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Biological Control of Red Imported Fire Ant (*Solenopsis invicta*) Using *Pseudacteon* (Diptera: Phoridae) in Central Arkansas

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Biological Control of Red Imported Fire Ant (*Solenopsis invicta*) Using *Pseudacteon* (Diptera:
Phoridae) in Central Arkansas

Biological Control of Red Imported Fire Ant (*Solenopsis invicta*)
Using *Pseudacteon* (Diptera: Phoridae) in Central Arkansas

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Entomology

by

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This thesis is approved for recommendation to the Graduate Council.

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Abstract

Red imported fire ants are major pests in the southeastern United States. As a part of an integrated pest management strategy, a biological control program has been implemented which includes *Pseudacteon* decapitating flies. These flies are parasitoids of fire ant workers and two species of *Pseudacteon* are established in Arkansas: *Pseudacteon tricuspis* and *Pseudacteon curvatus*. *Pseudacteon cultellatus* and *P. obtusus* were released in the spring and fall of 2013. Despite sampling throughout 2014, establishment cannot be confirmed. Two phorid fly sampling methods, hand aspirating and sticky traps, were studied. A vacuum aspirator was compared with two sticky trap configurations. Sticky traps used one of three bait types: live fire ants, dead fire ants, and foraging fire ants. Both *P. curvatus* and *P. tricuspis* were captured, but many more *P. curvatus* were caught than *P. tricuspis*. Foraging fire ant-baited traps caught relatively few flies. In contrast, live ant and dead ant-baited traps captured many flies, with dead ant-baited traps performing better overall. A lab study was performed to investigate possible competition among flies that were released in Arkansas because *P. curvatus* has been reported to competitively displace *P. tricuspis* in other states. *Pseudacteon curvatus* was paired with *P. obtusus* and *P. cultellatus* in parasitization trials. Host size, sex ratios, species ratios, and development times were recorded to evaluate possible competitive interactions between species. Due to a shortage of *P. cultellatus* at the Florida Department of Agriculture rearing lab, this species was not analyzed. *Pseudacteon curvatus* host sizes did not vary by sex but *P. obtusus* hosts were smaller for males than for *P. obtusus* females. Sex ratios varied between species, but competition treatments did not influence sex ratios or rates of parasitism.

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I also thank the students and professors of the Entomology Department for the support and sound advice throughout my time in the program.

Dedication

This thesis is dedicated to my parents who encouraged my interest in insects from the beginning, and to my wife for her patience and unwavering support despite her personal aversion to insects.

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Chapter 1. Review of Literature about *Solenopsis invicta* and *Pseudacteon* Phorid Flies

Introduction

Solenopsis invicta, the red imported fire ant, is a major pest in tropical and subtropical regions outside of its native range including Taiwan, mainland China, and Australia (Wilson 1951, Ascunse et al. 2011). Principally it is a nuisance pest, causing painful stings to those that disturb its nests (Vinson 1997). However, these stings can lead to serious medical conditions, either through secondary infections or through severe allergic reactions (DeShazo et al. 1990). In addition, fire ants cause agricultural damage through yield loss and equipment repair. *Solenopsis invicta* can aggregate in electrical equipment when individual ants contact open wires and get electrocuted, thus releasing pheromones which attract other ants. Eventually, the aggregation of ants can cause short-circuits resulting in power outages (Slowik et al. 1996). Ecologically, they are the dominant species in infested areas and can alter biodiversity (Allen et al. 2004). They are of economic importance, costing the United States over \$6.2 billion annually through damage and through control measures (Lard et al. 2006). The most effective control measure is chemical control which is not sustainable as a stand-alone measure (Vander Meer et al. 2007). For this reason, researchers have suggested biological control might be a viable aspect of the integrated control of *S. invicta* (Feener and Brown 1992).

Natural History of *Solenopsis invicta*

The red imported fire ant (RIFA) is identified by its 10-segmented antennae, red body with a black gaster, and two petiole segments (Fisher and Cover 2007). In addition, the RIFA can be distinguished from other species in the genus *Solenopsis* by the following characteristics: 1) four

mandibular teeth, 2) antennal scape as long or longer than the vertex above the eye, 3) the presence of a median clypeal tooth, 4) lack of a petiolar process (Jacobson et al. 2006), and 6) a striated mesopleuron (Vinson 1997). These diagnostic characters require magnification, preferably with a dissecting scope, and there is some variation which may lead to misidentification (Jacobson et al. 2006). Colonies can be easily identified by the dome-shaped mound the ants erect and their aggressive swarming behavior when disturbed (Loftin and Hopkins 2007). Since fire ants are eusocial insects, the colony is made up of separate castes – the worker caste which maintains the colony and the reproductive males and females (i.e. alates) including the queen (Vinson 1997). Female alates and queens are much larger than the worker ants and have large heads with ocelli (Tschinkel 2006). Male alates are black rather than reddish and have small heads. Both male and female reproductives possess wings. These adults, along with the brood make up the constituents of the colony.

The life cycle of any ant species is best measured at the colony level (Holldobler and Wilson 1994). In the case of *Solenopsis invicta* the cycle begins with a newly mated alate female. After mating up to 1000 feet in the air, the newly mated queen locates a disturbed area such as a grazing pasture (Tschinkel 2006), breaks off her wings, and begins to excavate a short vertical tunnel (Markin et al. 1972). Soon she begins laying and tending eggs. These first offspring are very small (~ 0.5 mm), and are called minims or nanitic workers (Porter and Tschinkel 1986). The queen feeds and rears these first workers solely from the reserves in her body (Taber 2000). Once mature, the minims tend the queen and forage for the small colony. As the number of workers increases, the size of the colony grows, as does the size variation of workers. After about five years, the colony is mature and can contain as many as 220,000 workers (Tschinkel 1988).

The colony begins to produce the alates once the workers can provide sufficient resources. Ants exhibit haplodiploidy (Holldobler and Wilson, 1994), in which females are diploid and arise from fertilized eggs, whereas males are haploid and arise from unfertilized eggs (Taber 2000). Inside the colony, the alates do not contribute to foraging, brood care, or upkeep (Holldobler and Wilson 1990). However, on warm days after a rain, the alates are led out of the mound by the workers and up onto any nearby tall objects. From here, the alates take flight and ascend to just below 1000 feet, where mating occurs (Taber 2000). The males die, and the newly mated queens continue the cycle.

When *Solenopsis invicta* first colonized the United States, most of the colonies were monogyne, having only one functional queen per colony. However, polygyne colonies which have multiple queens were discovered in Mississippi (Glancey and Lofgren 1988), followed by other states throughout the southeast (Fletcher 1983 and Lofgren and Williams 1984). Polygyne colonies differ from monogyne colonies in two key ways. First, areas infested with polygyne colonies have much higher colony density (Mirenda and Vinson 1982). Secondly, the average worker size is smaller (Greenberg et al. 1985). Infestation by polygyne colonies has several implications in addition to these two factors. Polygyne colonies are more effective at outcompeting native ants due to the increased numbers of ants, which can monopolize food sources (Porter and Savignano 1990). Also, as a result of the higher mound densities, they can be more problematic in recreational areas (Porter et al. 1991). In general, queens from polygyne colonies disperse differently than those from monogyne colonies. Rather than dispersing via mating flights, polygyne queens disperse by “budding,” in which they form sister colonies nearby (Porter et al. 1988). Polygyny also results in decreased aggression against workers from other polygyne colonies (Vander Meer and Morel 1998). Other implications (which influence management

practices) include a resistance to the microsporidian *Kneallhazia* (= *Thelohania*) *solenopsae* Knell, Allen, and Hazard, a possible biological control agent (Oi 2006), and decreased efficacy of single mound chemical treatments (Porter et al. 1991).

The underlying mechanism that causes polygyny has been found to be the presence of a “supergene,” which is a non-recombinant group of closely associated loci (Mather 1950). This supergene is associated with the Gp-9 gene which influences pheromone-binding proteins in the cuticle (Krieger and Ross 2002). Gp-9 is very large, consisting of 1700 base pairs. Aside from its strong association with polygyny (Ross 1997), the gene also carries other consequences for the ants. For example, the homozygous recessive (Gp-9bb) genotype is lethal in queens (Ross 1997). In addition, polygyne queens are in general smaller than those from monogyne colonies (Porter et al. 1988). In addition, males with the recessive genotype are infertile (Lawson et al 2012). This leads to a higher proportion of uninseminated queens in areas infested by polygynous colonies. However, the success of polygynous colonies helps to counteract these drawbacks and maintains the recessive trait in the population (Ross 1997).

The success of *S. invicta* is partly due to its ability to efficiently exploit resources (Vinson 1997). Dietary preference is primarily responsible for this quality. *Solenopsis invicta* is omnivorous feeding on other arthropods (Hays and Hays 1959) and sugary liquids from plants (Green 1952, Tennant and Porter 1991). They may also tend aphids and mealybugs to provide carbohydrates from honeydew, which the aphids produce as waste (Hays and Hays 1959, Tschinkel 2006). They are effective foragers, using a wide variety of food sources (Hays and Arant 1960). Foraging territories vary depending on colony size, and may range from 50 to 1,200 ft² (Wilson et al. 1971). Only the oldest workers forage, and they share the food they acquire with their nestmates through trophallaxis, or the mouth-to-mouth exchange of food (Taber 2000). Any

solid foods that are brought into the mound are given to the fourth-instar larvae, which is the only stage at which the ants can ingest solids (Petralia and Vinson 1978). The preference of foraging workers for oils and solids holds implications for how toxicant baits are formulated (Hooper-Bui et al. 2002).

***Solenopsis invicta* in the United States**

The red imported fire ant was accidentally introduced into the United States from South America in the early 1930's possibly through dirt ballast (Buren et al. 1974). However, it was preceded by the black imported fire ant, *Solenopsis richteri* Forel (Loding 1929). *Solenopsis richteri* is only found in parts of Tennessee, Arkansas, Mississippi, and Alabama (Taber 2000), and has not reached the wide distribution of *S. invicta*. These species spread naturally through reproductive flights, but also artificially through transport on vehicles (Lofgren 1986a), or in nursery stock and other agricultural products (Lofgren 1986b). The most current range for the red imported fire ant is difficult to determine, but the annual United States Department of Agriculture, Animal and Plant Health Inspection Service quarantine map (Fig. 1.1) is a good indicator of the extent of the infestation (USDA APHIS 2014). The quarantine map reflects the approximate range of red imported fire ants and often does not include isolated infestations that occur in non-quarantined areas. Although within the last 80 years the red imported fire ant has spread across the southeastern United States, it has not yet reached its ultimate range in the United States. Korzukhin et al. (2001) provided a model which, based on climatic variables, predicts the range limits to extend to the northernmost counties in Arkansas, Oklahoma, Tennessee, and Virginia, and into Oregon, northern California, Delaware, and Maryland. With increasing average temperatures related to climate change, the range limits may expand (Morrison et al. 2005).

Considering the wide range *S. invicta* may inhabit, an increasing number of the human population will experience the multiple ways in which it is a pest. The red imported fire ant is primarily a nuisance pest, but also causes medical, agricultural, and ecological damages (Lofgren et al. 1975). Approximately 30% of humans in fire ant-infested areas are stung annually (Adams and Lofgren 1981). Although most stings are not severe, some individuals may experience a severe allergic reaction requiring medical care (DeShazo et al. 1990). In agriculture, damage can occur directly through plant destruction (Vander Meer and Shatters 1999) or through damage to harvesting equipment (Banks et al. 1990). *Solenopsis invicta* may also tend aphids which can increase feeding damage by aphid populations (Reilly and Sterling 1983). Red imported fire ants may also disrupt the natural enemy assemblage in some crops (Eubanks 2001). Ecologically, the red imported fire ant reduces native ant diversity and richness (Kaspari 2000, Morrison 2002) along with other arthropods (Porter and Savignano 1990). Red imported fire ants also negatively impact vertebrate fauna such as reptiles, amphibians, birds, and mammals (Allen 2004). Impacts on reptiles include neonatal alligators (*Alligator mississippiensis*) which exhibit decreased weight gain in fire ant-infested nests (Reagan et al. 2000). The effects of fire ants on bobwhite quail (*Colinus virginianus*) has been subject of much debate (Allen 2004). Bobwhite quail population decline has been linked to fire ant infestation, although several other factors play a role in this decline (Allen et al. 1995). Finally, small mammals such as pygmy mice (*Baiomys taylori*) are negatively impacted by fire ants (Killian and Grant 1993). The monetary cost of controlling *S. invicta* places it among the most important pests in multiple settings. Lard et al. (2006) estimated that the annual economic impact of red imported fire ants in the United States is over \$6 billion. Arkansas' share of this sum is over \$128 million.

Although it is clear that the red imported fire ants are a definitive pest in the United States, situations exist in which they are beneficial. One notable example of *S. invicta* playing a beneficial role is in sugarcane production, where imported fire ants are the most important predator of the sugarcane borer, *Diatraea saccharalis* F. (Reagan et al. 1972). In addition, tick populations decreased in fire ant infested sites (Burns and Melancon 1977). Fire ants have also been shown to reduce horn fly (*Haematobia irritans* L.) larval populations (Summerlin et al. 1977). Despite these benefits, most Americans view fire ants as a pest.

Historically, state and federal agencies have attempted wide-scale control and eradication efforts against the imported fire ant. Although imported fire ants first arrived in 1918, the first coordinated fire ant eradication was not initiated until 1937 in Alabama using calcium cyanide dust, DDT, and other chemicals as individual mound treatments (Eden and Arant 1949). The onset of World War II led to the suspension of the control effort, but after the war, in 1948, the use of chlordane was implemented in Alabama and Louisiana. Chlordane was very effective, but fire ants continued to spread because fire ants quickly moved back into uninhabited areas (Williams et al. 2001). In 1957, after years of research, the U.S. Congress enacted a cooperative control program (Williams 1983) through the appropriation of \$2.4 million for eradication. This cooperative program funded much of the fire ant research in the following decades. During this time, the predominant chemicals used were heptachlor and dieldrin granules applied aerially over infested areas (Williams et al. 2001). They were effective, but the extensive area-wide use led to environmental concerns related to aquatic vertebrate toxicity and other non-target effects (Lofgren 1986a). As a response, Mirex was selected for area-wide fire ant control (Williams 1983). Mirex was effective against fire ants and was initially considered to be more environmentally friendly than its predecessors (Williams et al. 2001). Later, however, Mirex was

found to have serious drawbacks. Its residual activity as a bait was short and required frequent reapplications. It effectively eliminated fire ants from treated sites, but it also opened up the treated areas for recolonization from fire ants outside of treated areas. (Markin et al. 1974). In addition, the active ingredient in Mirex persisted in the environment for years (Carlson et al. 1976), had negative effects on estuarine organisms (Bookout et al. 1972), and was carcinogenic (Ulland et al. 1976). For these reasons, it was banned from use in 1970 (Lofgren 1986b). With the removal of Mirex, the eradication program for imported fire ants ended, and the paradigm shifted from eradication to management.

Current Management of Red Imported Fire Ant

Although eradication failed, the most effective means of control in most settings is still chemical control (Lofgren 1986b). The most current and effective approach to chemical control for a homeowner is the “two-step” method (Drees and Gold 2003). In this technique, fire ant bait is spread (broadcast application) over the area of concern. Fire ant baits are made up of a carrier, usually corn cob grit, solvent oil, and the active ingredient (Banks et al. 1985). Active ingredients are either insect growth regulators or toxins. Baits are the least expensive chemical formulation and are effective because they are attractive to the ants, exhibit delayed toxicity thereby allowing the chemical to be distributed throughout the colony via trophallaxis, and have minimal impact on the environment (Williams 1983). Several days after the bait application, the second step is to individually treat problem colonies that need immediate control with fast acting drenches or powders. Some non-chemical control methods are relatively effective. First, boiling water works well as an individual mound treatment (Tschinkel and Howard 1980) for small areas where the use of chemicals may not be preferred, or where the infestation is minor. However,

the use of large amounts of boiling water is labor intensive and transporting the water is can be dangerous if it is spilled on the users (Tschinkel and King 2007).

The most widely studied alternative to chemical control is the biological control of *Solenopsis invicta*. Porter et al. (1992) suggested that imported fire ants are pests in the United States because they are free from the influence of natural enemies. In South America, *S. invicta* are rarely pests (Porter et al. 1997a), and there is a large complex of natural enemies present.

Pathogens such as viruses, microsporidians, nematodes, parasitoids, and even a social parasite ant are all known natural enemies (Briano et al. 2012). The USDA Agricultural Research Service has been conducting research on classical biological control since 1998, in collaboration with state and university agencies throughout the southeast and Texas (Williams and Brenner 2001).

Three RNA viruses have been found in fire ants: SINV-1, SINV-2, and SINV-3 (Briano et al. 2012). All of these viruses are positive-strand RNA viruses (Valles et al. 2009). All three have been detected both in Argentina and in the United States at varying frequencies. Transmission occurs both vertically and horizontally. The effects for each virus vary. *Solenopsis invicta* workers infected with SINV-1 were less effective at defending against competing ant species, while SINV-2 is asymptomatic and SINV-3 causes worker and brood die-off and even colony collapse (Valles 2012). There is potential for development of biopesticides using these viruses, but more work needs to be done in this area.

Kneallhazia (= *Thelohania*) *solenopsae* and *Varimorpha invicta* Jouvenaz and Ellis are microsporidians which infect *S. invicta* (Briano et al. 2012). *Kneallhazia solenopsae* infects larvae, pupae, workers, and queens (Knell 1977) and results in decreased brood production

(Williams et al. 1999) and mound densities (Briano 2005). Both species are highly specific to *Solenopsis* (Briano et al. 2002) and have been shown to reduce mound densities up to 100% in South America (Briano 2005). *Kneallhazia solenopsae* is present in the United States (Williams and Oi 1998) and *V. invicta* has not been detected in the U.S. but has been suggested as a possible biological control agent (Oi et al. 2012) and is undergoing the approval process for release (Solter et al. 2012).

In addition to viruses and microsporidia, some nematode species have been discovered which parasitize *S. invicta* (McInnes and Tschinkel 1996). Most recently, *Allomermis solenopsae* Poinar, Porter, Tang, and Hyman has been shown to have detrimental effects on worker ants, including initiating inability to sting, lack of aggression, and shortened lifespan (Briano et al. 2012). Application of these nematodes as a biological control agent is possible, especially in areas with access to open water because the nematodes reproduce in water. However, more research on their basic biology, including host specificity, is needed (Poinar et al. 2007).

Much of the work has focused on a genus of decapitating parasitoid flies in the family Phoridae. In South America, twenty-four known species in the genus *Pseudacteon* attack fire ants (Porter 1998a), and several have been studied and released in the United States (Callcott et al. 2011).

Natural history of *Pseudacteon* Phorid Flies

Pseudacteon phorid flies are small, dipterans in the family Phoridae which are parasitoids of the *Solenopsis saevissima* complex of fire ants (Disney 1994). There are at least 18 species of *Pseudacteon* flies in this group (Porter 1998a), which can be distinguished by the morphology of the ovipositor (Porter and Pesquero 2001), a character which probably relates to how the flies insert their eggs into the ants (Wuellner et al. 2002a).

Biological information for *Pseudacteon* spp. is reviewed by Porter (1998a). Flies are attracted to active *S. invicta* workers, either during foraging, mound disturbance, or mating flights. The flies respond to alarm pheromones that the ants emit during these activities (Morrison and King 2004). Female flies search for a suitable host by hovering several millimeters above the ants. Then, they inject a single egg into the ant's thorax. Upon being struck by a fly, the host ant exhibits a defensive response in which the gaster curls under the thorax and the head is raised (Feener and Brown 1992). Once the egg hatches the larva begins to feed on the hemolymph of the ant. Parasitized ants exhibit lower activity and do not exit the colony as often as unparasitized workers (Henne and Johnson 2007). After approximately 3-4 days, the larva moves into the head of the ant, but does not immediately kill it (Porter et al. 1995a). For 10-60 days, the larva feeds on the hemolymph of the ant. Shortly before pupation, the larva releases an enzyme that digests the connective tissue of the head and detaches it from the thorax, killing the ant (Porter 1998a). Next, over the course of six to eight hours, the larva consumes the entire soft tissues of the head (Porter 1998a). When it begins pupation, it pushes the ant's mouthparts out of the oral cavity and forms a sclerotized cap. The pupal stage lasts from 17 to 29 days, depending on the species and the temperature (Folgarait et al. 2002a). During eclosion, the adult fly pops open the puparium and exits through the oral opening of the head capsule. For some species like *P. curvatus* Borgmeier and *P. cultellatus* Borgmeier mating occurs immediately after emergence (Wuellner et al. 2002b). However, for *P. obtusus* Borgmeier and *P. tricuspis* Borgmeier mating does not occur until they are attracted to ants (Orr et al. 1997, Porter et al. 1997b). In this case, males circle several centimeters over the activity searching for mates. When they find a female fly, they grab her and mate in the air. They often fall to the ground for a moment before they resume flight (Porter et al. 1997b). Though a mechanism that is not

understood sex determination in some species occurs, based on host size, in which female flies develop in larger hosts (Morrison et al. 1999).

As parasitoids, the life history of *Pseudacteon* is closely linked to their hosts. The relatively wide variety of species associated with a single host – *S. invicta* worker ants – is the result of evolutionary niche separation (Fowler 1997). Four factors contribute to the partitioning of their host. First, time of day varies among *Pseudacteon* spp. Some species are active during the morning and evening, such as *P. litoralis* Borgmeier, while others are active midday, such as *P. tricusps* (Pesquero et al. 1996) and *P. curvatus* (LeBrun et al. 2012). Second, in addition to circadian activity, phenological patterns also separate sympatric species, in which peak parasitization activity varies from month to month. For example, *P. curvatus* reaches peak activity in November in its native range while *P. tricusps* is most active in March (Folgarait et al. 2003). Third, parasitization strategy varies among species. For example, *P. obtusus* parasitize workers that are foraging while *P. curvatus* parasitize during mound disturbances or mating flights (Orr et al. 1997). Finally, host partitioning occurs by host size preference. Since *S. invicta* workers are of varying sizes within a colony, each *Pseudacteon* spp. parasitizes workers of a particular range of sizes (Morrison et al. 1997). For instance, *P. obtusus* prefer larger workers (~0.9 mm) (Folgarait et al. 2005) while *P. cultellatus* prefer smaller workers (~0.6 mm) (Folgarait et al. 2002b). These mechanisms allow for the separation of species seen today.

As in any natural enemy used in classical biological control, host specificity is of major concern (Simberloff and Stiling 1996). Also, there are multiple native species of fire ants in North America such as *S. geminata* Forel and *S. xyloni* McCook, as well as native ants of other genera. For this reason, multiple studies have investigated the host specificity of *Pseudacteon* phorid flies that parasitize *Solenopsis saevissima* complex of fire ants. Field tests by Porter et al.

(1995b) showed that *P. tricuspis* and *P. litoralis* were not attracted to *Solenopsis geminata*, while *P. wasmanni* Schmitz and *P. pradei* Borgmeier were only slightly attracted. In another study, no *P. tricuspis*, *P. litoralis*, *P. wasmanni*, *P. pradei*, nor *P. curvatus* made oviposition attempts on *S. geminata* (Porter 1998b). In no-choice lab studies, *P. litoralis*, *P. wasmanni*, and *P. tricuspis* rarely attacked *S. geminata*, while *P. curvatus* did successfully parasitize them. However, no adults emerged from the parasitized *S. geminata* ants (Gilbert and Morrison 1997). Morrison and Gilbert (1999) used the same methods to test the host specificity of *P. obtusus* and *P. borgmeieri* and found that *P. obtusus* never parasitized *S. geminata* while *P. borgmeieri* Schmitz did attack them. *Pseudacteon cultellatus*, *P. nudicornis* Borgmeier, and *P. nocens* Borgmeier, did attack *S. geminata* at very low rates (Estrada et al. 2006). Another lab study tested the host specificity for *P. tricuspis*, *P. litoralis*, and *P. wasmanni*, and found that *P. tricuspis* could successfully develop in *S. geminata* (Porter and Alonso 1999). However, this occurred in trials where *S. geminata* were mixed with freeze-killed *S. invicta* workers, which induced the attack. The conclusion of these specificity tests was that *Pseudacteon* phorid flies are very host specific, and although there may be some concern for non-target effects, they were so rare that they would unlikely occur in a field setting.

Further risk analysis for *P. curvatus* by Porter and Gilbert (2005) showed that in no-choice lab trials, *P. curvatus* did successfully develop in *S. geminata* and *S. xyloni* hosts but at much lower rates than in *S. invicta*. In paired choice tests, there was a significantly greater preference for *S. invicta*, although there were instances of parasitism of the native ant species. However, adult flies that emerged from *S. geminata* and *S. xyloni* still showed strong preference for *S. invicta*. Subsequent field studies showed negligible instances of non-*S. invicta* parasitism by *P. curvatus* (Vazquez and Porter 2005). In addition to host specificity, Porter and Gilbert (2004) investigated

possible attraction to food, as that may lead them to become a nuisance. They found that there was no significant attraction to any foods or moist tissues. The authors also noted that the flies were never attracted to peoples' faces nor did they land on their hands. The results of this study showed that *Pseudacteon* spp. show no indication of becoming nuisances to people.

The aforementioned studies show that *Pseudacteon* spp. are candidates for classical biological control agents. In addition to these reports confirming high host specificity, there is little reason to expect any host shift. First, *Pseudacteon* are phylogenetically specific as ant parasitoids (Disney 1994). Secondly, *Pseudacteon* flies are morphologically dependent on ants because their pupa is evolved to fit inside the head of worker ants (Porter and Gilbert 2005). Third, *Pseudacteon browni* Disney and *P. crawfordi* Coquillett are parasitoids of *S. geminata* and are native to North America, yet they have not transferred to *S. invicta* in the 80 years since imported fire ants were introduced in the United States (Folgarait et al. 2002a). Even if they did switch hosts, it would likely take place over thousands of years and occur in isolated populations (Porter and Gilbert 2005). Also, many of these flies rely on polymorphic hosts because sex is determined by the size of the host (Morrison et al. 1999). Any ant species that do not have polymorphic workers would likely not support *Pseudacteon* phorids (Gilbert and Morrison 1997).

Mortality due to parasitization is only 1-3% in the field (Morrison et al. 1997, Morrison and Porter 2005a). Instead, phorid flies have a greater indirect effect on imported fire ants rather than through population reduction. Red imported fire ants have evolved multiple defensive responses to the presence 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). As a result, colonies are less efficient at bringing in resources. This weakens

the colonies and allows native ants to compete for food more effectively (Mehdiabadi and Gilbert 2002). This indirect effect together with direct impacts may result in population-wide suppression of *S. invicta* in the United States (Morrison et al. 1997). Imported fire ants have been characterized like invasive weeds rather than conventional invasive insect pests because they are stationary, take multiple years to reach reproductive maturity, and collect resources using non-reproductive workers (Porter and Gilbert 2004). As with weeds, guilds of natural enemies are often assembled to offer control on multiple fronts (McEvoy and Coombs 1999). Current efficacy studies of *P. tricusps* did not indicate any significant impact on fire ants. It is likely that additional natural enemies are necessary for successful biological control of imported fire ants (Morrison and Porter 2005b).

History of Phorid Release in the United States

The use of phorid flies as biological control agents of fire ants is currently the only mobilized program in the United States (Lebrun et al. 2012). The history of this program begins with the discovery of *Pseudacteon* flies in South America by Borgmeier (1921), who described the majority of the species that parasitize fire ants. Wasmann, Borgmeier, and Smith observed the parasitic behavior of phorid flies in Holland, Brazil, and the United States respectively (Porter 1998a). Feener (1981) observed that foraging activity was reduced in *Pheidole dentata* Mayr workers when in the presence of their parasitoid phorid flies. Later, in 1992, Feener and Brown documented similar responses with *Solenopsis geminata* and suggested the potential for biological control of *S. invicta*. The phorid fly biological control program has been led by two groups: The Breckenridge Field Laboratory in Austin, Texas and USDA Agricultural Research Service in Gainesville, Florida and has been carried out by numerous state agencies across the southeastern United States (Callcott et al. 2011). The release effort has continued for over

fifteen years, and has resulted in the establishment and spread of *Pseudacteon* phorid flies in nine states and Puerto Rico.

Pseudacteon release attempts began in the United States with *Pseudacteon tricuspis* (Porter et al. 2004). *Pseudacteon tricuspis* was selected for release as a sustained biological control of imported fire ants for three reasons: 1) it was the first species to be successfully reared in the laboratory, 2) it is very host specific, being able to produce viable offspring only through *Solenopsis invicta* (Porter et al. 1995b), and 3) it is one of the three most common species in the fire ant native range (Porter et al. 2004). Successful *P. tricuspis* releases took place in 1997 in Florida (Porter 2004) and 1999 in Alabama (Graham et al. 2001). In 1995, the Breckenridge Field Laboratory in Austin, Texas began releasing *P. tricuspis* in sites in Texas (Gilbert and Patrock 2002). However, due to severe droughts, these releases failed. It was not until 2002, when the drought broke, that *P. tricuspis* finally began to establish and spread in Texas (Gilbert et al. 2008). *Pseudacteon tricuspis* releases began in 1999 in Louisiana and resulted in establishment (Henne et al. 2007). Releases in Tennessee did not result in establishment of *P. tricuspis*, while in Mississippi, *P. tricuspis* releases in 2000 did result in establishment (Callcott et al. 2011). In 2001, field releases in Arkansas were successful for *P. tricuspis* (Clemons et al. 2003). By 2003, *P. tricuspis* had been established in nine states and Puerto Rico (Callcott et al. 2011). *Pseudacteon curvatus* has also been released through this program. This species was initially rejected for release by the Breckenridge Field Lab because initial host specificity tests indicated some targeting of native fire ants (Gilbert et al. 2002), but additional host range studies led them to accept the species for releases (Porter and Gilbert 2004, Gilbert et al. 2008). The rearing facility in Gainesville first released a strain of *P. curvatus* that originated from Buenos Aires, Argentina. These flies had a strong preference for black imported fire ants (*Solenopsis*

richteri) and *S. richteri* x *S. invicta* hybrid fire ants. They were released in Alabama, Florida, and Tennessee but did not establish in Florida because black and hybrid fire ants are not present there (Graham et al. 2003a). Releases in Mississippi were successful (Thead et al. 2005). A different strain of *P. curvatus* from Formosa, Argentina was released beginning in 2003 in North Florida (Porter 2010). This strain had a much stronger preference for *S. invicta*. It was released in 2003 and was successful at quickly spreading across the state. Since then the Formosan strain of *P. curvatus* has been introduced and established into Alabama, Arkansas, Georgia, Louisiana, North Carolina, Oklahoma, Puerto Rico, South Carolina, and Texas (Callcott et al. 2011).

In addition to these two species, the following species have been established in the United States: *Pseudacteon obtusus* in Texas (Gilbert et al. 2008) and Florida (Porter and Calcaterra 2013), *Pseudacteon litoralis* in Alabama (Porter et al. 2011), *Pseudacteon nocens* in Texas (Plowes et al. 2012), and *Pseudacteon cultellatus* in Florida (Porter et al. 2013). Release attempts for *P. obtusus* were made in Arkansas in 2008 (Kelly Loftin, personal communication) but were unsuccessful, probably due to drought conditions at time of release. *Pseudacteon litoralis* is only present in Alabama (Porter et al. 2011) and is suited best to monogyne colonies (Lebrun et al. 2012).

Throughout the phorid release program, multiple release strategies have been used. For *P. tricuspis* (Graham et al. 2003b, Porter et al. 2004, Henne et al. 2007) and *P. litoralis* (Porter et al. 2011) releases, adult flies were released over disturbed fire ant mounds so that they would parasitize workers. A more refined technique, in which worker ants were transported to quarantine labs for parasitism and returned to their original colonies, was implemented for *P. curvatus* releases (Graham et al. 2003a, Vazquez et al. 2006, Gilbert et al. 2008), *P. cultellatus* (Porter et al. 2013), and *P. obtusus* (Porter and Calcaterra 2013). An attempt was made to use a

greater proportion of collected workers by exposing them to both *P. curvatus* and *P. tricuspis*, but severe drought after the release prevented establishment from taking place, except for *P. curvatus* in an irrigated section (Gilbert et al. 2008). Another alternative method, in which phorid pupae were buried in insulated emergence boxes, was used to release *P. nocens* and was determined to be an effective method for species with small lab cultures (Plowes et al. 2012). However, *P. cultellatus* releases that implemented this method were not as successful (Porter et al. 2013).

The outcomes for releases, both failed and successful, are the result of several factors, such as stochastic events, location, release strategy, and total numbers of individuals released (Grevstad 1999). Weather events such as drought have been used to explain failure to establish after releases (Graham et al. 2003a, Gilbert et al. 2008). Also, latitude was a significant factor in the rate of establishment of *P. tricuspis*, with lower rates of establishment in more northerly latitudes. Total number of individuals released was not a significant factor in establishment at release sites (Callcott et al. 2011).

Techniques used in *Pseudacteon* detection

In biological control, it is necessary to track the spread of the species to determine establishment (LeBrun et al. 2008). In these situations, *Pseudacteon* flies may be difficult to detect because initial population densities can be very low (Gilbert et al. 2008). Some *Pseudacteon* species exist in low densities either in their native range (Folgarait et al. 2007) or after establishment in the United States (LeBrun et al. 2009). In either case, inefficient or ineffective sampling may result in underestimating true phorid distributions. Therefore, it is important to use a sampling method that is sensitive enough to detect rare species without requiring a large sampling effort,

which might negatively impact the population if many individuals are captured. The two most common means for detecting phorid flies are through aspirating (Porter et al. 2004, Vazquez et al. 2006) or through sticky traps. Aspirating is accomplished by disturbing ant mounds and searching for phorids as they parasitize workers. Aspirating is time consuming and is subject to observer biased because the flies are very small (Lebrun et al. 2008). Although they have not been used in *Pseudacteon* sampling, vacuum aspirators are often used in mosquito research (Aldridge et al. 2012) and in natural enemy studies in crop settings (Hossain et al. 2008). This technique may be more easily standardized than conventional aspirating because effort can be consistent over the time allotted for sampling. Sticky traps require less effort and are more efficient (Puckett et al. 2007). Lebrun et al. (2009) used a modified food storage box lined with sticky fly tape. Another widely used sticky trap design by Puckett et al. (2007) uses a Tanglefoot-coated pizza tri-stand. For sticky traps, *S. invicta* are used to attract flies. However, some studies used dead ants (Puckett et al. 2007) and others used live ants (LeBrun et al. 2009, Farnum and Loftin 2011). Studies that investigated the effects of phorids on fire ant foraging used a food source as a bait to attract *S. invicta* workers (Feener and Brown 1992, Folgarait and Gilbert 1999). *Pseudacteon obtusus* has been characterized as a parasitoid that parasitizes foraging workers (Orr et al. 1997), so a trap that is baited with foraging ants may be more effective for detecting this and similar species. All methods used are effective for capturing phorid flies, and trap efficacy studies have been done (Puckett et al. 2007, LeBrun et al. 2008, Puckett et al. 2013). However, these studies only compared variations of their respective author's design. No study to date directly compares Puckett's pizza tri-stand sticky trap with LeBrun's sandwich box sticky trap, nor between aspirating and trapping.

Competitive Interactions among *Pseudacteon* spp.

Despite successful establishment and spread of both *P. tricuspis* and *P. curvatus* throughout the southeast (Callcott et al. 2011), there have been indications that *P. tricuspis* has been displaced due to interspecific competition with *P. curvatus*. In Texas, population sampling in the field indicated that within months of *P. curvatus* establishment *P. tricuspis* populations began to decline (LeBrun et al. 2009). Similar observations were made in Florida following the establishment of *P. curvatus* (Porter et al. 2013). It has been suggested that this occurred for two reasons: 1) the flies directly compete for the host resource and 2) that they indirectly compete by one species preempting the other to the host resource thereby making the host resource unavailable (LeBrun et al. 2009). This may occur among different species and within species (Chirino et al. 2012). Many *Pseudacteon* spp. exhibit sexual selection relating to host size (Porter 1998a, Folgarait et al. 2005). By one species exploiting a particular size class, it is possible to skew the sex ratios of another species thereby disrupting the population of that species. Lebrun et al. (2009) found this to occur with *P. tricuspis* in areas occupied by *P. curvatus*, although this alone was not sufficient to explain the decline of *P. tricuspis* in these areas. An additional factor influencing parasitism is the social form of *S. invicta*, in which sex ratios change (Chirino et al. 2009, Chirino et al. 2012). This is probably due to the variation of size classes between the social forms, in which polygyne workers tend to be smaller (Greenburg et al. 1985). Multiple species of *Pseudacteon* successfully coexist in their native range (Fowler 1997), but in the context of *P. tricuspis* and *P. curvatus* in the United States this may not to be the case for multiple reasons. First, the sources for the flies that were released in the United States were in different parts of South America (Vazquez et al. 2006, Porter et al. 2004), so the biotypes released may not be

sympatric. Biotype does influence host preference, as the Las Flores biotype of *P. curvatus* was released in the United States but failed to establish because they prefer *S. richteri* (Graham et al. 2003a). However, the Formosan biotype of *P. curvatus* was released and quickly spread throughout the southeastern United States (Callcott et al. 2011). Two biotypes of *P. obtusus* which vary by size were found to be sufficiently genetically distinct that it was recommended they be considered separate species (Kronforst et al. 2007). No research has been done to support the hypothesis that displacement is a result of incompatible biotypes.

Another explanation for the displacement of *P. tricuspis* by *P. curvatus* is variation in climate throughout the introduced range in the United States. Phorid species are adapted to various climates; therefore different species are better suited to different parts of the imported fire ant's range in the United States (Folgarait et al. 2007). *Pseudacteon tricuspis* established more often in southern latitudes with > 100 cm rainfall annually (Callcott et al. 2011). Conversely, *P. curvatus* is abundant and widely distributed in Arkansas (Farnum and Loftin 2011). However, both species have been characterized to exist in the same ecoregion in South America (Folgarait et al. 2005).

The effects of competition between *P. tricuspis* and *P. curvatus* indicate that although species are sympatric in their native habitat, they may not coexist in the introduced area. For this reason, it is necessary to better understand the underlying mechanisms that drive competition with additional species.

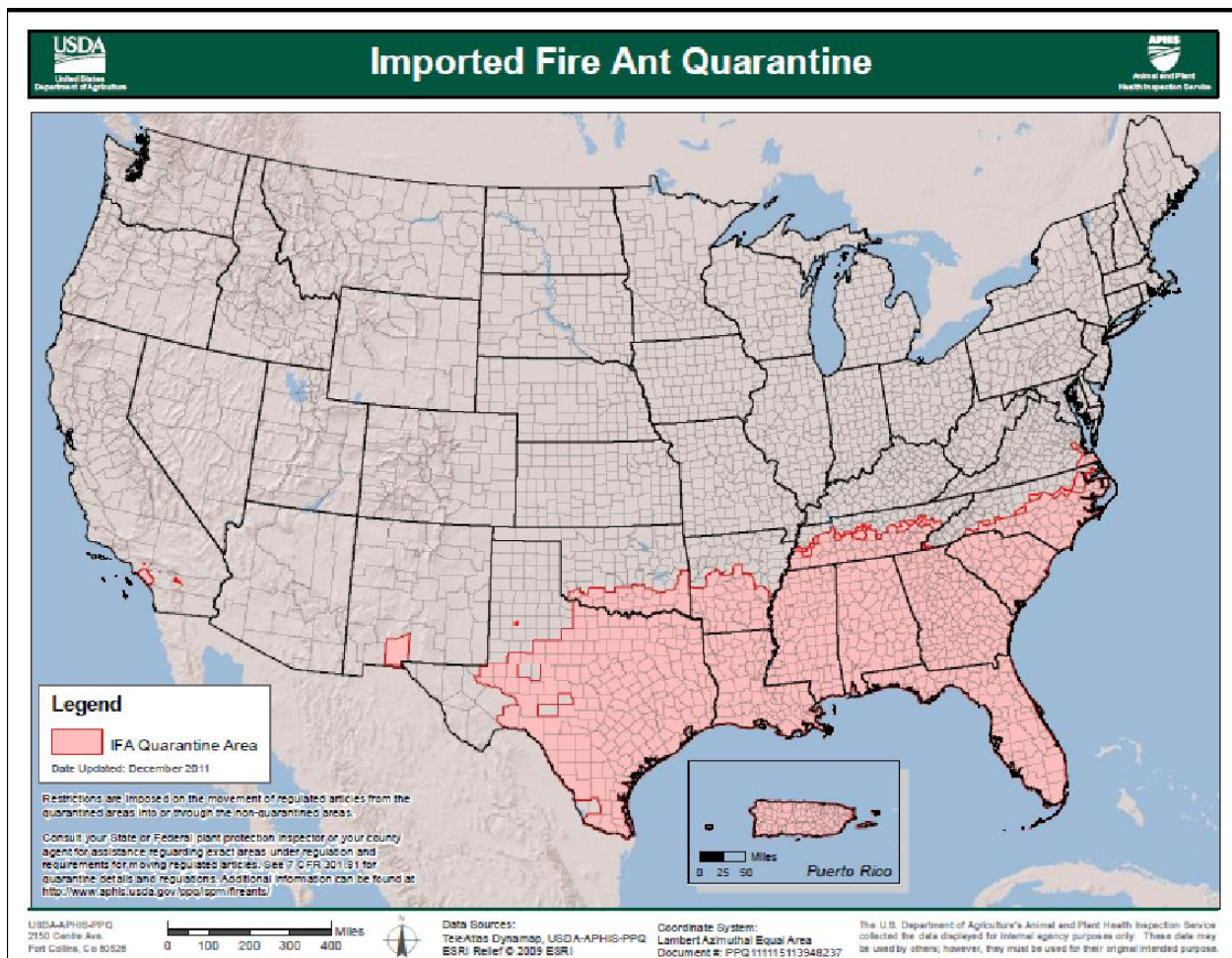


Figure 1.1 Current Animal and Plant Health Inspection Service quarantine map updated 2011 (USDA, 2014).

**Chapter 2. Releasing *Pseudacteon cultellatus* and *Pseudacteon obtusus* (Diptera: Phoridae)
for Biological Control of Red Imported Fire Ant, *Solenopsis invicta* (Hymenoptera:
Formicidae) in Central Arkansas**

Introduction

Solenopsis invicta Buren, the red imported fire ant, is a major pest throughout the tropical and subtropical regions of the world, including the southeastern United States (Ascunse et al. 2011). *Solenopsis invicta* is native to South America, but was inadvertently introduced into Mobile, Alabama in the 1930s (Buren et al. 1974). *Solenopsis invicta* is primarily a nuisance pest, but causes damage in several ways. It inflicts painful stings when disturbed. In rare cases, the stings can lead to anaphylaxis or secondary infection (DeShazo et al. 1990). It causes agricultural losses, either through decreased crop yield or through damage to equipment (Banks et al. 1990). *Solenopsis invicta* is an effective colonizer of disturbed habitat and can alter biodiversity in infested areas (Allen et al. 2004). Densities of *S. invicta* are much higher in North America than in their native range. It has been proposed that escape from natural enemies allowed for higher densities outside of their native range (Porter et al. 1997a).

Current management practices consist primarily of chemical control either through individual mound treatments or as a broadcast bait application (Drees and Gold 2003). However, these approaches are not cost effective in pastures and most other agricultural settings (Vander Meer et al. 2007). For this reason, a classical biological control program including the release of *Pseudacteon* spp. has been implemented as an additional control measure. *Pseudacteon* phorid flies are parasitoids of red imported fire ants and are currently being used in the biological control program for *S. invicta* (Lebrun et al. 2012). These flies parasitize worker ants by injecting an egg into the thorax. As the larva matures, it moves into the head capsule and decapitates the ant (Porter 1998a). Although *Pseudacteon* flies do cause mortality through parasitism, their greatest impact is by altering the behavior of the ants by reducing foraging and

mound repair (Folgarait and Gilbert 1999). These impacts could give native ants a competitive advantage over *S. invicta* (Mehdiabadi and Gilbert 2002).

There are at least 18 known species of *Pseudacteon* that parasitize the *Solenopsis saevissima* complex of fire ants (Porter 1998a). This diversity of species for a single host is made possible through host partitioning by size preference, among other factors (Morrison et al. 1997).

The phorid release program began in 1994 and has resulted in the establishment of six species in the southeastern United States. At this time, there has not been a measured effect of phorid flies on fire ants populations in the United States. Additional biological control organisms may be necessary if effective biological control of *S. invicta* is to be achieved. This includes a variety of viruses, microsporidia, and additional species of phorid flies (Mehdiabadi and Gilbert 2002).

There are currently two *Pseudacteon* spp. established in Arkansas. *Pseudacteon tricuspis* Borgmeier was the first to become established in southwest Arkansas in 2002 followed by *P. curvatus* Borgmeier in 2005. *Pseudacteon curvatus* has spread throughout the fire ant-infested regions of the state (Farnum and Loftin 2011). Two additional species that have established in other states are *P. obtusus* Borgmeier (Porter and Gilbert 2004) and *P. cultellatus* Borgmeier (Porter et al. 2013). These species differ from the already established flies in that they prefer different sizes of workers. The establishment of *P. cultellatus* and *P. obtusus* may lead to improved biological control of *S. invicta* in Arkansas.

Traditionally, individual *Pseudacteon* spp. are released into sites by collecting fire ant workers from the site and sending them to a rearing facility where they are parasitized by the flies. The ants are then returned to their original colony so that the adult flies will emerge in the new location (Callcott et al. 2011). Because phorid flies have different host size preferences (Fowler

1997), some of the workers that are collected from the colony are not parasitized. This method might be improved by exposing worker ants to multiple phorid species. If effective, these combined releases could make releases more efficient. The objectives of this study are to release *P. cultellatus* and *P. obtusus* in Central Arkansas and to compare establishment success between the traditional individual release method and the proposed combined release method.

Materials and Methods

Releases took place at Little Rock Air Force Base (LRAFB) in Jacksonville, and Camp Robinson (CR) in North Little Rock and occurred May-June 2013 and October 2013. In order to compare establishment success between combined and individual releases, only one of the two species were released at each site at LRAFB; whereas both species were released at CR release sites. Sites had a minimum of 50 mounds per hectare (20 mounds per acre), which was determined by counting the number of active fire ant mounds (mounds with more than 25 ants active after disturbing the colony) in a 0.1 ha (0.25 acre) circular plot. In the release sites, large mounds were inspected for worker sizes that corresponded to the host size preference of the *Pseudacteon* flies that were being released. *Pseudacteon obtusus* prefer large workers (0.9 mm ant head width) (Folgarait et al. 2005), and *P. cultellatus* prefer small workers (<0.6 mm ant head width) (Porter et al. 2013). Therefore, mounds with a high proportion of large ants were selected at *P. obtusus* release sites and mounds with many small workers were selected at *P. cultellatus* release sites. For the combined releases, mounds with a mixture of sizes were selected.

Live ants were collected by disturbing a mound and placing a PVC pipe (~ 11cm diameter x ~28 cm height) on the mound (Fig. 2.1). Ants climbed up the pipe and then were knocked into a bucket for collection. These ants were then weighed and packaged in 18 x 13 cm lock-lid

storage boxes for shipping (Fig. 2.2). The boxes also contained test tubes which were filled with water, a cotton ball, and moistened dental plaster to maintain humidity in the boxes. A moist cotton ball was also provided as a water source (Graham et al. 2003). The boxes were shipped to the Florida Department of Agriculture in Gainesville, Florida, where they were exposed to the phorid flies for parasitization following the method by Porter et al. (2004). Combined release colonies differed from individual releases in that ants were exposed to one species of *Pseudacteon* for the parasitization period then exposed to the other species for an additional time period. After being parasitized, the weights of the parasitized ants were recorded. Then they were shipped back to Arkansas where they were released back into their respective colonies. In 10 of the 100 release colonies, ant colonies had moved after being collected so the nearest active colony was used for release. If a nearby colony could not be found, the ants were placed in the original colony. On hot dry days the mounds were spritzed with water before the ants were released. Based on the weights of parasitized ants, it is possible to estimate the number of flies that were released (Table 2.1). The first two sets of combined releases were field parasitized, in which the ants were placed outside the Florida Department of Agriculture facility in Gainesville. Without knowing which *Pseudacteon* spp. actually parasitized these ants, these collections were removed from consideration in this study. Additional collections were made for the combined release site. Sampling took place in August 2013 using four modified Puckett sticky traps (Farnum and Loftin 2011) at the release sites. Then, for each month in April, June, and August 2014, five traps were placed at each release site in order to detect an overwintering population. Temperatures on all sampling days were above 21°C.

Results and Discussion

One of four traps from Camp Robinson captured one *P. obtusus* female in August of 2013.

Spring sampling in April and June 2014 did not detect any phorid flies in release sites at Camp Robinson or Little Rock Air Force Base. However, traps placed in August 20 2014 did capture *P. curvatus* in all release sites. Detection of overwintering *P. obtusus* and/or *P. cultellatus* would have been an important step in determining establishment. However, trapping conducted in 2014 revealed no *P. obtusus* or *P. cultellatus* flies. This region of Arkansas experienced a colder than average winter which may have negatively impacted fire ant and phorid fly populations (Table 2.2). The coldest mean monthly temperature was -4°C during the winter of 2013-2014, and there were four consecutive days with a high of less than 1.1 °C. These temperatures were similar to those noted in winter survivability studies in Tennessee (Callcott et al. 2000). Comparisons between fire ant mound densities in 2013 and 2014 indicated an average of 82% reduction of fire ant mounds (Table 2.3). Previous *P. curvatus* release studies reported drought to have negatively impacted fire ant colonies (Gilbert and Patrock, 2002), disrupting *Pseudacteon* establishment. However, remaining populations of fire ants near water successfully maintained the fly population. This may not be possible when very cold weather is responsible for decreased ant populations. Future studies may reveal that lower than average temperatures affect the success of fly population regardless of rainfall and/or proximity to water sources. Populations of *P. curvatus* that were found in August 2014 indicate that *Pseudacteon* flies survived the cold winter. Because *P. curvatus* is abundant in the region the population may have survived despite significant mortality. However, populations of *P. obtusus* and *P. cultellatus* may have been subject to the Allee effect in which severe winter conditions, coupled with reduced ant

populations resulted in population densities that were below a minimum threshold, leading to extinction (Liebhold and Tobin, 2008).

Comparisons between phorid fly presence and/or abundance at individual and mixed release sites are not yet possible. Additional monitoring will be necessary to determine whether or not *P. cultellatus* and *P. obtusus* releases were successful at either combined or individual release sites. Future studies tracking the spread of the species will be necessary to compare between release methods. Although traps failed to detect *P. cultellatus* and *P. obtusus* in 2014, it is possible that *P. obtusus* and *P. cultellatus* are present in the release sites, but populations are too low to detect. As a result, the established population may not have overlapping generations due to the small population size which would decrease the likelihood of successfully detecting the flies. Weekly monitoring would improve the odds of sampling when adults are present, and could possibly illustrate this effect.



Figure 2.1 PVC Pipe used to collect fire ants from mounds.



Figure 2.2 Lock-lid storage box used for shipping ants.

Table 2.1 Estimated release numbers for summer and fall 2013¹

| Release site | Estimated flies released |
|---|--------------------------|
| LRAFB – <i>P. obtusus</i> site | 8,000-12,000 |
| LRAFB – <i>P. cultellatus</i> site | 26,000-38,000 |
| CR – Combined release – <i>P. obtusus</i> | 6,500-10,000 |
| CR – Combined release – <i>P. cultellatus</i> | 36,000-54,000 |

¹Based on: total ants parasitized * # ants/gram * 20-30% lab parasitism rate

Table 2.2 Winter weather data for years 2010-2014

| Winter | Lowest mean monthly min temp (°C) | Lowest mean monthl y max temp (°C) | Consecutive winter days with max temp at or below 0°C | Most consecutive winter days with max temp at or below 1.1°C | # Sets of consecutive winter days with max temp at or below 1.1°C | Mean mound density at release sites* (per ha) |
|-----------|--|---|---|--|---|--|
| 2010-2011 | -1 | 10 | 2 | 5 | 3 | |
| 2011-2012 | 1 | 13 | 0 | 0 | 0 | |
| 2012-2013 | 1 | 11 | 1 | 1 | 0 | 170 |
| 2013-2014 | -4 | 10 | 3 | 4 | 3 | 30 |

* Mound densities were estimated in June of 2013 and 2014.

Table 2.3 Mound density estimates for June 2013-2014

| Location | Release Type | 2013 Count (per ha) | 2014 Count (per ha) | Percent Change |
|----------------------------|-----------------------|--------------------------------|--------------------------------|---------------------------|
| LRAFB | <i>P. cultellatus</i> | 306 | 79 | -74 |
| LRAFB | <i>P. cultellatus</i> | 296 | 79 | -73 |
| LRAFB | <i>P. cultellatus</i> | 198 | 59 | -70 |
| LRAFB | <i>P. obtusus</i> | 168 | 0 | -100 |
| LRAFB | <i>P. obtusus</i> | 119 | 0 | -100 |
| LRAFB | <i>P. obtusus</i> | 207 | 0 | -100 |
| LRAFB | <i>P. obtusus</i> | 128 | 0 | -100 |
| LRAFB | <i>P. obtusus</i> | 296 | 10 | -97 |
| LRAFB | <i>P. cultellatus</i> | 316 | 198 | -38 |
| LRAFB | <i>P. cultellatus</i> | 99 | 0 | -100 |
| LRAFB | <i>P. cultellatus</i> | 99 | 79 | -20 |
| CROB | Simultaneous | 89 | 30 | -67 |
| CROB | Simultaneous | 326 | 10 | -97 |
| CROB | Simultaneous | 188 | 0 | -100 |
| CROB | Simultaneous | 69 | 10 | -86 |
| CROB | Simultaneous | 109 | 30 | -73 |
| CROB | Simultaneous | 49 | 0 | -100 |
| Mean Percent Change | | | | -82 |

Chapter 3. Comparing Sampling Methods Used in Detection of *Pseudacteon* spp. (Diptera: Phoridae)

Introduction

Since accidental introduction in the 1930s, the red imported fire ant, *Solenopsis invicta* Buren, has become a major pest in the southeastern United States. *Solenopsis invicta* inflicts painful stings when disturbed, damage crops and farm equipment (Banks et al. 1990), and alter the biodiversity in infested areas (Allen et al. 2004). Due to the negative impact of *S. invicta*, a biological control program using parasitoid phorid flies in the genus *Pseudacteon* was created 1994. This program has involved the cooperation of federal and state institutions to release the flies in states throughout the southeast (Callcott et al. 2011).

Pseudacteon phorid flies are parasitoids of the *Solenopsis saevissima* complex of fire ants (Disney 1994). They are known as decapitating phorid flies because of their unique life cycle in which female flies use their specialized ovipositors to inject an egg into the thorax of fire ant workers. The larva hatches and feeds on the ant but does not kill it until shortly before pupation. At that time, the larva moves into the head capsule and releases enzymes which dissolve the membranes of the head causing it to detach. The larva then uses the head capsule of its deceased host to pupate (Porter 1998a).

Over the course of the phorid release program, six fly species have established in the United States (Porter et al. 2013). The progress of the releases was tracked using various methods. Initially, aspirating was used to capture flies as they came to disturbed mounds (Porter et al. 2004). This technique is labor-intensive and time consuming. It may also be subject to observer bias because the flies are small and difficult to see (Lebrun et al. 2008). Alternatively, sticky traps have been used. These traps use fire ants as bait to attract phorid flies. As they search for potential hosts, they land on the sticky surface or fly into the trap (Puckett et al. 2013). Two trap

designs have been used. Lebrun et al. (2009) used a fly-paper lined sandwich storage box in which the sticky tape is suspended over the bottom of the box with #2 rubber stoppers. Another design by Puckett et al. (2007) uses a Tanglefoot® coated pizza tri-stand that is held over a 15 cm Petri dish. Both traps use *S. invicta* as bait. Some studies used midden, which is the collection of dead ants and refuse, from lab colonies (Puckett et al. 2013) while others used live ants (Farnum and Loftin 2011). Some *Pseudacteon* spp. parasitize workers as they forage (Orr et al. 1997), so foraging ants have been used as bait (Folgarait and Gilbert 1999). Trap efficacy studies show that all sampling methods can be effective (Puckett et al. 2007, LeBrun et al. 2008, Puckett et al. 2013); however, there has not been a study directly comparing the various trap designs, or between aspirating and trapping.

There are currently two *Pseudacteon* species in Arkansas. *Pseudacteon tricuspis* Borgmeier was the first established species followed by *P. curvatus* Borgmeier (Farnum and Loftin 2011). *Pseudacteon tricuspis* is rare and has a limited distribution in the state while *P. curvatus* is abundant and widespread. Two new species, *P. cultellatus* Borgmeier and *P. obtusus* Borgmeier, have been released but establishment has not been confirmed. It is important to know which trap type and number might be necessary to detect rare species such as *P. tricuspis* or either of the two recently released species. The objectives of this study are to compare bait/trap combinations and to determine which sampling technique is most effective for sampling phorid flies in Arkansas.

Materials and Methods

Phorid sampling methods were studied the Terrell and Petty farms in Pike County near where *P. tricuspis* had been previously released (Clemons et al. 2003). Each location had at least 50 fire

ant mounds per hectare (20 mounds per acre), which was determined by counting the number of mounds in 0.1 ha circular plots. The study was conducted on June 1, 2, 16, 17, and October 11, 19, 20, and 21. The two trap designs used were the sandwich sticky trap used by Lebrun (2009) and a modified Puckett sticky trap used by Farnum and Loftin (2011) (Fig. 3.1). For each trap, three different attractants were used: 500-800 confined live ants (~1 g), 500-800 dead ants (~1 g), and foraging fire ants. Ant numbers are based on the wet weight for both live ant dead ants. A 1 cm sq. corn meal based bait cube (Jones et al. 2004) was used to attract foraging ants to traps. The foraging ant bait was included in this study because some *Pseudacteon* spp. prefer to parasitize foraging workers (LeBrun et al. 2012) and because *P. curvatus* had been observed parasitizing foraging fire ants in Central Arkansas (author's observation). Ants used for the dead ant treatment were collected at the trapping site and were killed either by freezing the night before, crushing, or with a microwave oven. Live ants were collected by disturbing a mound and placing a PVC pipe (~ 11cm diameter x ~28cm height) on the mound. Ants climbed up the pipe and then were knocked into a bucket so that they could be distributed to the traps. Traps using live ants as baits were shaded with a 22 cm Styrofoam plate. A moist cotton ball was placed in the trap to maintain live ants throughout the sampling period of one day. In the October sampling days, shade was also provided in the foraging ant baited traps. The edges of the Petri dishes were coated with Fluon to prevent ants from escaping (Petri dishes containing dead ants were not Fluon coated). Puckett traps using foraging ants as bait were placed directly on the ground with a bait cube on the base of the trap. Foraging ant baited sandwich sticky traps contained a bait cube which was placed in the center of the sandwich box. Slots were cut into the bottom of the trap so that ants could move freely into and out of the trap while they were foraging. The trap/bait combinations evaluated were Puckett/dead ants (PD), Puckett/live ants (PL), Puckett/foraging

ants (PF), sandwich/dead ants (SD), sandwich/live ants (SL), and sandwich/foraging ants (SF) (Fig. 3.1). Traps were placed in a five-transect grid with one of each trap/bait combination per transect. Tall grass was trimmed so that traps could be placed directly on the ground. The traps were spaced 10m apart and were randomized within each transect. If a fire ant mound was in a trap position, the trap was placed next to the mound so that the mound would not be disturbed. All traps were deployed by 9:30 AM or after dew had evaporated. Traps operated until dusk.

Aspirating occurred at three separate intervals (morning, midday, and evening) on the days that traps were placed. A battery-powered vacuum aspirator from Bioquip® (Model 2820 B) was used in lieu of the conventional hand aspirator because it could provide consistent sampling effort for the time that it was operated. This standardized the sampling effort for aspirating and it decreased the possibility of observer bias. The aspirator used interchangeable chambers which were modified by adding a fine mesh screen (0.3 x 0.3 mm) to prevent phorids from passing through. For each sampling period, five mounds were selected, which were at least 10 meters apart. Each mound was disturbed by prodding with a stick. Some workers were crushed by hand and placed on the mound to increase the release of alarm pheromones. The mound was then aspirated for one minute, then the chamber was exchanged, and the next mound aspirated. This was repeated five times so that each mound was aspirated for a total of five minutes. This process took about one hour. Ants were commonly captured along with the phorid flies, making it necessary to cool the chambers to prevent ants from destroying any captured flies. This was accomplished by storing the chambers in a portable cooler with ice-packs while in the field. Once the samples had been frozen they were transferred to 1 dram pill vials for storage. Even those flies damaged by the ants could still be identified. All samples were counted and identified with a dissecting scope and recorded for analysis. The number of flies of each species was

analyzed. Presence/ absence data is often used to determine the establishment and spread of *Pseudacteon* spp. (Farnum and Loftin, 2011). Therefore, the mean number of positive observations for the flies was also analyzed. This allowed for the comparison between aspirating and trapping methods despite the differences between them. To simplify this analysis, each trap/bait combination and aspirating period was considered as a separate treatment.

Analysis took place in SAS Version 9.4 (© SAS Institute Inc.). A mixed-effects model analysis of variance was done to compare trap/bait combinations with Tukey-Kramer adjustment for multiple comparisons. For presence/absence data, a logistic regression analysis was used with Tukey-Kramer adjustment for multiple comparisons.

Results

Pseudacteon curvatus and *P. tricuspis* were present at both Terrell and Petty Farms.

Pseudacteon curvatus was more abundant than *P. tricuspis* with 2,644 *P. curvatus* captured compared to only 20 *P. tricuspis* over the course of all eight sampling periods. Due to the low numbers of *P. tricuspis*, comparative statistical analyses were not done for this species. The results of *P. tricuspis* sampling are summarized in Table 3.1.

Summer Sampling Results

The Puckett/foraging ant-baited traps did not capture any flies, and thus were not included in the analysis. The overall analysis of variance for trap and bait as separate variables revealed a four-way interaction between location, day, trap, and bait ($p=0.0034$). To account for this, bait and trap were combined into single treatments, and separate analyses were done for each location (Fig. 3.2). The confounding effect of day was then confined to the Terrell sampling days.

For traps at Petty Farm, there were significant differences in mean numbers of *P. curvatus* captured among treatments ($p=0.0228$). However, the differences were between sandwich/dead ant traps and sandwich/foraging ant traps. All other comparisons were non-significant. There were no differences among aspirating periods at Petty Farm. Daily high temperatures for sampling days at Petty Farm were 26°C and 29 °C for June 2 and June 17 respectively.

At Terrell Farm, there was an interaction between treatment and day ($p<0.0001$). However, separate comparisons could be made for each day. On June 1, sandwich/dead ant traps caught more *P. curvatus* than any other trap/bait combination followed by Puckett/dead ant traps ($p<0.0001$ for both treatments). There were no significant differences among trap/bait combinations for June 16. There were no significant differences among aspirating sampling periods for either day. The high temperatures were 26 °C and 31 °C for June 1 and June 16, respectively.

In order to compare between trapping and aspirating, each observation was assigned a value of “1” if it successfully detected *P. curvatus* and “0” if it did not. The relative frequency of success was then compared using a mixed linear model (Fig. 3.3). Analysis was separated as above to account for interactions. Frequency success for Petty Farm varied significantly for sampling method ($p<0.0001$). Trapping was more successful than aspirating with 100% success rate for Puckett/dead ants and sandwich/dead ants. Trapping and aspirating were not significantly different on June 1 sampling; although, there were significant differences between treatments. Though there was a 100% success rate for both aspirating and some traps, only sandwich/dead ant traps were still 100% successful on June 16 sampling.

Fall Sampling Results

October 11 was the only successful fall sampling day. Average high temperatures were 4°C cooler on October 19, 20, and 21 compared to the monthly mean (18°C for sampling days, 22°C for the month) which likely resulted in only 41 *P. curvatus* and three *P. tricuspis* caught over the course of those days, compared to 360 total *P. curvatus* and three *P. tricuspis* caught on October 11. Analysis for fall sampling was only done for October 11 data. For that day, mean temperature was 21 °C. Only traps with dead and live ants captured flies despite the presence of foraging ants at foraging-ant baited traps. Foraging-ant baited traps were not included in analysis. There were significant differences among trap types ($p=0.028$). There were also significant differences among aspirating periods ($p=0.01$) with midday aspirating capturing the most flies (Fig. 3.4). Trapping and aspirating frequency varied significantly for fall sampling ($p<0.0001$). It is not possible to compare between traps for fall data due to the lack of *Pseudacteon* captured.

Discussion

Inconsistent results for sampling days suggest that trap efficacy varies by ambient conditions. June 16 was warmer and windier compared to other sampling days which could explain the low number of flies captured. In the analysis, day and location were difficult to separate because each location was sampled on different days.

Overall, foraging traps were unsuccessful at capturing *P. tricuspis* and *P. curvatus*. For summer sampling, traps were too hot for foraging ants. All baits were actively foraged on fall sampling days, but no flies were captured. This may be because *P. tricuspis* and *P. curvatus* are known to

parasitize workers at disturbed colonies (Gilbert and Porter 2004). Foraging ant-baited traps may be more effective for detecting *P. obtusus* which attack foraging workers.

The results of this study demonstrate that all sampling methods can capture *Pseudacteon* flies. On one of the sampling days sandwich box/dead ant-baited traps captured more *P. curvatus* than any other trap/bait combination, but this was not consistent through the entire experiment. In addition, sticky traps captured more flies than aspirating.

Mean success rate for *P. curvatus* detection varied among trap/bait combinations.

Sandwich/dead ant-baited traps were the only combination in which every trap used in the study successfully detected *P. curvatus*. It was also the combination that detected *P. tricuspis* most often. This is likely due to the additional surface area that the sticky fly tape provides compared to the arms of the pizza tri-stand.

Most sampling for *Pseudacteon* spp. takes place after introducing new species to a fire ant-infested area with the objective of tracking the establishment and spread of the species. In these cases, it is necessary to use a sampling method that is effective at detecting rare species. The study sites in Arkansas were optimal because the two species present represent two extremes in abundance. *Pseudacteon curvatus* is widely present in fire ant-infested sites while *P. tricuspis* is rare and locally present. The results of this study will influence the sampling method used in future release, including *P. obtusus* and *P. cultellatus*, which were recently released in Central Arkansas.

Some important considerations when selecting a sampling technique for *Pseudacteon* research are the cost of materials and efficiency of use. Puckett et al. (2013) did a cost analysis for the traps considered in the comparison study and found that the Puckett trap configuration used in

this study cost \$1.07 per trap. Based on the materials used to make the sandwich sticky traps, each trap cost \$2.94 per trap. Unlike Puckett traps, sandwich sticky traps can be reused after replacing the fly tape. Although sandwich/dead ant-baited traps performed better than other trap types in some situations, they took longer to make and to process compared to Puckett traps. In addition, collecting enough dead ants to use in traps is time consuming and difficult to provide enough ants for a large-scale sampling effort. The optimal method for a sampling study is ultimately a compromise between the sensitivity needed and the feasibility of use.



Figure 3.1 Bait and trap combinations for sticky traps.

Table 3.1 *Pseudacteon tricuspis* captured over all sampling dates.

| Sampling Method | # Positive Observations/Total | Total <i>P. tricuspis</i> Captured |
|------------------------|-------------------------------|------------------------------------|
| Puckett/Dead Ants | 3/40 | 3 |
| Puckett/Foraging Ants | 0/40 | 0 |
| Puckett/Live Ants | 1/40 | 1 |
| Sandwich/Dead Ants | 8/40 | 10 |
| Sandwich/Foraging Ants | 0/40 | 0 |
| Sandwich/Live Ants | 1/40 | 1 |
| Morning Aspirator | 4/40 | 4 |
| Midday Aspirator | 1/40 | 1 |
| Evening Aspirator | 0/40 | 0 |

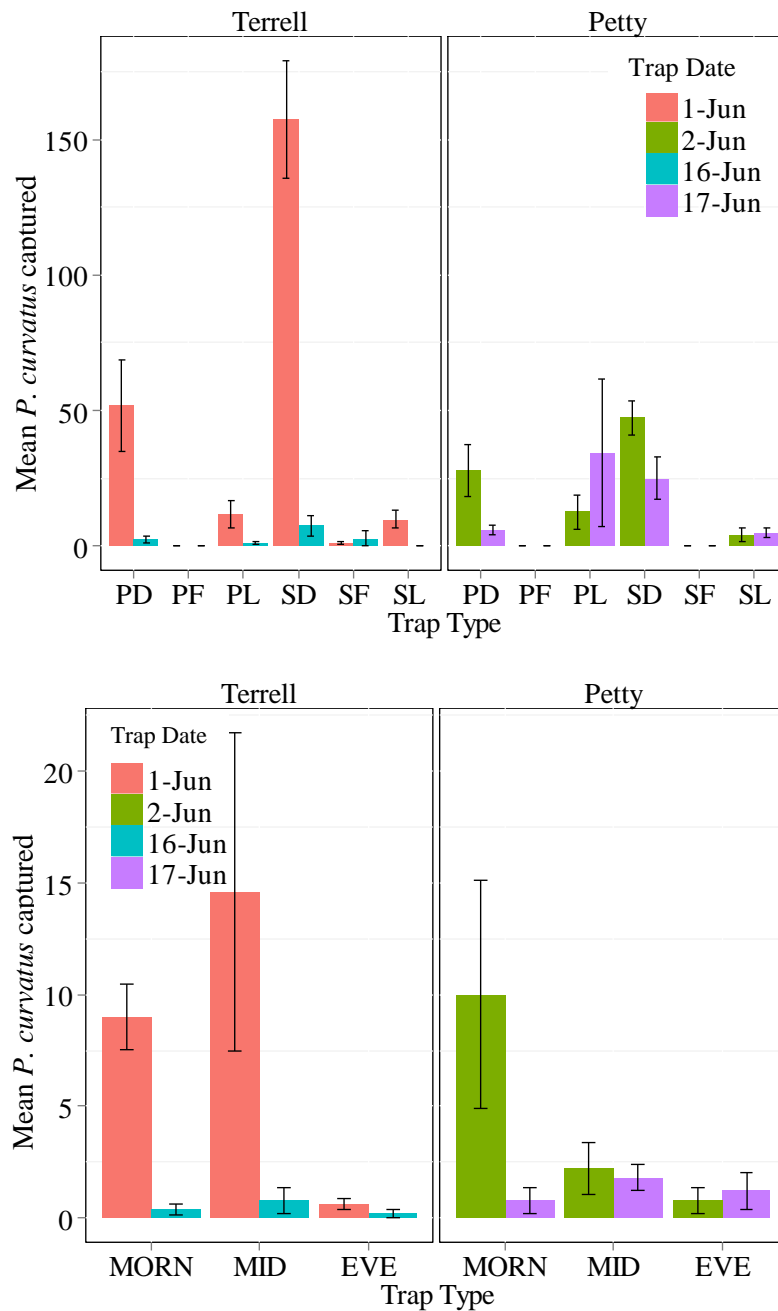


Figure 3.2 Mean *P. curvatus* captured by sticky trap (top) and by aspirating (bottom) for summer sampling dates. Each bar is the mean of five traps/samples. Sticky trap/ bait configurations included Puckett/dead ants (PD), Puckett/live ants (PL), Puckett/foraging ants (PF), sandwich/dead ants (SD), sandwich/live ants (SL), and sandwich/foraging ants (SF). Aspirating took place during the morning (MORN), midday (MID), and evening (EVE). Error bars signify one standard error of the mean.

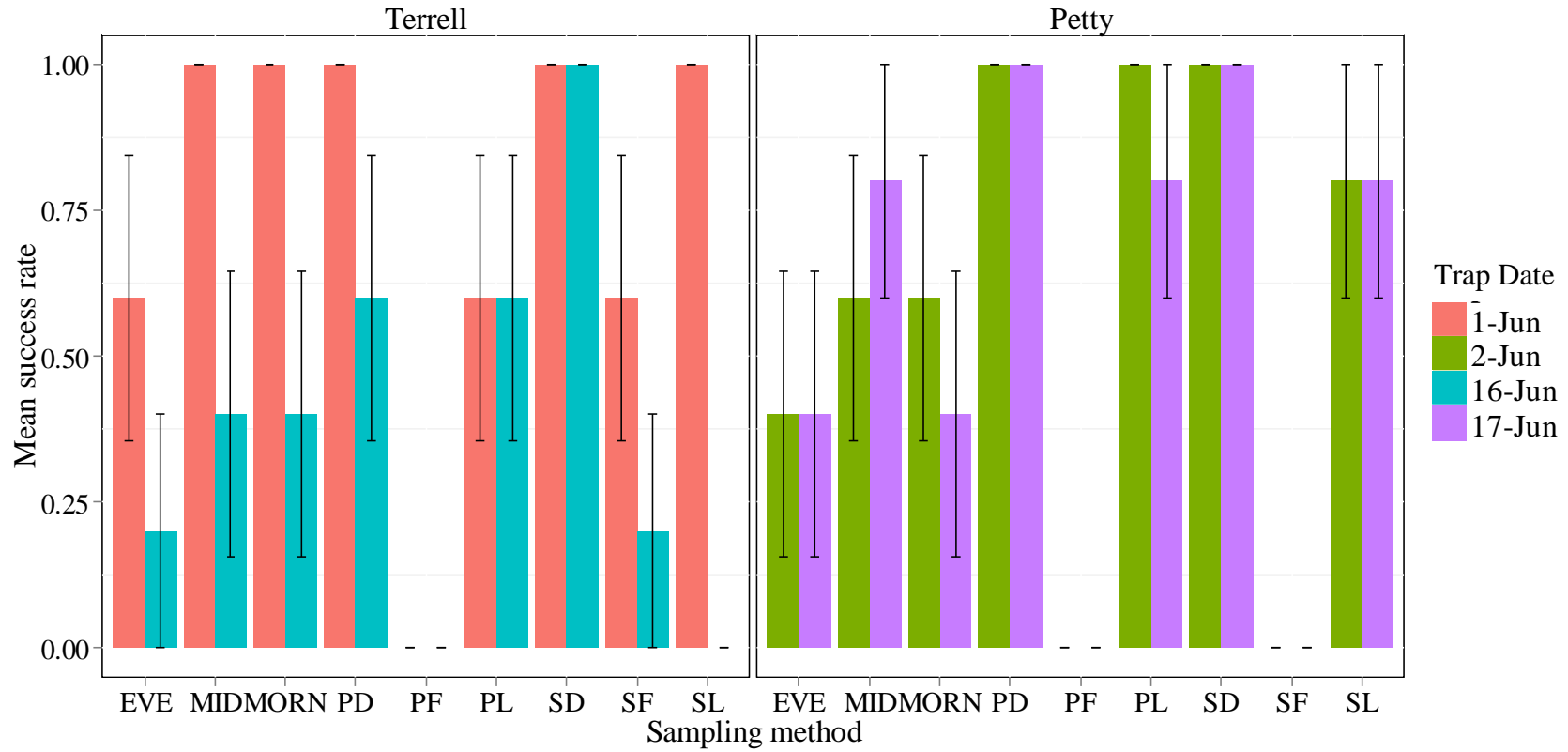


Figure 3.3 Mean capture success rate for all sampling methods on summer sampling dates. A score of “1” means that all samples contained *P. curvatus*. Each bar is the mean of five traps/samples. Error bars signify one standard error of the mean.

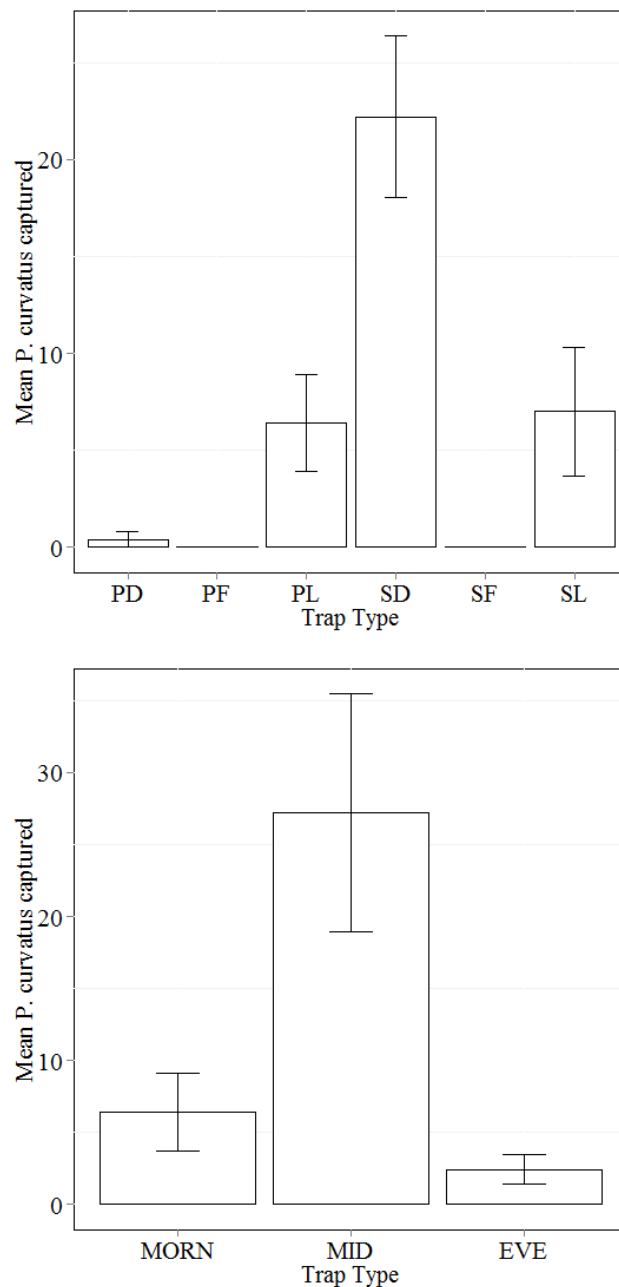


Figure 3.4 Mean *P. curvatus* captured by sticky trap (top) and by aspirating (bottom) for October 11 at Terrell Farm. Each bar is the mean of five traps/samples. Sticky trap/ bait configurations included Puckett/dead ants (PD), Puckett/live ants (PL), Puckett/foraging ants (PF), sandwich/dead ants (SD), sandwich/live ants (SL), and sandwich/foraging ants (SF). Aspirating took place during the morning (MORN), midday (MID), and evening (EVE). Error bars signify one standard error of the mean.

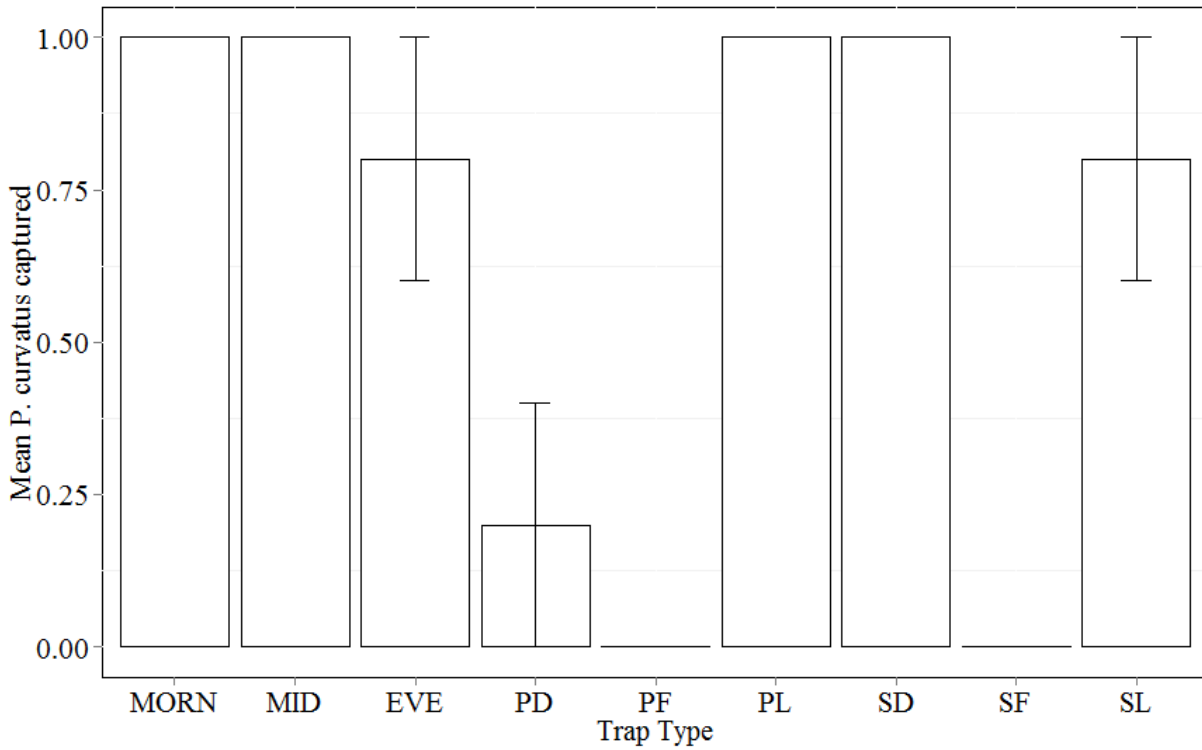


Figure 3.5 Mean capture success rate for all sampling methods on October 11. A score of “1” means that all samples contained *P. curvatus*. Each bar is the mean of five traps/samples. Error bars signify one standard error of the mean.

Chapter 4. Interspecific Competition among *Pseudacteon* spp. (Diptera: Phoridae) in a Laboratory Setting

Introduction

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), originates from South America and is a major pest in the United States, Puerto Rico, Australia, China, and Taiwan (Ascunse et al. 2011). In an effort to provide sustained economical control, a biological control program was initiated in 2001 by the United States Department of Agriculture Animal Plant Health Inspection Service (USDA-APHIS) involving the cooperation of public, state, and federal agencies (Callcott et al. 2011). *Solenopsis invicta* have natural enemies that include microsporidia, viruses, nematodes, and *Pseudacteon* phorid flies (Briano et al. 2012).

Pseudacteon spp. (Diptera: Phoridae) parasitize worker ants and are called decapitating phorid flies because they use the detached heads of their hosts as a puparium. These flies are excellent candidates for biological control because of their host specificity (Gilbert and Porter 2005).

Currently, they are the only group of organisms used in classical biological control against an ant pest (Lebrun et al. 2012). There are 18 species of *Pseudacteon* that parasitize *S. invicta* (Disney 1994). The wide variety of species for a single host is the result of evolutionary niche separation (Fowler 1997). Each species of phorid fly partitions its host through phenological cycles (Folgarait et al. 2003), circadian activity (Pesquero et al. 1996), parasitization strategy (Orr et al. 1997), and host size preference (Morrison et al. 1997). *Solenopsis invicta* workers are polymorphic, and range in size from 2-6 mm (Porter and Tchinkel 1985b). As a result, multiple *Pseudacteon* spp. will be required for effective biological control of red imported fire ants (Morrison and Porter 2005b).

Since the beginning of the phorid biological control release program in 1998, six *Pseudacteon* spp. have established over nine states throughout the southeastern United States (Porter et al.

2013). *Pseudacteon tricuspis* Borgmeier was the first species to establish, followed by *P. curvatus* Borgmeier (Callcott et al. 2011).

Pseudacteon tricuspis populations began to decline in some areas that *P. curvatus* colonized due to competitive displacement (Lebrun et al. 2009). *Pseudacteon tricuspis* is sexually dimorphic, in which females are larger, and emerge from larger hosts (Chirino et al. 2012). It was found that while *P. curvatus* prefer smaller worker ants, a host-size overlap occurred with *P. tricuspis* males (Lebrun et al. 2009). *Pseudacteon curvatus* arrives at mounds before *P. tricuspis* and, due to *S. invicta* behavioral response, alters the size distribution of worker ants so that *P. tricuspis* was less successful at locating hosts for female offspring. This resulted in skewed *P. tricuspis* sex ratios and eventual displacement.

Future parasitoid releases may be negatively impacted by interspecific competition if species are incompatible. For example, *P. obtusus* Borgmeier and *P. cultellatus* Borgmeier were recently released into Central Arkansas. *Pseudacteon obtusus* prefer larger workers (~0.9 mm host ant head width) (Folgarait et al. 2005) than *P. curvatus* (~0.6 mm host ant head width) (Chirino et al. 2009) while *P. cultellatus* prefer smaller workers (<0.6 mm host ant head width) (Folgarait et al. 2002b). There is potential for competition to occur between species within this group. The objectives of this study are to investigate the mechanisms of successful parasitism among these three species by comparing host size ranges, abundance ratios, and sex ratios when in competition with one another.

Materials and Methods

Pseudacteon obtusus, *P. cultellatus*, and *P. curvatus* were used in the study. *Pseudacteon obtusus* and *P. cultellatus* were selected because they were recently released in Central Arkansas, and *P. curvatus* was selected because it is abundant throughout the state (Farnum and Loftin 2011) and because it has been suggested that through interspecific competition it displaced *P. tricuspis* in Texas (LeBrun et al. 2009). It was hypothesized that competition between the two species resulted in skewed sex ratios because *P. tricuspis* is preempted to mound disturbances by *P. curvatus* and because of a host size overlap between *P. curvatus* and *P. tricuspis* males. It is possible that the establishment of the recently released species may be disrupted if *P. curvatus* has a similar effect on them. Conversely, *P. curvatus* may be adversely affected if it is outcompeted by one or both species. Because *P. obtusus* prefer large workers and *P. cultellatus* prefer the smallest workers, it is not expected that they would have a negative interaction; however, *P. curvatus* host size preference may overlap with one or both species. To evaluate this, *S. invicta* workers were exposed to pairings of *Pseudacteon* spp. for parasitization.

Pseudacteon curvatus was paired with one of the two other species in competition trials. The treatments were split into three categories: low competition which consisted of six females of each species, high competition which consisted of 12 of each species, and controls which consisted of six females of one species. Each treatment was repeated three times. *Pseudacteon obtusus* mate over fire ants, so equal numbers of males were included with the female flies.

Flies were shipped from the Florida Department of Agriculture and Consumer Services in Gainesville, Florida as pupae. They were stored in an incubation chamber at an average temperature of 27°C and 90% RH until they emerged. On May 15, it was discovered that the incubation chamber was set at 25°C and was adjusted to 27°C. Flies emerged in the morning and

were given three to five hours to mate before being collected with an aspirator. The flies were held at 5°C for three minutes. They were then placed on a cold table at ~4°C so that they could be counted and sorted for trials.

Fire ants used in the study were collected in Texarkana, Arkansas and near Lamar, Arkansas. They were collected by excavating mounds and placing them into five-gallon buckets. They were then transported back to the laboratory where they were given at least 24 hours to rebuild their galleries. The ants were separated from the soil by using the floatation method (Banks et al. 1981). Water was dripped into the bucket for 4-8 hours. This initiated instinctual rafting behavior by the fire ants which floated to the top of the water so that they could be removed and placed into Fluon® - coated plastic shoe-boxes. These shoeboxes were provisioned with 13 x 150 mm culture tubes containing water, a cotton ball, and moistened Castone ® blocks. These tubes provided a humid, enclosed shelter for the ants. Other culture tubes contained 1.5 M sucrose solution as a food source. Crickets were also fed daily to colonies. The colonies were stored at 63% average humidity and at 24 °C.

To provide a consistent size range to the flies, ants were sorted using test sieves. First, alates and brood were separated from workers. This was accomplished by anesthetizing the ants with ether. As the ants resumed activity, they clung to construction paper while the brood was knocked off. Alates were removed using forceps. Colonies with a large proportion of alates were filtered through a #16 (1,190 µm) sieve to remove alates. To separate worker ants into size groups, a #18 (1,000 µm) sieve was used to isolate the major workers, #20 (850 µm) for the medium workers, #25 (710 µm) for the small, and ants that passed through the #25 were minor workers. For each replicate, approximately 250 workers in each of the four groups were placed into a 29 x 21 cm lock-lid storage box. Rather than counting the ants, the average weight for 250 ants of

each size class was used (0.8 g for major workers, 0.5 g for medium workers, 0.5 g for small workers, and 0.3 g for minor workers). Boxes were provisioned with a Kimwipe ® laboratory napkin soaked in 1.5 M sucrose solution, a Castone ® plaster block, and a cricket. No single colony was used more than once per treatment.

In all replications, flies were allowed to parasitize ants for 5 hours or until their attack activity ceased. To keep ants moving in the arena, a manual cup-lever system was used (Fig. 4.1).

Approximately 1 gram of brood was also included with the ants to encourage movement between the cups. The cups were alternated every five minutes which exposed the ants to the flies. At the end of the exposure period, the flies were removed from the box and ants were placed in the incubation chamber. Starting at 10 days after parasitism, midden – the dead ants and refuse – was taken from the boxes daily and fly pupae were removed. The pupae were then placed ventral side up on AlphaScents ® yellow sticky cards so that emerging flies would not escape. Once flies emerged, they were identified to species and sex. Female flies were identified based on structure of their ovipositors (Porter and Pesquero 2001). Males were identified based on the overall length of the anal tube, thickness of the setae, and shape and length of their antennae (Porter et al. 2013). Host ant head width was measured at the width across the eyes (Chirino et al 2012). Emergence date was recorded for flies that pupated after the temperature adjustment on May 15.

Host ant head width was analyzed by comparing the average of the replicates for each species and sex among treatments. A mixed-effect model analysis of variance was used for mean host ant head width analysis. For sex and species ratios, logistic regression analysis was used. Sex ratio analyses were modelled as the probability that emerging flies were female for each treatment. For species ratio analysis, the probability that emerging flies were *P. curvatus* was

modelled. Mixed-effects analysis of variance was also used for development period. Pairwise comparisons were done for significant effects. To account for unequal variance, the Tukey-Kramer adjustment was made. Analyses were done in SAS Version 9.4 (© SAS Institute Inc.), and graphs were drafted in R.

Results

Because the Florida Department of Agriculture experienced a shortage of *P. cultellatus*, treatments with this species were not used in analysis. Instead, the experiment focused on possible competition between *P. curvatus* and *P. obtusus*. Overall, 645 adult *Pseudacteon* emerged from treatments (309 *P. curvatus* (113 females, 196 males) and 336 *P. obtusus* (127 females, 209 males). Total emergence for each replicate ranged from 17-99 individuals. Emergence rates ranged from 44% to 79%.

Host Size

Mean host head capsule widths are presented in Table 4.1. Based on the analysis of variance, host ant head width varied significantly between species ($p < 0.0001$), but no significant differences were found among treatments. *Pseudacteon curvatus* females did not emerge from one replication in each of the competition (high and low) treatments. As a result, the unequal sample size made it difficult to make means comparisons. This was remedied by running an additional analysis of variance which ignored treatment effects. This analysis found that *P. curvatus* males and females did not use different host sizes, but that *P. obtusus* males and females did ($p < 0.0001$). *Pseudacteon obtusus* flies emerged from larger hosts than *P. curvatus*. Fig. 4.2 shows the host size distributions based on species and sex.

Sex and Species Ratios

For sex ratios, there was an interaction between treatment and species ($p=0.0023$). Sex ratios did not significantly vary for either species. However, sex ratios did vary between species. In the low competition experiment, mean sex ratios were 2.7:1 (m:f) for *P. curvatus* and 3.5:1 for *P. obtusus* ($p=0.01$). In addition, sex ratios differed significantly between control replications (1.3:1 for *P. curvatus* and 7.1:1 for *P. obtusus*) (0.0169). By removing the two replications where *P. curvatus* females did not emerge, sex ratios only differed significantly between control replications ($p=0.015$, unadjusted p-value) (Fig. 4.3). The Tukey adjusted p-values showed no significant differences in sex ratios. For species ratios, there was no significant difference among treatments ($p=0.075$) (Fig. 4.4).

Development Period

Analysis for development period only includes replications that took place after May 15. The analysis of variance showed a three-way interaction between species, sex, and treatment ($p=0.002$). Development period for each treatment is presented in Table 4.1. Means comparisons showed that development period varied by treatment but not across treatments for each species (Table 4.2).

Discussion

Mean host head width distributions varied significantly between species. Only in *P. obtusus* did females emerge from larger hosts than males. An overlap occurred between both sexes of *P. curvatus* and *P. obtusus* males. This observation was also made for *P. tricuspis* and *P. curvatus*

in field tests by LeBrun et al. (2009) and was suggested to be a contributing factor to the competitive displacement of *P. tricuspis*.

The disruption of sex ratios was another contributing factor to the competitive displacement of *P. tricuspsis* (LeBrun et al. 2009). If sex ratios had been significantly different between individual treatments and the competition treatments for the two species, then interspecific competition may have an effect in nature; however, the results of this study show no significant effect. For *P. curvatus*, sex ratios did vary by treatment, but not within species. This indicates that the two species operate with different sex ratios, but no disruption was detected in this study. Another explanation for the variation in *P. curvatus* sex ratios relates to the variation among replications. Female *P. curvatus* did not emerge in two of the competition replications. By ignoring these replications, the sex ratios were more consistent. LeBrun et al. (2009) suggested that the main mechanism for competitive displacement of *P. tricuspsis* was that *P. curvatus* preempted them from their host. There was no way to directly measure this in the study presented here; however, the results of this study did not show any competition between *P. curvatus* and *P. obtusus*.

Development time differed between species. The presence of other species did not seem to have any significant impact on development time. However, the difference in development time between species may have an impact on the population dynamics of the two species. For instance, *P. obtusus* has a shorter development time than *P. curvatus*, which may lead to a faster population growth rate over time. This might have a stabilizing effect on stochastic events, such as drought because affected populations could recover more quickly. However, the difference in development times observed here may not be enough to have a significant effect on the number of generations for these species.

Pseudacteon obtusus sex ratios did not vary significantly among treatments, but a wide variation in the number of females emerging from replications occurred. Although sorting took place to ensure that equal numbers of each size class were present, there may not have been sufficient numbers of the largest female *P. obtusus* workers available. If this were the case, then females emerging from such colonies might emerge from smaller hosts. There were no differences in *P. obtusus* female host size among treatments or replications in this study. Therefore, this does not seem to be the case.

Competitive displacement may occur if species ratios are skewed in favor of a particular species. There were no significant differences in species ratios among treatments in this study. Therefore, the results of this study indicate that *P. curvatus* and *P. obtusus* should be able to coexist based on the conditions of our laboratory test; however other factors are almost certainly involved in the field.

The results of this study are consistent with what was found in field studies by others. In field studies in Florida, *P. obtusus* competes well with *P. curvatus*. Porter and Calcaterra (2013) suggested that the lack of competition between species is due to the different host size preference and that *P. obtusus* is attracted mostly to foraging ants while *P. curvatus* is attracted to mound disturbances. Plowes et al. (2011) successfully established *P. obtusus* in Texas, but found that establishment was 35 times more successful in areas that are uninhabited by other *Pseudacteon* spp. It was suggested that competition may influence successful establishment. Because establishment outcomes vary by region (Gilbert et al. 2008), climate (Folgarait et al. 2005b), and host social form (Chirino et al. 2012), further field studies in other areas are needed to confirm what allows the two species to exist in sympatry when this does occur.

The biological control of red imported fire ants requires multiple natural enemies (Morrison and Porter 2005), as is the case for many other invasive pests. In these cases, it is possible for unforeseen competition among natural enemy guilds to affect the success or failure of a program. For this reason, it is important to investigate the mechanisms of intraguild competition so that science-based decisions can be made when releasing natural enemies.



Figure 4.1 Manual cup-lever system used in replications. Cups were alternated by reaching into the quarantine box and rotating the arms of the lever.

Table 4.1 Mean head capsule width, sex ratio, and mean fly emergence.

| Treatment | <i>P. curvatus</i> | | <i>P. obtusus</i> | |
|---------------------|-----------------------------------|-------------------------------------|-----------------------------------|-------------------------------------|
| | M/F Head Capsule Width (mm) | M:F Sex Ratio (total # flies) | M/F Head Capsule Width (mm) | M:F Sex Ratio (total # flies) |
| Control | 0.75/0.76 | 1.52 (67) | 1.02/1.28 | 7.12 (23) |
| Low competition | 0.82/0.76 | 3.42 (15) | 1.03/1.21 | 3.53 (51) |
| High Competition | 0.82/0.77 | 1.25 (30) | 1.06/1.26 | 5.57 (34) |

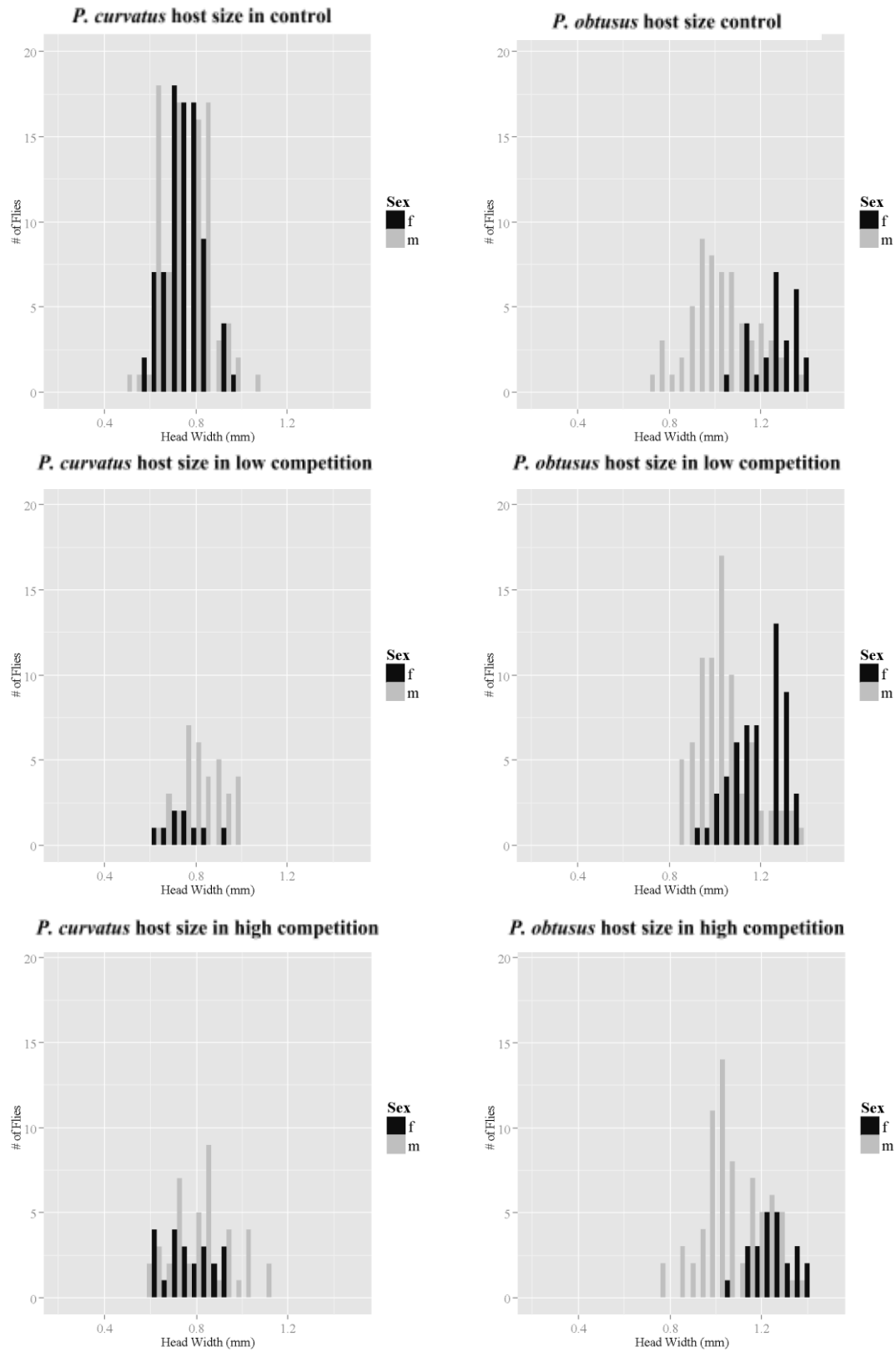


Figure 4.2 Host size distributions for *P. curvatus* and *P. obtusus* for treatments.

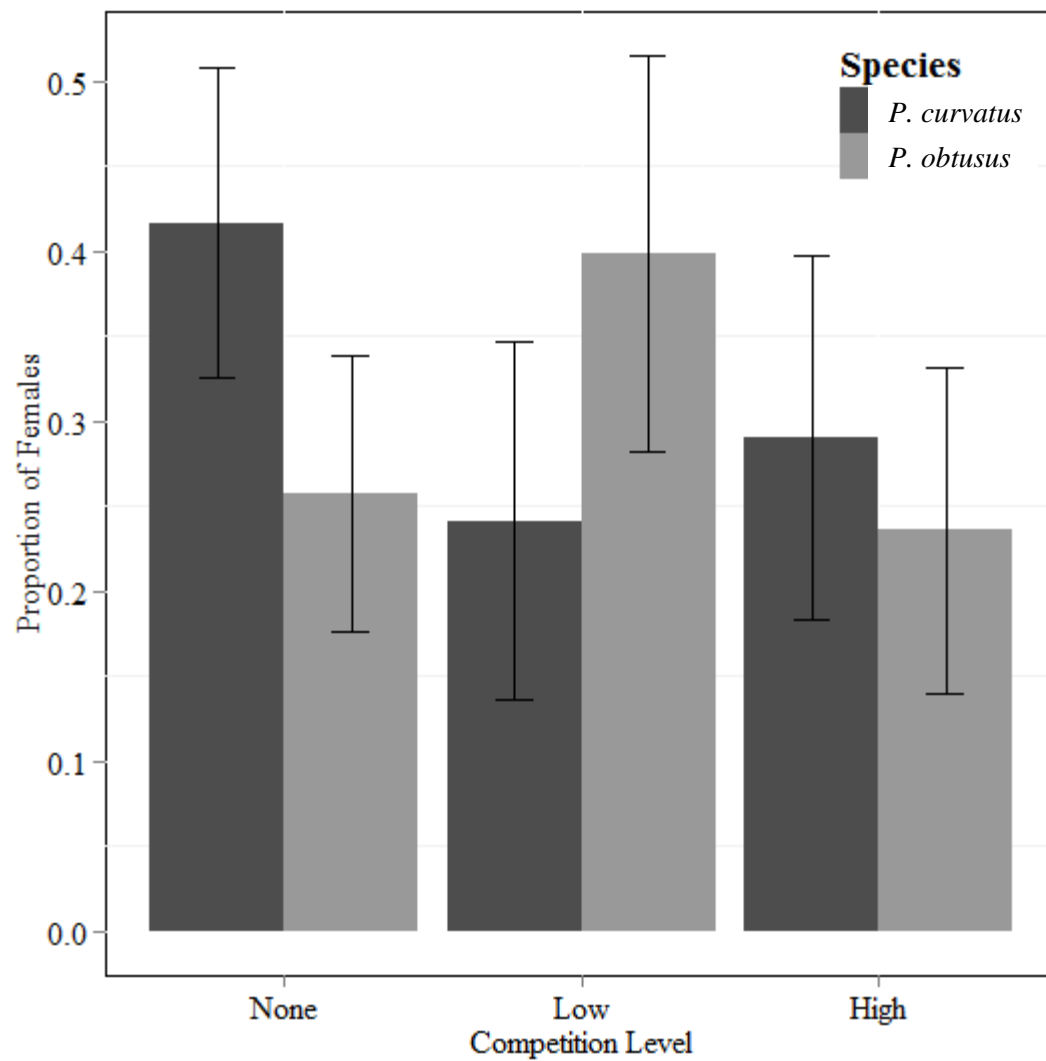


Figure 4.3 Proportion of females for each *Pseudacteon* species and competition level. Dark bars represent *P. curvatus* and light bars represent *P. obtusus*. Error bars signify one standard error one the means.

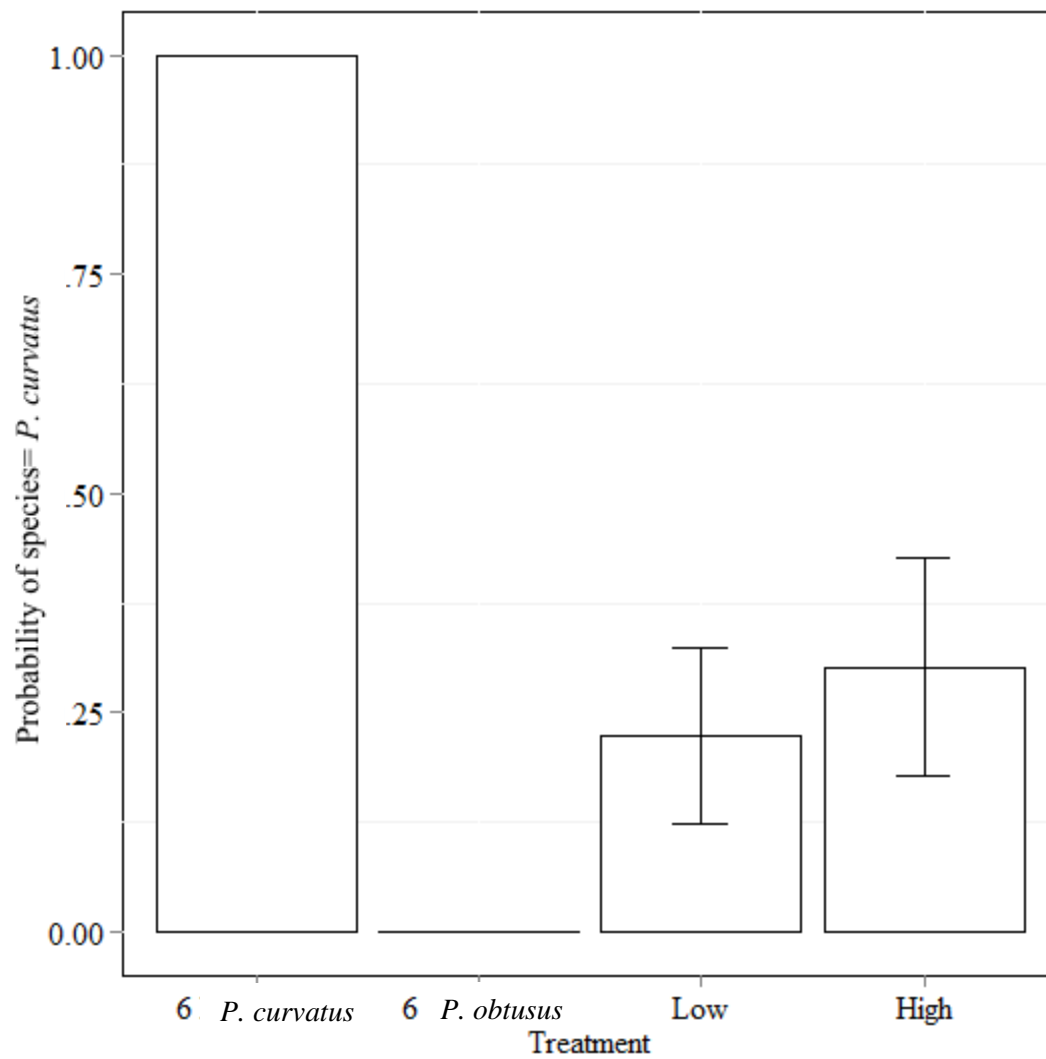


Figure 4.4 Mean probability that emerging flies will be *P. curvatus* for each treatment. Error bars signify one standard error of the mean.

Table 4.2 Significant comparisons for development period analysis.

| <i>P. curvatus</i> | | | <i>P. obtusus</i> | | | |
|----------------------------|-----|--------------------------------|-------------------|-----|--------------------------------|---------|
| Treatment | Sex | Mean Development Period (days) | Treatment | Sex | Mean Development Period (days) | p-value |
| High Competition | f | 29.2 | High Competition | f | 27.5 | 0.0106 |
| High Competition | f | 29.2 | High Competition | m | 26.5 | <0.0001 |
| High Competition | m | 29.3 | High Competition | f | 27.5 | 0.0006 |
| High Competition | m | 29.3 | High Competition | m | 26.5 | <0.0001 |
| <i>P. curvatus</i> Control | m | 29.2 | High Competition | m | 26.5 | 0.0102 |
| <i>P. curvatus</i> Control | f | 29.3 | High Competition | m | 26.5 | 0.011 |
| Low Competition | m | 28.5 | High Competition | f | 27.5 | 0.0193 |
| Low Competition | f | 29.2 | Low Competition | f | 27.8 | 0.04 |

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