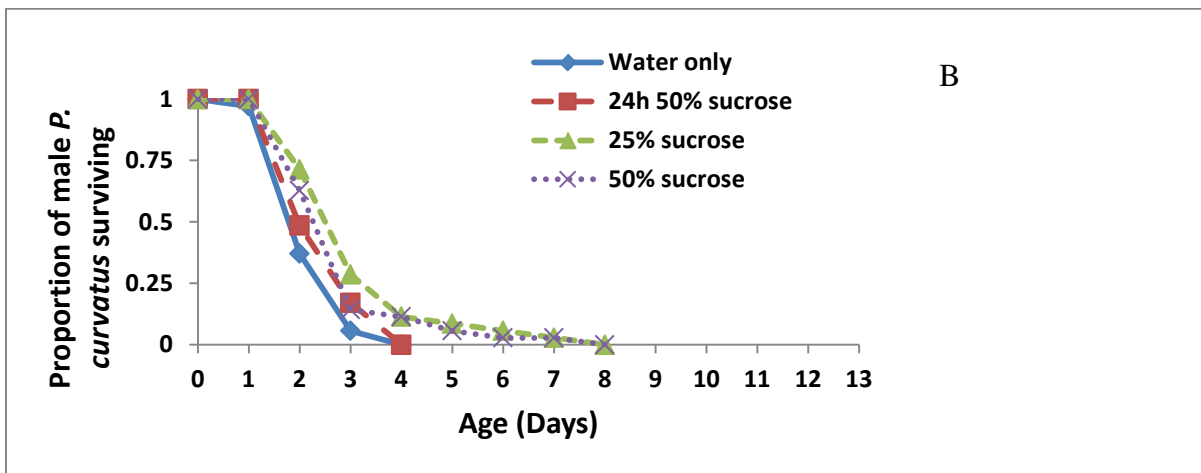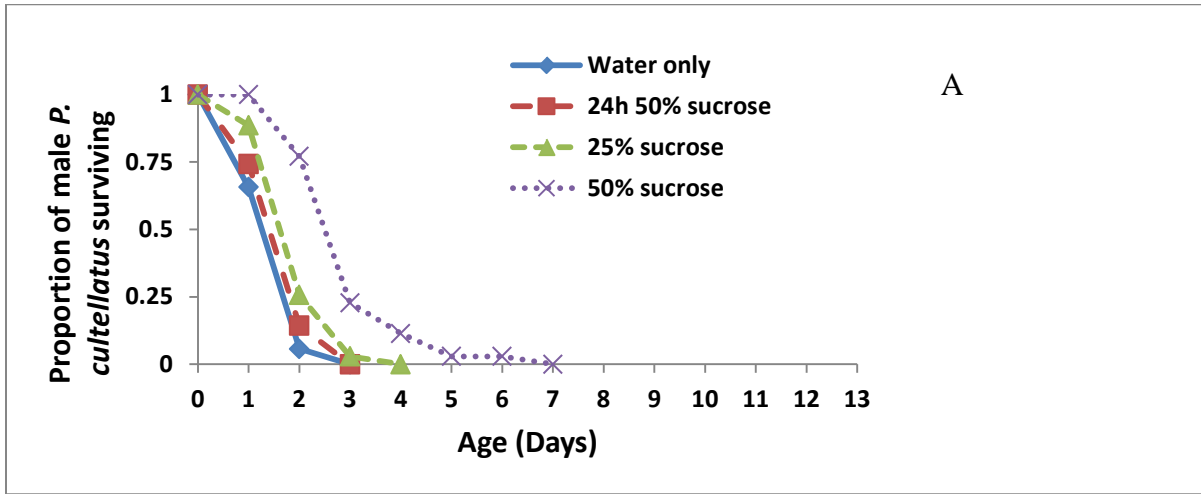
Figure 1.

Figure 2.

Figure 3.

.

Conclusions

This study demonstrates the importance of sugar-rich food on the longevity of

Pseudacteon parasitic flies. In general, the flies' survival was significantly greater when they

were sugar-fed than when sugar-starved. Sucrose concentrations of 25% and 50% were clearly

beneficial for the survival of *Pseudacteon* parasitic flies, and most sugar sources in the field

where these flies are being released have sucrose concentrations ranging from 20-50% (van

Handel et al. 1972). This finding suggests that not only are these flies capable of utilizing sugar

sources from plants in the field but that sugar sources are also within the range suitable to the

flies. In most cases, the survival of individuals provided 50% sucrose for only one day after

emergence, was significantly different from that of sugar-starved flies, suggesting that even

limited sugar availability can significantly increase adult lifespan of *Pseudacteon* parasitic flies.

There was a clear relationship between fly size and longevity of the *Pseudacteon* parasitic flies

tested, with large sized flies living longer than small sized flies in all diet treatments. This

finding suggests that longevity may be a function of fly size in *Pseudacteon* parasitic flies.

Overall, the increase in life span that resulted from sugar feeding enhances the efficacy of

Pseudacteon parasitic flies as biological control agents by allowing the parasitoids more time for

suitable fire ant host location.

The results from anthrone tests indicate that *P. curvatus* utilizes sugar sources in the field.

Although the gut sugar levels in sucrose-fed flies were significantly higher than those found in

sucrose-starved individuals, those of field-collected flies and sucrose-fed flies were similar.

There was only a marginal (approximately 0.2µg) but insignificant increase in gut sugar level of

field-collected flies when compared with that of sucrose-starved flies. The results show significant effect of diet on gut sugar level. Although there was no significant effect of the level of body sugars on the diet treatments, there were marginal (approximately 0.1µg) but insignificant increases in body sugar levels of field-collected flies when compared with those of sucrose-starved flies. The body sugar levels of both field-collected and sugar-fed flies were the same. The effect of diet on glycogen levels showed similar trend to body sugar levels. Lipid levels were significantly higher in field-collected flies than in both sugar-fed and sugar-starved individuals. In general, the results suggest moderate utilization of sugar sources and significant utilization of lipid sources in the field by *P. curvatus*. Sugars and lipid are essential for meeting the energetic and reproductive needs of these parasitoids.

All specimens of newly emerged *P. obtusus* analyzed by light microscopy showed post-vitellogenic oocytes inside the ovaries. The lack of typical follicles (oocyte-nurse cell complexes) in all specimens suggests that oogenesis occurred during the pre-adult stage, likely the pupal stage, indicating that *P. obtusus* is pro-ovigenic. Similar results were reported for *P. formicarum*, *P. solenopsidis* and *P. wasmanni* (Wasmann 1918, Zacaro and Porter 1997, Zacaro and Porter 2003), which may indicate an adaptation to maximize fecundity in these short-lived *Pseudacteon* flies.