Physiology and Chemical Ecology of *Solenopsis* Fire Ants: Interactions with Biotic and Abiotic Factors in Their Environment

by

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Abstract

Interactions between organisms and the environment affect the survival and distribution of species. Biotic and abiotic factors impact the adaptations of *Solenopsis* fire ants (Hymenoptera: Formicidae) in the southeastern United States. This study investigated the physiology and chemical ecology of some native and introduced *Solenopsis* fire ant species in relation to some aspects of their adaptations to biotic and abiotic factors in the southeastern United States. These aspects include desiccation tolerance, use of volatiles emitted by the ants as host location cues, and the interspecific variation in venom alkaloid components in queen fire ants.

In chapter I, I introduced the topic and discussed pertinent literature on physiology and chemical ecology of *Solenopsis* species of fire ants in the southeastern United States, identified specific aspects of their adaptations to biotic and abiotic factors that could limit their survival and distribution, and provided rationale for the current study.

In chapter II, I tested the prediction that tropical/sub-tropical fire ants (*S. invicta* and *S. geminata*) will have lower cuticular permeability values and tolerate higher levels of desiccation (i.e., greater %total body water loss) than temperate fire ants (*S. richteri* and *S. invicta* × *S. richteri*). This study compared the water relations of temperate and tropical/sub-tropical fire ant workers across different latitudes in southern USA and is the first report of cuticular permeability (CP) and desiccation tolerance of *S. invicta* × *S. richteri* hybrid workers. The data supported my hypothesis, as CP value of live workers of a temperate fire ant species, *S. richteri* is significantly greater than that of a tropical species, *S. invicta*. The data indicate that *S. invicta* is significantly less vulnerable to desiccation stress than *S. richteri*. This may partially explain the predominant location of each of these species, as *S. invicta* are found in lower latitudes and tropical/subtropical region in their native South America, while *S. richteri* are found in higher latitudes and temperate regions in their native South America. In addition, the climate in their current location in southeast U.S.A. is similar to that of their native distribution. This suggests that CP

plays a role in the distribution patterns of invasive ants, and adaptation to the climate of their abundant location.

In chapter III, I quantified and compared the salient characteristics of the DGC in *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata*. The goal was to make comparative DGC for ants occupying different habitats. *Solenopsis invicta* and *S. geminata* seem to be xeric while *S. richteri* and *S. invicta* × *S. richteri* are more of mesic ants. The DGC frequency in workers and alates of *S. invicta* in my results are similar to published results. Results showed that the DGC was pronouncedly expressed in the alate caste (irrespective of temperature) in all tested *Solenopsis* species. This may be explained by the hygric genesis hypothesis that if the DGC was primarily a mechanism for conserving respiratory water, it would be preferentially expressed in the species and castes subject to more desiccating conditions, and alates are exposed to desiccating environments, especially during season of fire ant mating flights. Altogether, the DGC seems to influence the adaptation of these *Solenopsis* species of fire ants in the southeastern USA.

In chapter IV, I hypothesized that certain compounds in the venom alkaloid profiles of fire ant species mediate preference in their hosts, *Pseudacteon* phorid flies (Diptera: Phoridae) for imported versus native fire ants. Two host phorid flies, *P. curvatus* and *P. obtusus*, were tested for their preference to *cis*- and *trans*- fractions of fire ant venom alkaloids in behavioral bioassays. Results from the behavioral experiments provide evidence for the involvement of venom alkaloids in mediating host preference of *Pseudacteon* species between native and imported *Solenopsis* fire ants. In the olfactometer bioassays, both *P. curvatus* and *P. obtusus* exhibited stronger attraction to *cis* + *trans* venom alkaloids of imported *S. invicta* and *S. richteri* fire ants over those of native fire ants. Altogether, the results indicate that fire ants venom alkaloids are involved in host preference in parasitic phorid flies. However, host preference and performance of a parasitoid are not always tightly linked.

In chapter V, I compared venom alkaloid profiles of queens of different reproductive status among invasive and native species in both *S. saevissima* and *S. geminata* complexes. I hypothesized that the abundant piperidine alkaloids (*cis*-C_{11} and *trans*-C_{11}), unique to the venom of *Solenopsis* fire ants, encode information on reproductive status in queens. In *S. invicta*, proportion of *cis* piperidines (the less abundant geometric configuration in workers) was 1.5-fold more abundant in reproductive than non-reproductive queens. There were differences in profile of *cis* piperidines between reproductive and non-reproductive *S. invicta* queens suggesting that this chemical is a signal that their workers use to recognize and discriminate between queens of the two reproductive status. Such behavioral discrimination becomes apparent only when the queen becomes sexually mature. The results supported previous studies that suggests that total alkaloids and ratio of *cis*-C_{11} to *trans*-C_{11} in queen *Solenopsis* fire ants are associated with reproductive status and taxonomic classification into species complexes.

In chapter VI, I summarized the major findings of my dissertation research and discussed areas of future studies in insect ecological adaptation research. Investigation of expressions of candidate genes that likely influence desiccation resistance in terrestrial insects was identified as one of areas that merits further consideration. Future studies should investigate the effect of combination of other venom alkaloid components of *Solenopsis* fire ants on host preference in *Pseudacteon* phorid flies, in order to better understand the mechanisms underlying host preference in these parasitoids. In addition, the effect of genes that determine monogyny or polygyny in *Solenopsis* fire ants (such as *Gp-9* genes) should be investigated on fertility signaling in several species of Solenopsis fire ants in both the *S. saevissima* and *S. geminata* complexes.

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CHAPTER 1

INTRODUCTION

1.1 Biology of *Solenopsis* Fire Ants

The genus *Solenopsis* (Hymenoptera: Formicidae) currently contains about 185 described species, including ~20 species of fire ants that are native to the Americas (Trager 1991, Pitts et al. 2005; Tschinkel 2006; Chen and Fadamiro 2018). They are so-called fire ants because of the burning sensation produced when workers stung and inject toxin into intruders of their nests. The stinging results to formation of pustules in many human victims. Ants in this genus belong to the subfamily, Myrmicinae, the largest subfamily of ants with300 North American species, and its members can usually be recognized by the fact that the pedicel of the metasoma has two segments. Ants of the genus *Solenopsis* may be distinguished from other myrmicine ants by the fact that the antennae are 10-segmented with a 2-segmented club. The members in this genus are widely distributed and vary considerably in habits (Triplehorn and Johnson 2005).

Eusociality is observed in *Solenopsis* fire ants, which is typically characterized by a division of labor into groups called castes (Wilson and Hölldobler 2005). Worker caste is non-reproductive, while the reproductive castes are male alates and female alates, and each colony contains at least a reproductive queen. The workers are 3–6 mm long and reddish brown. Workers are sterile females, lacking ovaries, and provide the labor for all of colony life including building the nest, rearing the next generation of ants, foraging, processing and distribution of food, defending the nest, caring for the queen, and sanitation services Tschinkel 2006). Colonies of *Solenopsis* spp. may contain > 100,000 workers (Markin et al. 1973; Mirenda and Vinson 1981; Tschinkel 1988b; 1993a). The workers are polymorphic, having large variation in sizes. The tasks carried out by large, medium, and small workers differ, although they frequently overlap.

There is territoriality in colonies of fire ants. Workers recognize nestmates by their cuticular hydrocarbons as well as from the food the colony eats and the soil they dwell in. Fire ants are holometabolous, they undergo complete metamorphosis.

Sex determination in fire ants is through a mechanism called haplodiploidy, where fertilized eggs become female ants, while unfertilized eggs develop into males (arrhenotokous parthenogenesis). During late spring and early summer, sexual alates participate in mating flights (Markin et al. 1972, Tschinkel 1993a). Newly mated queens dealate, dig chambers and rear the first workers either by themselves or in cooperation with other newly mated queens (Markin et al. 1972, Tschinkel and Howard 1983, Tschinkel 1993b). Most of the southeastern USA is occupied by colonies with single queens, but some areas in Texas harbor enclaves in which colonies have multiple queens (Ross et al. 1988; 1996a; 1996b; Tschinkel 2006). The tolerance of multiple queens by workers in a colony is associated with allelic variation at the gene *Gp*-9 (Gotzek and Ross 2007). Reproductive queens have swollen abdomens with large ovaries and are the only egg laying caste in the colony. A single reproductive queen can lay an egg about every 30 seconds, without pause and emits semiochemicals that control the eventual sex of larvae and the behavior of workers. one or more queens.

Fire ant nests are mounded and are made by workers. The nests are hard-crusted and may sometimes be as large as a meter high and a meter across. The mound encloses a maze of tunnels and warms faster than the surrounding soil. These nests contain both adult ants as caretakers and the colony's brood.

1.2 Origin and Spread of Native and Imported Fire Ants in Southeast USA

The black imported fire ant, *Solenopsis richteri* Forel, and the red imported fire ant, *S. invicta* Buren, were accidentally introduced into the United States of America from South America in the 1920s and 1930s, respectively, through Mobile, Alabama (Lofgren et al. 1975; Patterson 1994). Since their introduction, they have spread over much of the Southeast U.S. from the Carolinas to Texas. *S. invicta* has displaced native fire ants (Vinson 1997), including *S. geminata* (Fabricius). Since their introduction, both *S. richteri* and *S. invicta* have formed an extensive zone of hybridization in their invaded region, producing hybrid *S. invicta* × *S. richteri* species (Diffie et al. 1988; Vander Meer et al. 1985). However, hybridization has not been found in their native South America (Vander Meer et al. 1985; Cohen and Privman 2019).

The introduced species belong to a group of ants taxonomically known as the *Solenopsis saevissima* complex, while the native species belong to the *S. geminata* complex (Trager 1991; Ross and Trager 1990). Members of the two complexes are considered distant from each other. *Solenopsis richteri* occurs at slightly higher latitudes than *S. invicta* both in the USA and in their native South America, while *S. invicta* × *S. richteri* occur between and intermixed with pure species populations (see maps in Taber (2000), pp 27 and 59) (Buren et al. 1974; Tschinkel 2006). *Solenopsis invicta* and *S. geminata* are usually found in the tropical/subtropical USA, while *S. richteri* are usually found in more temperate areas in the USA (Buren et al. 1974; Morrison et al. 2005). The pattern of spread of *S. invicta* and *S. richteri* in the invaded southeastern USA, has been predicted to take similar shape to that of their native South America (Morrison et al. 2005). In their native habitat, *S. invicta* and *S. richteri* have not been observed to co-occur often, with few overlaps at the borders of their occupied ranges (Tschinkel 2006).The introduced *S. richteri* and *S. invicta* have remained phenotypically constant since the time of their introduction to North America (Buren 1972).

Solenopsis invicta has spread throughout the southern United States via nuptial flights of newly mated queens and by human commerce. This species now occupies much of southern USA and has established in California, West Indies, New Zealand, Australia, the Caribbean, and parts of Asia (Callcott and Collins 1996; Davis et al. 2001; Mescher et al. 2003; Ascunce et al. 2011). Much of the tropical and subtropical regions of the world have been predicted to be at risk of *S. invicta* infestation (Morrison et al. 2004), which was validated by the recent invasions of Taiwan and southern China (Chen et al. 2006; Zhang et al. 2007; Chen and Fadamiro 2018). Once established in a new environment, fire ant colonies spread rapidly in disturbed habitats and are often associated with disruption of established arthropod communities (King and Tschinkel 2008; Chen and Fadamiro 2018).

1.3 Economic Importance and Cost of Control of *Solenopsis* species

Solenopsis species are well represented in the southeastern USA and some are considered important pests. They are pests in agricultural, urban, and natural habitats; and are also medically important pests. For example, *S. invicta* is considered one of the world's most invasive alien species (Lowe et al. 2000), causing damage to natural ecosystems and (Gotelli and Arnett 2000; Stiles and Jones 1998; Stuble et al. 2009; Chan and Guénard 2020) agricultural land (Adams 1986; Zhang et al. 2007). This species has displaced native ant species, caused population collapses of numerous arthropods, and poses significant threats to biodiversity and urban environments (Allen et al. 2004; Tschinkel 2006; Vinson 1997; Wojcik et al. 2001; Chen and Fadamiro 2018). In the USA alone, *S. invicta* is responsible for a decline in agricultural production through direct predation on different plant parts (e.g. roots, fruits, flowers, stems) (Adams1986; Chan and Guénard 2020). Moreover, this species also causes indirect damage to crop production through their mutualism with sap-sucking insects such as aphids and mealybugs, and limits the efficiency of biological control programs through the predation of auxiliary insect predators or parasitoids (Eubanks 2001; Hill and Hoy 2003; Parys and Johnson 2012). As a result, the presence of *S. invicta* costs the agricultural industry an estimated $6 billion per year in the USA with$200 million of that applied to control the population (Pimentel et al. 2005; Drees and Lard 2006). This significant financial loss is the result of the mounds causing damage to agricultural machinery, and labor loss as workers are reluctant to work in areas where they may encounter their painful stings. The nests may be constructed on agricultural and, home sites, school yards, and recreational areas.

Furthermore, fire ants impact public health directly, through their aggressive stinging behavior, the injected venom can result in multiple medical problems such as pustules, pain, nausea and anaphylaxis (Kemp et al. 2011; Chan and Guénard 2020; Vinson 2013; Chen and Fadamiro 2018). Generally, imported fire ants (i.e., *S. invicta*, *S. richteri*, and their hybrid) are considered one of the most important drivers of ecosystem dynamics in their introduced range (Holway et al. 2002; Chen and Fadamiro 2018). A number of highly effective insecticides are available for fire ant control (Williams et al. 2001; Chen and Fadamiro 2018).

1.4 Biotic factors Impacting *Solenopsis* fire ants

Natural enemies of fire ants constitute a group of biotic factors that impacts the survival, reproduction, and consequently the spread of *Solenopsis* species. One of these enemies is *Pseudacteon* (phorid) flies (Diptera: Phoridae). *Pseudacteon* flies are koinobiont, solitary parasitoids of worker ants (Feener and Brown 1997; Chen and Fadamiro 2018). Phorid flies were introduced from their native South American range into the USA as classical biological control agents of imported *Solenopsis* fire ants. The adult flies live only a few days in the laboratory, but their life span outside of captivity is unknown (Morrison 2000; Porter 1998; Chen and Fadamiro 2018). Sugar feeding can considerably enhance the life span of adult phorid flies for approximately 4–15 days in the laboratory (Ajayi and Fadamiro 2016; Chen et al. 2005; Fadamiro et al. 2005; Chen and Fadamiro 2018). Under field conditions, phorid flies may encounter and feed on nectar and/or honeydew sources, as they are capable of utilizing cotton aphid honeydew with an approximately 1-day increase in adult longevity (Chen and Fadamiro 2006; Fadamiro and Chen 2005; Henneberry et al. 2000; Chen and Fadamiro 2018). Host-specific *Pseudacteon* (Diptera: Phoridae) flies are promising classical biocontrol agents for fire ant populations (Morrison 2012; Porter and Alonso 1999; Chen and Fadamiro 2018).

In the past two decades, six phorid fly species have been imported from southern South America and released in many parts of the southern United States for fire ant control (Callcott et al. 2011; Gilbert et al. 2008; Plowes et al. 2012; Porter et al. 2011; Porter et al. 2013; Chen and Fadamiro 2018). At least 22 species of *Pseudacteon* flies oviposit in worker fire ants of the *Solenopsis saevissima* complex, which includes *S. invicta* and *S. richteri* (Folgarait et al. 2007b; Chen and Fadamiro 2018). Whereas at least 4 species of *Pseudacteon* flies oviposit in *S. geminata* workers, which belong to the *Solenopsis geminata* complex (Morrison et al. 1999). The same species that are known to attack *S. geminata*, do not attack *S.*

invicta, suggesting host partitioning among *Pseudacteon* flies (Feener 1987; Disney 1991; Chen and Fadamiro 2018). *Pseudacteon curvatus* was first released in parts of the southeastern US in 1999 and has since become well established in several parts of the region (Graham et al. 2003). *Pseudacteon obtusus* was first released in 2006 in Texas, and subsequently in other parts of the southeastern US (Plowes et al. 2011). Another parasitoid of *S. invicta* is the male *Caenocholax fenyesi* (Strepsiptera: Myrmecolacidae) (Triplehorn and Johnson 2005).

Solenopsis invicta displaces other *Solenopsis* species of fire ants, thus constituting a biotic factor that affects the distribution of *Solenopsis* species. Venom is utilized to achieve this displacement. Ant venoms contain a diversity of proteins, alkaloids and other classes of compounds (Fry et al. 2009). There is large interspecific and intraspecific variation in ant venom components leading to disparity in venom use (Brand et al. 1973a). Utilization of venoms by ants include prey capture, self- and kin-defense, and antimicrobials (Blum 1985). Furthermore, the structure of ant communities has been suggested to be mediated by venom (Andersen et al. 1991; Westermann et al. 2015), and it serves as a means of biotic resistance against invaders (Sorrells et al. 2011).

1.5 Abiotic factors Impacting *Solenopsis* fire ants

Temperature is a key abiotic factor impacting terrestrial insects such as fire ants as it directly affects survival (Angilletta 2009). Most terrestrial insects experience substantial variation in body temperature which can drive changes in metabolic rate and subsequent oxygen consumption (Gillooly et al. 2001; Irlich et al. 2009). For fire ants and some other insects, it has been shown that higher body temperatures are associated with higher ventilatory frequencies or burst frequencies during discontinuous gas exchange (Lighton 1994; Davis et al. 1999; Chappell and Rogowitz 2000; Vogt and Appel 2000; Harrison et al. 2012). Many studies have demonstrated that the respiratory burst volume decreases with increased temperature in ants (Lighton 1988a; Quinlan & Lighton 1999; Vogt & Appel 2000). Temperature plays a crucial role as the essential modifier of the intensity of metabolic processes in poikilothermic organisms, including ants (Vogt and Appel, 2000), also the degree of the organism's acclimatization to environmental conditions affects the intensity of its metabolic processes (Nielsen et al. 1999).

Relative humidity is another abiotic factor that can impact fire ants. Distribution of terrestrial insects including fire ants is limited by tolerance to abiotic stressors such as temperature and relative humidity (Deutsch et al. 2008; Sunday et al. 2014). Desiccation (high temperature and low relative humidity) impacts insect water balance, and tolerance to this stress plays an important role in the geographic distribution of insects (Danks 2000; Chown and Nicolson 2004; Kleynhans and Terblanche 2011). Understanding native distributions and ecologies of invasive insect species is useful in predicting their potential spread in new areas of introduction (Morrison et al. 2005; Weldon et al. 2016). Desiccation tolerance is an inherent trait and is expected to be constantly expressed phenotypically between generations (Alpert 2005). There are apparent physiological and ecological limits to desiccation tolerance which may be underlain by phylogenetic or phenotypic limits to tolerance (Alpert 2000; Alpert 2005; Alpert and Oliver 2002).

The high surface area to volume ratio of terrestrial insects such as fire ants makes them highly susceptible to desiccation via water loss through the cuticle (Appel et al. 1991). Cuticular permeability is usually the most important factor limiting water loss (Edney 1977; Appel et al. 1991; Hadley 1994; Mazer and Appel 2001). Cuticular permeability is significantly affected by size and status (live or dead) of terrestrial insects. The cuticular water pump, located in the epidermal cells, actively restricts water loss in living insects (Winston 1967; Winston and Beament 1969; Berridge 1970; Appel et al. 1991). These variabilities and factors are expected to play a role in limiting species geographical distribution patterns. Cuticular permeability of terrestrial insects is influenced by climatic factors such as temperature, relative humidity, and saturation deficit in their habitat (Appel et al. 1991; Mazer and Appel 2001). These climatic factors vary from latitude to latitude (Manabe et al. 1991; Fields et al. 1993; Peng et al. 2002) and are being negatively impacted by climate change (Cunze et al. 2013; Jagadish et al. 2012). Dramatic variation in water availability among habitats is another important factor in species distribution.

A number of investigations have compared cutaneous water relations within and across some *Solenopsis* spp. and have identified a number of physiological traits related to cutaneous water loss. Workers of *S. invicta* were more tolerant to desiccation stress than *S. richteri* workers despite the significantly higher body water content in *S. richteri* than *S. invicta* (Chen et al. 2014). This may be attributed to phenotypic differences between the two species. Size also plays a role in cuticular permeability, the rate of water loss from the cuticle, in *Solenopsis* spp. For example, despite the higher total body water content (%TBW) in small than large *S. invicta* workers, cuticular permeability was found to be higher in small than large *S. invicta* workers (Appel et al. 1991). In addition, Schilman et al. (2007) found that water-loss rates were significantly lower for large rather than small *S. xyloni* McCook workers. Interestingly, Li and Heinz (1998) indicated that desiccation resistance in polygyne *S. invicta* is not a function of body size and found low heritability of desiccation resistance in the tested population. Thus, several inheritable physiological traits impact the water relations of *Solenopsis* spp. Similar impacts could also be caused by environmental factors.

Several studies have revealed the degree of impact and relation of extreme abiotic factors to cutaneous water loss within and across some *Solenopsis* spp. Braulick et al. (1988) compared lethal times (LT) in hours at zero percent relative humidity and high temperatures for workers of *S. aurea*, *S. geminata*, *S. invicta*, and *S. xyloni*. In general, workers survived progressively longer periods of time as temperature decreased; and major workers survived two to four times as long as minor workers at the same temperatures. Munroe et al. (1996) compared the effect of desiccation on survival times in workers of *S. invicta*, *S. geminata*, and *S. xyloni*, and found that *S. invicta* had lower LT_{50}-values than *S. geminata* and *S. xyloni*. Wendt and Verble-Pearson (2016) found that major and medium *S. invicta* workers survived higher temperatures more often than did minor workers. Phillips et al. (1996) found that workers of *S. invicta* from xeric conditions were less prone to desiccation than were those from moist conditions. Li and Heinz (1998) found that *S. invicta* polygyne populations might be capable of adapting to arid habitats, therefore suggesting an advantage in dominance of polygyne over monogyne *S. invicta* populations in arid habitats. Martin and Vinson (2008) demonstrated that the ability to maintain a minimum viable level of body water could be a limiting factor to foraging range in *S. invicta* workers. Vogt et al. (2003) also showed that temperature is a significant predictor of foraging activity in *S. invicta* workers. Thus, abiotic and biotic factors impact the water relations of *Solenopsis* species. However, more insight is needed on how these factors impact the chemical ecology, physiology, survival, and distribution of various imported and native *Solenopsis* species in the southeastern USA. Insight on this mechanism will aid in the management of these pests by prioritizing management efforts and prediction of potential spread of the ants within and across their current range in the southeastern USA.

This dissertation focuses on (a) the comparative cutaneous water loss and desiccation tolerance of temperate and tropical/sub-tropical fire ants, *Solenopsis* spp., in the southeastern USA, and their implications for distribution patterns; (b) discontinuous gas exchange in invasive and native *Solenopsis* fire ants in the southeastern USA, and the effects of differences in species, caste, and temperature; (c) host preference in parasitic phorid flies, and the response of *Pseudacteon curvatus* and *P. obtusus* to venom alkaloids of native and imported *Solenopsis* fire ants; and (d) queen venom alkaloids in *Solenopsis* species, its interspecific variation and association with fertility.

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CHAPTER 2

COMPARATIVE CUTANEOUS WATER LOSS AND DESICCATION TOLERANCE OF TEMPERATE AND TROPICAL/SUB-TROPICAL FIRE ANTS, *SOLENOPSIS* SPP., IN THE SOUTHEASTERN UNITED STATES: IMPLICATIONS FOR DISTRIBUTION PATTERNS

2.1 Introduction

Distribution of terrestrial insects is limited by tolerance of abiotic stressors such as temperature and relative humidity (Rogers et al. 2004; Detsch et al. 2008; Sunday et al. 2014). Desiccation impacts insect water balance, and tolerance to this stress plays an important role in the geographic distribution of insects (Danks 2000; Chown and Nicolson 2004; Kleynhans and Terblanche 2011). Understanding the native distribution of invasive insect species is useful in predicting their potential spread in newly introduced areas (Morrison et al. 2005; Weldon et al. 2016). Desiccation tolerance (i.e., %Total Body Water lost at death) could be expressed phenotypically between generations (Alpert 2005). There are also apparent morphological and ecological limits to desiccation tolerance such as phylogenetic limits to tolerance or physical or physiological constraints (Alpert 2005; Alpert 2000; Alpert and Oliver 2002).

The high surface area to volume ratio of terrestrial insects makes them highly susceptible to desiccation mainly through the cuticle (Appel et al 1991). Terrestrial insects conserve water by a relatively water impermeable cuticle covered with a thin layer of epicuticular lipids (particularly hydrocarbons) which serve as the primary mechanism to limit loss of water across the insect’s exoskeleton (Chown and Nicolson 2004; Beament 1961; Hadley 1994). Cuticular permeability is usually the most important factor limiting water loss (Beament 1961; Hadley 1994; Edney 1977; Mazer and Appel 2001), and is often measured as the amount of water lost (µg) per unit surface area (cm^2) per unit time (h) per unit saturation deficit (mmHg, Torr, or kPa) (Beament 1961; Edney 1977; Mazer and Appel 2001; Manabe et al 1991). Higher cuticular permeability values represent greater rates of water loss and faster desiccation. Cuticular permeability of terrestrial insects is influenced by climatic factors such as temperature, relative humidity, and saturation deficit in their habitat (Appel et al. 1991; Mazer and Appel 2001). These climatic factors vary between latitudes (Manabe et al. 1991; Fields et al. 1993; Peng et al. 2002) and are being negatively impacted by climate change (Cunze et al. 2013; Jagadish et al. 2012). Dramatic variation in water availability between habitats is another important factor of species distribution. Cuticular permeability is also significantly affected by the condition (live or dead) of terrestrial insects because the cuticular water pump, located in the epidermal cells, actively restricts water loss in living insects (Appel et al. 1991; Winston 1967; Winston and Beament 1969; Berridge 1970). These factors and variabilities are expected to play a role in limiting species geographical distribution. However, little is known about how desiccation tolerance is related to the distribution and success of invasive insect species in their introduced environment as compared with their native range (Human et al. 1998).

The black imported fire ant, *Solenopsis richteri* Forel, and the red imported fire ant, *S. invicta* Buren, were accidentally introduced into the United States of America from South America in the 1920s and 1930s, respectively, through Mobile, Alabama (Lofgren et al. 1975; Patterson 1994). Since their introduction, both *S. richteri* and *S. invicta* have become serious pests and *S. invicta* has displaced native fire ants (Vinson 1997), including the tropical fire ant, *S. geminata* (Fabricius). *Solenopsis richteri* and *S. invicta* have formed an extensive zone of hybridization in the USA (Cohen and Privman 2019), but hybridization has not been found in their native South America (Buren 1972; Tschinkel 2006). Colonies of *Solenopsis* spp. may contain > 100,000 workers of varying size and are likely to be exposed to a variety of environmental stressors, including high temperature and low relative humidity, during their lifetimes, especially when foraging (Appel et al. 1991; Mirenda and Vinson 1981). *Solenopsis richteri* occurs at slightly higher latitudes than *S. invicta* both in the USA and in their native South America (see maps in (Taber 2000), pp 27 and 59) (Buren et al. 1974; Tschinkel 2006), while *S. invicta* × *S. richteri* occur in the hybrid zone in the USA, but may mix with populations of its parental species. Furthermore, *S. invicta* and *S. geminata* are usually found in the tropical/subtropical USA, while *S. richteri* are usually found in more temperate areas in the USA (Morrison et al. 2005; Buren et al. 1974). The introduced *S. richteri* and *S. invicta* are expected to have each remained phenotypically constant since the time of their introduction from South America (Buren et al. 1972). The potential range of expansion for *S. invicta*, based on current temperature and rainfall patterns, has been modelled for the continental USA (Korzukhin et al. 2001) and worldwide (Morrison et al. 2004). *Solenopsis invicta* now occupies much of southern USA and has established in California, West Indies, New Zealand, Australia, and parts of Asia (Callcott and Collins 1996; Davis et al. 2001; Mescher et al. 2003; Ascunce et al. 2011).

A number of investigations have compared water use within and across some *Solenopsis* spp. and have identified several physiological traits related to water loss. Workers of *S. invicta* were more tolerant to desiccation stress than *S. richteri* workers despite the significantly greater body water content in *S. richteri* (Chen et al. 2014). Size also plays a role in cuticular permeability in *Solenopsis* spp. For example, despite the greater percent total body water content (%TBW) of small *S. invicta* workers, cuticular permeability was greater in these individuals as compared to large *S. invicta* workers (Appel et al. 1991). This had also been demonstrated in *S. xyloni* (McCook) workers (Schilman et al. 2007). Interestingly, Li and Heinz (1998) indicated that desiccation resistance in polygyne *S. invicta* is not a function of body size and found low heritability of desiccation resistance in the tested population.

In addition to size, cuticular permeability was shown in *S. invicta* workers to be influenced by death from exposure to cyanide gas, which increased cuticular permeability by about 1.5-fold, and death by hexane extraction, which increased cuticular permeability by about 1.8-fold (Appel et al. 1991). When comparing *S. invicta*, *S. richteri*, and *S. invicta* × *S. richteri*, Xu et al. (2018) found that *Solenopsis* species with the highest water loss transition temperature (T_c) and highest melting point ($T_{m\text{-}max}$) of cuticular hydrocarbons (CHC's) retain more water under relatively higher temperatures, and consequently are able to occupy warmer environments. Thus, several heritable physiological traits impact the water relations of *Solenopsis* spp. Similar impacts could also be caused by acclimation and environmental factors.

Studies within and across some *Solenopsis* spp. have revealed the degree of impact and relation of extreme abiotic factors to cutaneous water loss. Braulick et al. (1988) compared lethal times (LT) in hours at 0 %RH and high temperatures for workers of *S. aurea* Wheeler, *S. geminata*, *S. invicta*, and *S. xyloni*. In general, workers survived progressively longer periods as temperature decreased; and major workers survived 2 – 4 times longer than minor workers at the same temperatures. Munroe et al. (1996) compared the effect of desiccation on survival times in workers of *S. invicta*, *S. geminata*, and *S. xyloni*, and found that *S. invicta* had lower LT_{50}-values than *S. geminata* and *S. xyloni*. Wendt and Verble-Pearson (2016) found that major and medium *S. invicta* workers survived higher temperatures more often than did minor workers. Phillips et al. (1996) found that workers of *S. invicta* from xeric conditions were less prone to desiccation than were those from moist conditions. It was suggested that this observation may be the result of natural selection at the population level or a physiological modification (acclimatization) by *S. invicta* workers as a consequence of continued exposure to more stressful environmental conditions. Li and Heinz (1998) found that polygyne populations of *S. invicta* might be capable of adapting to arid habitats, therefore suggesting an advantage in dominance of polygyne over monogyne *S. invicta* populations in arid habitats. Martin and Vinson (2008) demonstrated that the ability to maintain a minimum viable level of body water could be a limiting factor to foraging range in *S. invicta* workers. Vogt et al. (2003) also showed that temperature is a significant predictor of foraging activity in *S. invicta* workers. Thus, several environmental as well as biological factors impact the water relations of *Solenopsis* spp.

Although there have been several water relations studies on some *Solenopsis* species, there are no documented studies on the *S. invicta* × *S. richteri* hybrid, and there are none comparing multiple tropical and temperate *Solenopsis* species. Our objectives were to determine the total body water content (%TBW content), rate of mass loss, rate of total body water loss (%TBW loss), and cuticular permeability of introduced and native *Solenopsis* spp. of fire ants in the southeastern USA. In addition, we determined the desiccation sensitivity of these species. We hypothesized that tropical/sub-tropical fire ants (*S. invicta* and

S. geminata) will have lower cuticular permeability values and tolerate higher levels of desiccation (i.e., greater %TBW loss) than temperate fire ants (*S. richteri* and *S. invicta* × *S. richteri*). This study compares the water relations of temperate and tropical/sub-tropical fire ant workers across different latitudes in southern USA and is the first report of cuticular permeability and desiccation tolerance of *S. invicta* × *S. richteri* hybrid workers.

2.2 Materials and Methods

2.2.1 Study Species and handling. All fire ant colonies were collected in March 2017. *Solenopsis invicta* workers were obtained from mounds on the Auburn University campus, Lee County, Alabama. *Solenopsis richteri* workers were obtained from mounds in Hohenwald, Lewis County, and Mount Pleasant, Maury County, Tennessee. Workers of *S. invicta* × *S. richteri* hybrids were obtained from mounds in Cullman, Cullman County, and Hollywood, Jackson County, Alabama. Workers of *S. geminata* were obtained from mounds in Gainesville, Alachua County, Florida. GPS coordinates of ant collection locations are in Table 1. The identity of each species was confirmed by gas chromatography of hexane extracts of *ca*. 50 workers using both venom alkaloid and cuticular hydrocarbon characters, (Ross et al. 1987; Chen et al. 2010) following methodology in Hu et al. (2018). Ants were used for experiments within the same week they were collected. Based on large variation in body sizes and behavioral differences among size classes of *Solenopsis* spp. workers, each species was categorized, using the range of their head width, as small (0.72 mm or less), medium (0.73 – 0.92 mm), or large (0.93 mm or more) (Wilson 1978; Hölldobler and Wilson 1990).

We used live and HCN killed ants in this study. Live ant data provides insight into desiccation tolerance, acceptable water loss for survival, and the abilities of the ants to actively regulate water loss, whereas dead insect data provides absolute cuticular permeability values. Live ants were separated by size and species and confined in a small glass jar with a screen-lid. Jars were placed in a 1 L glass chamber with ≈ 10 g NaCN and KCN. HCN gas was generated by adding ≈ 0.5 ml hydrochloric acid to the cyanide salts to produce HCN gas. Ants were exposed for approximately 3 min or until all ants were dead. Dead ants were removed from the killing chamber, weighed to the nearest 0.01 mg individually using an electronic digital analytical balance.

2.2.2 Total Body Water Content. Knowledge of the rate of percent total body water content (%TBW) loss and how much total water loss is lethal will enable the calculation of how long the ants may have to avoid or escape lethal environments. Total body water content of all ants was determined gravimetrically (see (Appel et al. 1991; Appel et al. 1983; Mack and Appel 1986)). Masses of individual workers were measured to the nearest 0.01 mg on a digital balance in preweighed plastic weighing boats coated on the inside with Fluon® to prevent escape of live ants. Ants were then transferred to glass vials ringed on the upper inside surface with Fluon® to prevent escape of live ants. Vials containing the ants were placed in an 11-L desiccating chamber containing approximately 0.5 kg anhydrous $CaSO_4$ (Drierite®, W.A. Hammond Drierite Co. LTD, Xenia, OH), resulting in 0 – 2 %RH. Maximal effectiveness of the Drierite was ensured by heating it at 230°C for at least 2 h prior to use to remove all water. The chamber was placed in an incubator and maintained at 30 ± 1°C. Temperature and %RH in the chamber were monitored at each weighing using a digital thermometer-hygrometer. Specimens were weighed and returned to the desiccator chamber as quickly as possible. The ants were weighed at 0, 2, 4, 6, 8, 10, and 24 h; dried in a 55°C oven for two days, weighed and then dried an additional two days. Ants were dried and weighed until two successive weighings did not differ by >0.01 mg. Mass loss was assumed to be due entirely to water loss. Percent total body water (%TBW) was calculated as follows:

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

where $M_{initial}$ is the initial fresh body mass and M_{dry} is the dry mass.

2.2.3 Cuticular Permeability. Cuticular permeability was calculated from the difference between initial and 2 h desiccated masses. Mass loss after 2 h was used to avoid confounds caused by variability in body shape and therefore surface area. Additionally, this period represented the maximum water gradient between the insect and the chamber, and thus the greatest water loss rate. Therefore, this period is the best estimate of absolute permeability (Appel et al. 1983).

$$\text{Cuticular permeability} = \frac{\text{water lost (µg)}}{\text{Surface area (cm}^2\text{)} * \text{time (h)} * \text{saturation deficit (mmHg)}}$$

Surface area (cm^2) was estimated by Meeh's formula,
Surface area (cm^2) = Initial mass $(g)^{2/3} * 12$ (Edney and McFarlane 1974).

Saturation deficit, which is the difference between the vapor pressure of water at a given RH and temperature and the vapor pressure of saturated air at the same temperature (Edney 1977), remained constant at 31.82 mmHg for 30°C and 0% RH (Haar et al. 1984).

Adjusted mass loss was calculated as the difference between initial and 2 h masses divided by initial mass (g) and the formula is as follows:

Adjusted mass loss = mg of H_2O lost ($T_0 – T_2$)/gram initial body mass (T_0)

where T_0 is initial mass and T_2 is mass after 2 h of desiccation.

2.2.4 Rates of Mass Loss, Water Loss, and Mortality. Hourly percentage of initial mean mass loss and %TBW loss of live and dead individuals of each species and size were plotted individually by time of desiccation; the analysis of these relationships is described below. The condition (live or dead) of live ants was recorded at each weighing.

2.2.5 Data Analyses. A randomized complete block design with body size, live/dead condition, and species as main effects and the size by species interaction was used to evaluate differences in cuticular permeability and %TBW among species within each size category. Analysis of variance followed by the Tukey–Kramer HSD comparison test ($P < 0.05$; (SAS Inc. 2013)) was performed on the initial mass, %TBW, and cuticular permeability data among and across the species. Linear regression was used to determine if cuticular permeability was related to initial live mass. Change in % mass loss or %TBW lost over time was analyzed using nonlinear regression (Systat Software Inc. 2014). A rectangular hyperbolic model was used for analysis of the change in % mass loss and %TBW lost overtime. The following function was fit to the hourly % mass loss and %TBW lost values:

$$Y = \frac{ax}{b + x}$$

where Y = % mass loss or %TBW lost, a = the maximum asymptotic value of % mass loss or %TBW lost, $b = (t_{max})/2$ or the period required for half the maximum value to be reached, and x = hour. This function was selected because it is the most parsimonious expression that contains a curvilinear increase and an asymptotic maximum. The maximum value would be obtained with complete dryness of the specimen. For % mass loss, the maximum represents %TBW; for %TBW loss, the maximum should approximate 100%, or complete loss of all body water. Probit analysis (Robertson et al. 2007) was used to estimate the median lethal time of ants exposed to desiccating conditions (*ca.* 30°C and 0-2% RH). Resulting LT_{50} values were used to estimate desiccation tolerance, or %TBW lost at death, from the nonlinear regression equations. Data are expressed as means ± SE; $\alpha = 0.05$.

2.3 Results

2.3.1 Body Mass and Water Content.

Initial body masses of *S. invicta* workers ranged from 0.44-0.81 mg for small workers, 0.84- 2.55 mg for medium workers, and 2.02-4.36 mg for large workers. Initial body masses of *S. richteri* workers ranged from 0.63-1.08 mg for small workers, 1.54-2.53 mg for medium workers, and 2.36-3.79 mg for large workers. Initial body masses for *S. invicta* × *S. richteri* workers ranged from 0.54-1.02 mg for small workers, 0.72-2.25 mg for medium workers, and 1.34- 4.72 mg for large workers. Initial body masses for *S. geminata* workers ranged from 0.46-1.9 mg for small workers, 1.11-2.91 mg for medium workers, and 3.32- 7.52 mg for large workers. Mean initial body masses for each size, and each species of live and dead ants are presented in Table 2.

2.3.2 % Total Body Water of Live Ants.

Small *S. invicta* workers had about 1.1-fold higher %TBW than *S. richteri*, while *S. geminata* was about 1.04-fold higher than *S. richteri* (Figure 1A). For all sizes, there were no significant differences in %TBW between tropical/sub-tropical (*S. invicta* and *S. geminata*) and temperate (*S. invicta* × *S. richteri*) (Figure 1A). Within each species, small workers had significantly greater %TBW than large workers (Figure 1B). In all the four *Solenopsis* species, ranking of %TBW of the worker sizes was small > medium > large (Figure 1B). Combining sizes, %TBW was not significantly different between tropical/sub-tropical and temperate species (Figure 1C).

2.3.3 % Total Body Water of Dead Ants. Combining sizes, %TBW was not significantly different between tropical/sub-tropical and temperate species (Figure 1C). When separated, %TBW of small, medium, and large workers were not significantly different among the four *Solenopsis* species (Figure 1D). In each of the sizes, *S. richteri* had the lowest in %TBW, whereas small and large *S. invicta* had the highest in %TBW (Figure 1D). There were no significant differences in %TBW among the sizes in all four *Solenopsis* species (Figure 1E).

2.3.4 Adjusted Mass Loss of Live Ants. Temperate *S. richteri* had significantly greater adjusted mass loss than the tropical *S. invicta* (Figure 2A). There was no significant difference in adjusted mass loss among the four species in each size (Figure 2C). Small workers of *S. invicta* × *S. richteri* and *S. geminata* had significantly greater mass loss than large workers (Figure 2E). Across all species, the ranking of adjusted mass loss was small > medium > large (Figure 2E).

2.3.5 Adjusted Mass Loss of Dead Ants. Combining sizes, temperate *S. invicta* × *S. richteri* had significantly greater adjusted mass loss, by *ca*. 1.3-fold, than sub-tropical *S. geminata* (Figure 2A). Adjusted mass loss of dead ants increased over that in live ants by *ca*. 1.2-fold and *ca*. 1.3-fold for *S. invicta* and *S. invicta* × *S. richteri* combined sizes, respectively (Figure 2A).

There were significant differences in adjusted mass loss between temperate (*S. richteri* and *S. invicta* × *S. richteri*) and sub-tropical (*S. geminata*) ant workers both in medium and large sizes (Figure 2B). Temperate *S. invicta* × *S. richteri* had significantly greater adjusted mass loss than tropical species *S. invicta* and *S. geminata* for both medium and large workers (Figure 2B). Ranking of species by adjusted mass loss of medium and large sizes was *S. invicta* × *S. richteri* > *S. richteri* > *S. invicta* > *S. geminata* (Figure 2B). There were significant differences in adjusted mass loss among the sizes within each species (Figure 2E). Adjusted mass loss of small workers was greater than medium, and that of medium was greater than large workers in all species except *S. invicta* × *S. richteri* (Figure 2E). Ranking of sizes by adjusted mass loss was small > medium > large for all the four *Solenopsis* species (Figure 2E).

2.3.6 Rates of % Initial Mass and % Total Body Water Loss. % initial mass loss and %TBW lost increased as rectangular hyperbolic function of desiccation time (Figures 3-6); rising from an intercept of zero and increased at a declining rate eventually reaching an asymptote. The asymptote for the % initial mass loss curves approximate %TBW; those for %TBW lost approximate 100%. The rectangular hyperbolic function was appropriate as all the regressions were highly significant ($P < 0.0001$) with r^2 values > 0.9 (Tables 3-6).

For live ants, % initial mass loss (Figure 3; Table 3) the maximum asymptotic value or ‘a’ coefficient ranged from 70- 107.9 for small *S. geminata* and large *S. invicta*, respectively. These asymptotic values (± 2 SE) overlap the %TBW (Figure 1). The period required for half the maximum value to be reached or ‘b’ coefficient ranged from 2.6 h for small *S. geminata* to 20.3 h for large *S. invicta* (Table 3). Percentage TBW lost by live ants increased similarly (Figure 4; Table 4) with the maximum asymptotic value overlapping 100% (representing 100% water loss) and the ‘b’ coefficient ranged from 2.4-20.2 h for small *S. geminata* and large *S. invicta*, respectively (Table 4). The ‘b’ coefficients for both % initial mass loss and %TBW lost increased with increasing ant size for all species.

For dead ants, % initial mass loss (Figure 5; Table 5) the ‘a’ coefficient or maximal asymptotic values were slightly lower than for live ants, ranging from 67.2 % for small *S. richteri* to 97.2 % for large *S. geminata* (Table 5). These values were similar to those for %TBW in Figure 2D. In all the four *Solenopsis* species, the ‘b’ coefficient or period required for half the maximum value to be reached ranged from 1.7- 16.2 h for small and large *S. geminata*, respectively (Table 5). Figure 6 illustrates the relationship between %TBW lost by dead ants and desiccation time. The maximum asymptotic value or ‘a’ coefficient overlaps or exceeds 100% (representing complete dryness). The period required for half the maximum value to be reached or ‘b’ coefficient ranged from 1.6-16.2 h for small and large *S. geminata*, respectively (Table 6). The ‘b’ coefficients for both % initial mass loss and %TBW lost increased with increasing ant size for all species.

2.3.7 Cuticular Permeability. Combining sizes, cuticular permeability of dead ants was significantly greater in *S. richteri* and *S. invicta* × *S. richteri* workers than in *S. geminata* workers ($P < 0.05$) (Figure 7A). Cuticular permeability was about 1.3-fold greater in temperate *S. invicta* × *S. richteri* than in sub-tropical *S. geminata* workers; and about 1.2-fold greater in temperate *S. richteri* than in sub-tropical *S. geminata* workers (Figure 7A). Cuticular permeability values of *S. richteri* and *S. geminata* were not significantly different from that of *S. invicta* (Figure 4A). Between temperate ants, cuticular permeability was significantly greater in *S. invicta* × *S. richteri* than *S. richteri* (Figure 7A). Calculated cuticular permeability of live *S. richteri* ants was significantly greater than that of *S. invicta* (Figure 7A).

There was no significant difference in cuticular permeability among small workers of the four *Solenopsis* species (Figure 7B). Medium *S. invicta* × *S. richteri* workers had significantly greater cuticular permeability than tropical/sub-tropical *S. invicta* and *S. geminata* workers, while tropical/sub-tropical *S. invicta* and *S. geminata* worker ants had similar cuticular permeability values (Figure 7B). Large temperate *S. richteri* and *S. invicta* × *S. richteri* workers had significantly greater cuticular permeability than tropical *S. geminata* (Figure 4B). Large temperate *S. invicta* × *S. richteri* workers had significantly greater cuticular permeability than tropical/sub-tropical *S. invicta* and *S. geminata* workers (Figure 7B). There were no significant differences in cuticular permeability among sizes for *S. invicta, S. richteri*, and *S. invicta* × *S. richteri* (Figure 7C). However, small workers had significantly greater cuticular permeability than both medium and large workers in *S. geminata* (Figure 7C).

2.3.8 Desiccation Tolerance. The LT_{50} values (h) for all live size-classes of *S. richteri, S. invicta* × *S. richteri, S. invicta, S. geminata* worker fire ants are shown in Table 7. All probit analyses were significant ($P < 0.05$) and LT_{50} values ranged between 1.5 h for small *S. geminata* workers to 8.5 h for large *S. invicta* workers. LT_{50} values increased with increasing ant size. Desiccation tolerance estimated as the percentage of total body water (%TBW) lost at the LT_{50} time is shown in Table 8. Median %TBW lost at death ranged from 35.7% for small *S. geminata* to 49.8% for large *S. invicta*. There were no significant differences in %TBW lost at death, based on overlap of the 95% confidence intervals, among the size classes of any of the *Solenopsis* spp. Mean %TBW lost at the LT_{50} time ranged from 42.5% for *S. geminata* to 45.8% for *S. invicta*.

2.4. Discussion

The data supports our hypothesis that tropical/sub-tropical fire ants (*S. invicta* and *S. geminata*) will have lower cuticular permeability values than temperate fire ants (*S. richteri* and *S. invicta* × *S. richteri*). However, the data do not support the general hypothesis that tropical/sub-tropical fire ants can tolerate greater levels of desiccation (i.e., greater %total body water loss). Comparing cuticular permeability values among dead ants (i.e., absolute permeability), tropical *S. invicta* and *S. geminata* had significantly lower cuticular permeability values than that of temperate *S. invicta* × *S. richteri*. Also, cuticular permeability values of dead *S. geminata* were significantly lower than those of *S. richteri* and *S. invicta* × *S. richteri*. Cuticular permeability values obtained for dead *S. invicta* were similar to those reported by Appel et al. (1991). Our data also suggests that large live *S. invicta* are significantly less vulnerable to desiccation stress than large live *S. richteri*. All these results may partially explain the predominant location of each of these species, as *S. invicta* are found in lower latitudes and tropical/subtropical region, while *S. richteri* are found in higher latitudes and more temperate regions in their native South America. In addition, the climate in their current distribution in the southeast U.S.A. is similar to that of their native distribution. This suggests that cuticular permeability and desiccation tolerance play a role in the distribution and adaptation of invasive ants. Considering the ranges of cuticular permeability values that we obtained, neither of the tropical/sub-tropical *S. invicta* nor *S. geminata* workers fell within the range for temperate *S. invicta* × *S. richteri* workers. This suggests that differences in habitat preference may exist between these species.

The body water content of 67.56 ± 0.90% for combined sizes of *S. invicta* in our data is similar to the 68.1% reported by Elzen (1986) and the 63% by Appel et al. (1991). The body water contents of *S. invicta* and *S. richteri* have been reported to be significantly different (Chen et al. 2014). However, our data show no body water content difference for combined sizes of workers of both the tropical/sub-tropical species (*S. invicta* and *S. geminata*) compared to the temperate species (*S. richteri* and *S. invicta* × *S. richteri*). The higher number of replicates in the study by Chen et al. (2014) may have contributed to the detection of significant differences in their study. The body water content values recorded for all four *Solenopsis* spp. in the current study are similar to those reported for workers of other ant species, including the desert ant *Pogonomyrmex rugosus* (Emery) (65.9%), *P. occidentalis* (Cresson) (63.4%), and *Messor pergandei* (Mayr) (64.7%) (see references in (Hadley 1994) [Table 2.1]). It is possible that temperate and tropical/sub-tropical fire ants may be similar in body water content. However, comparison based on the present data may be insufficient.

Large workers had significantly lower percent total body water loss (%TBW) values than small ones in all tested species. This is similar to results in Appel et al. (1991) where *S. invicta* small workers contained significantly more percent body water ($P < 0.05$) than large workers. Also, the values of %TBW in small *S. invicta* workers in our study (72.69 ± 1.65) was similar to that in Appel et al. (1991) (65.02 ± 1.30). %TBW values in large *S. invicta* workers in our study (63.93 ± 1.03) is also similar to that in Appel et al. (1991) (61.07 ± 0.49). In the present study, %TBW was approximately 1.14-fold greater in large than small *S. invicta* workers; 1.13-fold higher in large than small *S. geminata* workers; 1.11-fold higher in large than small *S. invicta* × *S. richteri* workers; and 1.08-fold higher in large than small *S. richteri* workers. Combining stages, %TBW was not significantly different between workers of the four species. Similarly, there was no significant difference in %TBW between *S. invicta* and *S. richteri* female alates but it significantly lower in workers of *S. invicta* than in *S. richteri* (Chen et al. 2014). Higher average %TBW for small workers may be due to the behavior of this stage in the colony. Small workers are more likely to care for brood than are other castes (Wilson 1978). Brood care in ants requires the availability of liquid food for trophalaxis, so samples of small workers could contain individuals with full and empty crops. In addition, small workers that forage are more likely to forage for liquid food (Appel et al. 1991; Mirenda and Vinson 1981).

Cuticular permeability values of dead ants gives us absolute comparisons to other arthropods. The cuticular permeability values obtained in the current study for *S. invicta* workers were: small (33.03 μg cm^{-2} h^{-1} $mmHg^{-1}$), medium (30.26 μg cm^{-2} h^{-1} $mmHg^{-1}$), and large (26.54 μg cm^{-2} h^{-1} $mmHg^{-1}$). These values are similar to those reported for other arthropods from xeric habitats, such as *Hadrurus hirsutus* (Wood); Scorpiones = 25 μg cm^{-2} h^{-1} $mmHg^{-1}$, *Locusta migratoria* (L.); Orthoptera = 22 μg cm^{-2} h^{-1} $mmHg^{-1}$ and *Hemilepistus reaumuri* (Milne-Edwards); Isopods = 23 μg cm^{-2} h^{-1} $mmHg^{-1}$. Cuticular permeability values for *S. geminata* workers (small = 35.14, medium = 27.97, and large = 23.34 μg cm^{-2} h^{-1} $mmHg^{-1}$) are also similar to those measured in arthropods of xeric habitats, such as *Venezillo arizonicus* (Mulaik and Mulaik); Isopods = 32 μg cm^{-2} h^{-1} $mmHg^{-1}$. Cuticular permeability values for *S. richteri* workers (small = 36.46, medium = 33.70, and large = 29.37 μg cm^{-2} h^{-1} $mmHg^{-1}$) are similar to those measured in arthropods of mesic habitats, such as *Lycosa amentata* (Clerck); Araneae = 28.3 μg cm^{-2} h^{-1} $mmHg^{-1}$. Similarly, cuticular permeability values for *S. invicta* × *S. richteri* workers (small = 38.03, medium = 40.99, and large = 36.20 μg cm^{-2} h^{-1} $mmHg^{-1}$) are similar to those measured in insects of mesic habitats, such as *Chortoicetes terminifera* (Walker); Orthoptera = 41 μg cm^{-2} h^{-1} $mmHg^{-1}$ (see references in (Edney 1977) [Table 6]). Even though there is an overlap in the range in cuticular permeability values of small workers between tropical (*S. invicta* and *S. geminata*) and temperate (*S. richteri* and *S. invicta* × *S. richteri*) in our study, there are no overlap in medium and large workers of these categories of species. This may be explained by the worker size of *Solenopsis* species that engages more in foraging. Medium and large workers engage in foraging more than the small workers (Wilson 1978). Furthermore, these data suggest that cuticular permeability of tropical/subtropical fire ants in our study exhibits a degree of adaptation to more xeric environments, whereas the cuticular permeability of temperate fire ants is more similar to arthropods adapted to mesic environments (Morrison et al. 2004).

Cuticular permeability values of dead workers (averaging across the three size classes) of the four species ranked as follows: *S. invicta* × *S. richteri* > *S. richteri* > *S. invicta* > *S. geminata*. Similarly, the cuticular permeability values of large workers ranked as follows: *S. invicta* × *S. richteri* > *S. richteri* > *S.*

invicta > *S. geminata*. This is similar to the ranking of initial mass of large workers. Cuticular permeability and initial mass tended to follow a similar trend in large workers of all species in our results. Thus, given the cuticular permeability of these *Solenopsis* species, a relatively larger rather than small body size may better tolerate water loss if water loss becomes a limiting factor when foraging. This result is similar to that of foraging harvester ants, *Pogonomyrmex rugosus* (Emery) (Lighton and Feener 1989).

In the present study, there was no significant difference between the absolute cuticular permeability values of small and large workers among the four *Solenopsis* species (except in *S. geminata*). However, a significant difference was detected between the cuticular permeability values of small and medium workers of *S. invicta* when more replicates were used by Appel et al. (1991). We speculate that significantly different results may have been detected in our study if more replicates were used, and if this were the case, the lower cuticular permeability of large workers may be related to the behavior of this stage in the colony.

Adjusted mass loss was used as a way to compare water loss from live and dead ants without making assumptions about the source of that water loss which could be through respiration, secretions, and feces in addition to the cuticle. If adjusted mass loss were greater for live compared with dead insects, and if cuticular permeability is the opposite, then differences would be due to respiratory, fecal, or secretory water loss. However, if adjusted mass loss was similar for live and dead insects, then it could be argued that these other routes of water loss are insignificant compared with cuticular water loss. Combining worker sizes, adjusted mass loss was similar between live and dead ants (Figure 2A). Mean values were slightly greater in dead *S. invicta* × *S. richteri* and *S. invicta* indicating that these species may have more active cuticular water “pumps”.

Meeh’s formula was used to estimate surface area in our study to calculate cuticular permeability because body size affects water loss (surface area to volume ratio). Linear regressions of cuticular permeability on body mass were performed to determine if Meeh’s formula had adequately compensated for the relationship between mass and surface area. The majority (>90%) of these regressions were not significant, indicating that Meeh's formula provided a reasonable estimate for surface area.

Our study also attempted to relate the desiccation tolerance of four species of *Solenopsis* fire ants with their geographic distributions. The ranking of the LT_{50} values of large workers in our results was *S. invicta* > *S. geminata* > *S. invicta* × *S. richteri* > *S. richteri* (Table 7). Large *S. invicta* workers had LT_{50} values approximately 2.3 times greater than those of *S. richteri*. Large workers are more frequent foragers than other sizes in fire ant colonies (Wilson 1978), thus our LT_{50} data suggests similarity in ranking of desiccation tolerance among these four species. This indicates that *S. invicta* large workers are more desiccation tolerant than *S. geminata*, *S. invicta* × *S. richteri*, and *S. richteri* large workers. Our data also suggests that foraging large *S. invicta* workers could tolerate exposure to desiccating conditions significantly longer than large *S. richteri* workers. Thus, *S. invicta* is expected to be able to forage in areas of high insolation longer than the other three species, perhaps contributing to their relative distributions (Hood and Tschinkel 1990). Temperate fire ants (*S. invicta* × *S. richteri* and *S. richteri*) have a lower LT_{50} than tropical and sub-tropical fire ants (*S. invicta* and *S. geminata*); this suggests that the ants may have more difficulty than tropical/sub-tropical fire ants surviving drier environmental conditions than those of their present range. In general, live large fire ants desiccate at a slower rate than live small fire ants. For instance, the increase in LT_{50} values for large over small workers were 5.23, 3.42, 2.58, and 1.65 times, in *S. geminata*, *S. invicta*, *S. invicta* × *S. richteri* and *S. richteri*, respectively. The desiccation rates between small and large fire ants in our results are similar to those reported by Munroe et al. (1996). These results support the suggestion by Edney (1977) that, in moisture-deficient situations, the amount of water loss in terms of total body water initially present is greater in small animals than large animals. The ranking of the LT_{50} values of large workers in our results was opposite to that of mass loss among the four live species, *S. richteri* > *S. invicta* × *S. richteri* > *S. geminata* > *S. invicta*. Our results may suggest that the reason large workers are able to carry a greater proportion of foraging responsibilities is that they are desiccation resistant and tolerant (Munroe et al. 1996; Porter and Tschinkel 1987).

The composition of epicuticular lipids enable tropical *S. invicta* to better cope with desiccation than temperate *S. richteri* (Chen et al. 2014; Xu et al. 2018). Our data showed that *S. invicta* is more desiccation tolerant than *S. invicta* × *S. richteri* and *S. richteri*. This agrees with the findings of Xu et al. (2018) in that the highest melting points of samples of cuticular hydrocarbons (CHCs) from *S. invicta* and *S. invicta* × *S. richteri* were significantly higher than that from *S. richteri*. Cuticular hydrocarbon profiles of *S. richteri* are characterized by significant amounts of short-chain (C_{23}–C_{27}) saturated and unsaturated hydrocarbons. In contrast, profiles of *S. invicta* consist primarily of long-chain (C_{27}–C_{29}) saturated hydrocarbons; unsaturated alkenes are completely lacking. The hybrid *S. invicta* × *S. richteri* shows intermediate profiles of the two parent species (Xu et al. 2018). Long-chain saturated waxy hydrocarbons are better at water proofing and have higher melting points than shorter chain unsaturated chains.

The task of foraging carried out by workers can expose ants to highly desiccating conditions, in contrast to the relatively cooler and more humid conditions in which temperate fire ant species inhabit. Tropical/sub-tropical fire ants may also benefit from the relatively cool and humid conditions in the deep parts of ant nests, where cuticular water-loss is likely to be minimal (Lighton and Feener 1989). As a result of these adaptations, we infer that water-loss rate directly affects colony fitness and plays an important role in limiting distribution of these terrestrial insects (Lighton and Feener 1989). Queen ants of different species vary in their desiccation tolerance (Mankowski 2001), and if this were the case among *Solenopsis* species as well, then we would expect queens of tropical *S. invicta* to be more desiccation tolerant than the queens of temperate *S. richteri*. Thus, this difference would play a role in colony establishment and limiting distribution among these species. Nevertheless, water vapor pressure deficits are likely to be low for claustral colony founder queens that are found deep in the nests (Lighton and Feener 1989).

Large workers are expected to be exposed to potentially desiccating conditions longer than other size classes when foraging (Wilson 1978). It is possible that there are qualitative and quantitative differences in the cuticular hydrocarbon profiles of small and large workers of these four *Solenopsis* species, as was found in *S. saevissima* (Fox et al. 2012). Foraging in ants tends to require the capacity to tolerate desiccating conditions (Hood and Tschinkel 1990), thus samples of large workers may include individuals with low cuticular permeability, enabling them to tolerate desiccating conditions better than non-frequent foragers such as the small workers. If this were the case, then it would be expected that more resources be invested into making the waxes on large workers that are more frequent foragers than small workers that engage more often in brood care within the nest (Wilson 1978). We speculate that small workers may possess greasier cuticular hydrocarbons while large workers have waxier cuticular hydrocarbons. Future studies should investigate if small workers have enough wax to not dry out immediately, and enough grease to readily sense pheromones among other semiochemicals. Thus, this may indicate if there exists a tradeoff between waterproofing wax and grease that allows better chemoreception.

In conclusion, this study illustrates differences in body mass, %TBW, cuticular permeability and desiccation tolerance of *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata*, important pests in the southeastern U.S. There are differences in the water relations of species adapted to temperate compared with tropical latitudes. Temperate *S. invicta* × *S. richteri* had significantly greater cuticular permeability than the tropical *S. invicta* and *S. geminata*. Live temperate *S. richteri* lost significantly more %TBW than tropical *S. invicta*. These results provide insights into differences in the water relations of these four *Solenopsis* species and help to explain the relative distribution of these species in the southeastern US. Capability of these four *Solenopsis* species to survive, limit cuticular water loss, and tolerate desiccation influences their distribution both in their native South America and in their introduced North American range. Extremes of hot and cold temperature and low relative humidity will limit the range of *S. invicta* and *S. richteri* (Morrison et al. 2005; Morrison et al. 2004; Munroe et al. 1996; Hung and Vinson 1978; Pimm and Bartell 1980; Moody et al. 1981). However, during global warming, the ranges of *S. invicta* and *S. richteri* are predicted to increase to the north of their present range (Morrison et al. 2004), and *S. invicta* to the west and east of its present range (Morrison et al. 2005; Munroe et al. 1996). Further studies including physiological and genetic analyses of desiccation tolerance in *Solenopsis* spp. are needed to determine what physiological and genetic attributes enable the tropical *S. invicta* to tolerate desiccation better than the temperate *S. richteri*.

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Table 1: Collection locations of *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri* and *S. geminata* fire ants in the U.S.A.

Species	**Colony**	**Town/City, State**	**Latitude**	**Longitude**
Solenopsis geminata	1	Gainesville, FL	29°34'19.49" N	82°27'22.89" W
	2	Gainesville, FL	29°34'24.92" N	82°27'30.49" W
	3	Gainesville, FL	29°34'25.23" N	82°27'29.59" W
S. invicta	1	Auburn, AL	32°37'31.47" N	85°30'07.78" W
	2	Auburn, AL	32°34'36.73" N	85°29'52.24" W
	3	Auburn, AL	32°36'59.58" N	85°30'27.14" W
S. richteri	1	Waverly, TN	36°05'01.41" N	87°48'31.32" W
	2	Hohenwald, TN	35°33'24.66" N	87°31'46.43" W
	3	Mount Pleasant, TN	35°36'38.52" N	87°15'46.40" W
S. invicta × *S. richteri*	1	Huntsville, AL	34°32'18.66" N	86°30'00.27" W
	2	Huntsville, AL	34°34'45.70" N	86°33'13.75" W
	3	Decatur, AL	34°31'47.41" N	86°54'02.96" W

Table 2: Initial mass (mg) of small, medium, and large live and dead workers of *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri* and *S. geminata* ants (mean ± SE).

Species	**Size-class**	**Initial mass (mg)**	
		Live	**Dead**
S. invicta	Small	0.59 ± 0.02cA	0.52 ± 0.02cB
	Medium	1.61 ± 0.13bA	1.41 ± 0.09bB
	Large	3.23 ± 0.19aB	3.52 ± 0.14aB
S. richteri	Small	0.89 ± 0.03bA	0.72 ± 0.03cA
	Medium	2.94 ± 0.03aA	1.47 ± 0.09bB
	Large	3.19 ± 0.12aB	3.31 ± 0.15aB
S. invicta × *S. richteri*	Small	1.18 ± 0.42bA	0.70 ± 0.02cA
	Medium	1.71 ± 0.16bA	1.49 ± 0.11bB
	Large	3.08 ± 0.27aB	3.21 ± 0.26aB
S. geminata	Small	0.63 ± 0.09cA	0.47 ± 0.02cB
	Medium	2.07 ± 0.14bA	2.23 ± 0.19bA
	Large	5.26 ± 0.35aA	5.63 ± 0.37aA

Means of the three size-classes within a species and within each live/dead status column followed by different lower-case letters are significantly different ($P < 0.05$).

Means of the four species within a size-class and within each live/dead status column followed by different upper-case letters are significantly different ($P < 0.05$).

N = 15

Table 3. Relationship between desiccation time (h) at 30°C and 0-2%RH and % initial mass loss by live small, medium, and large workers of *S. richteri*, *S. invicta* × *S. richteri*, *S. invicta*, *S. geminata* ants (mean ± SE). The significance level is 0.05 (Tukey HSD tests).

Species	**Stage**	**a ± SE**	**b ± SE**	**r^2**	**Df**	**F Values**	***p* Values**
S. richteri	Small	72.96 ± 3.20	2.76 ± 0.42	0.9895	6	473.22	<0.0001
	Medium	74.33 ± 2.03	3.73 ± 0.31	0.9966	6	1476.28	<0.0001
	Large	81.85 ± 3.34	8.38 ± 0.74	0.9961	6	1284.09	<0.0001
S. invicta × *S. richteri*	Small	75.44 ± 4.10	2.98 ± 0.55	0.9850	6	329.03	<0.0001
	Medium	80.31 ± 4.14	6.37 ± 0.79	0.9924	6	654.83	<0.0001
	Large	96.33 ± 5.49	13.61 ± 1.44	0.9954	6	1083.48	<0.0001
S. invicta	Small	79.99 ± 4.68	4.17 ± 0.70	0.9866	6	367.07	<0.0001
	Medium	83.94 ± 4.76	7.81 ± 0.99	0.9922	6	636.57	<0.0001
	Large	107.90 ± 11.30	20.26 ± 3.51	0.9905	6	520.30	<0.0001
S. geminata	Small	70.62 ± 3.04	2.60 ± 0.40	0.9896	6	474.02	<0.0001
	Medium	79.03 ± 3.66	7.17 ± 0.77	0.9942	6	854.19	<0.0001
	Large	101.54 ± 10.89	18.92 ± 3.42	0.9890	6	449.62	<0.0001

a = the maximum asymptotic value of % mass loss or % TBW lost; b = the period required for half the maximum value to be reached.

Table 4. Relationship between desiccation time (h) at 30°C and 0-2%RH and %TBW lost by live small, medium, and large workers of *S. richteri*, *S. invicta* × *S. richteri*, *S. invicta*, *S. geminata* ants (mean ± SE). The significance level is 0.05 (Tukey HSD tests).

Species	**Stage**	**a ± SE**	**b ± SE**	**r^2**	**Df**	**F Values**	***p* Values**
S. richteri	Small	108.05 ± 4.68	2.76 ± 0.42	0.9898	6	486.79	<0.0001
	Medium	113.77 ± 3.19	4.05 ± 0.33	0.9967	6	1499.02	<0.0001
	Large	131.26 ± 5.33	8.47 ± 0.75	0.9962	6	1310.94	<0.0001
S. invicta × *S. richteri*	Small	107.01 ± 6.18	3.14 ± 0.60	0.9837	6	302.43	<0.0001
	Medium	123.26 ± 6.39	6.41 ± 0.80	0.9924	6	650.27	<0.0001
	Large	150.98 ± 8.72	13.63 ± 1.46	0.9953	6	1055.96	<0.0001
S. invicta	Small	110.90 ± 6.68	4.25 ± 0.73	0.9860	6	351.11	<0.0001
	Medium	127.13 ± 7.09	7.78 ± 0.97	0.9924	6	655.62	<0.0001
	Large	167.94 ± 17.66	20.21 ± 3.52	0.9904	6	514.03	<0.0001
S. geminata	Small	100.82 ± 4.54	2.64 ± 0.43	0.9888	6	440.63	<0.0001
	Medium	121.52 ± 5.56	7.08 ± 0.75	0.9943	6	866.32	<0.0001
	Large	161.05 ± 17.08	18.55 ± 3.33	0.9890	6	447.94	<0.0001

a = the maximum asymptotic value of % mass loss or % TBW lost; b = the period required for half the maximum value to be reached.

Table 5. Relationship between desiccation time (h) at 30°C and 0-2%RH and % initial mass loss by dead small, medium, and large workers of *S. richteri*, *S. invicta* × *S. richteri*, *S. invicta*, *S. geminata* ants (mean ± SE). The significance level is 0.05 (Tukey HSD tests).

Species	**Stage**	**a ± SE**	**b ± SE**	**r^2**	**Df**	**F Values**	***p* Values**
S. richteri	Small	67.22 ± 1.47	2.08 ± 0.18	0.9969	6	1586.20	<0.0001
	Medium	70.59 ± 3.33	3.56 ± 0.52	0.9900	6	494.94	<0.0001
	Large	77.63 ± 6.51	6.95 ± 1.36	0.9820	6	273.24	<0.0001
S. invicta × *S. richteri*	Small	70.23 ± 2.91	1.89 ± 0.33	0.9884	6	424.35	<0.0001
	Medium	73.30 ± 3.37	2.74 ± 0.44	0.9886	6	435.28	<0.0001
	Large	75.86 ± 4.93	4.88 ± 0.86	0.9853	6	335.54	<0.0001
S. invicta	Small	73.71 ± 2.79	2.29 ± 0.33	0.9912	6	566.19	<0.0001
	Medium	72.89 ± 4.28	4.03 ± 0.69	0.9861	6	354.17	<0.0001
	Large	79.50 ± 7.11	8.49 ± 1.65	0.9829	6	288.05	<0.0001
S. geminata	Small	67.70 ± 1.92	1.72 ± 0.22	0.9942	6	850.63	<0.0001
	Medium	75.59 ± 5.02	6.01 ± 0.99	0.9870	6	381.06	<0.0001
	Large	97.21 ± 10.13	16.19 ± 2.98	0.9884	6	425.71	<0.0001

a = the maximum asymptotic value of % mass loss or % TBW lost; b = the period required for half the maximum value to be reached.

Table 6. Relationship between desiccation time (h) at 30°C and 0-2%RH and %TBW loss by dead small, medium, and large workers of *S. richteri*, *S. invicta* × *S. richteri*, *S. invicta*, *S. geminata* ants (mean ± SE). The significance level is 0.05 (Tukey HSD tests).

Species	**Stage**	**a ± SE**	**b ± SE**	**r^2**	**Df**	**F Values**	***p* Values**
S. richteri	Small	112.99 ± 2.37	2.03 ± 0.17	0.9971	6	1707.51	<0.0001
	Medium	121.06 ± 5.38	3.46 ± 0.48	0.9909	6	545.19	<0.0001
	Large	132.39 ± 10.78	6.81 ± 1.31	0.9827	6	283.82	<0.0001
S. invicta × *S. richteri*	Small	116.77 ± 5.06	1.92 ± 0.35	0.9874	6	392.31	<0.0001
	Medium	120.23 ± 5.55	2.77 ± 0.45	0.9886	6	434.45	<0.0001
	Large	122.97 ± 8.01	4.87 ± 0.86	0.9852	6	333.73	<0.0001
S. invicta	Small	117.12 ± 4.50	2.27 ± 0.34	0.9909	6	546.91	<0.0001
	Medium	124.96 ± 7.33	4.07 ± 0.70	0.9862	6	357.96	<0.0001
	Large	131.21 ± 11.79	8.64 ± 1.68	0.9831	6	290.85	<0.0001
S. geminata	Small	109.81 ± 3.16	1.62 ± 0.22	0.9938	6	802.42	<0.0001
	Medium	123.96 ± 8.34	6.00 ± 1.00	0.9867	6	371.49	<0.0001
	Large	158.00 ± 16.15	16.15 ± 2.91	0.9888	6	440.98	<0.0001

a = the maximum asymptotic value of % mass loss or % TBW lost; b = the period required for half the maximum value to be reached.

Table 7. LT_{50} values (h) for live small, medium, and large workers of *S. richteri*, *S. invicta* × *S. richteri*, *S. invicta*, *S. geminata* ants.

Species	Size	N	LT_{50} (h)	(95% CI)	Slope ± SE	χ^2	Df
S. richteri	Small	15	2.21	(1.59 – 2.71)	6.14 ± 1.75	0.09	4
	Medium	15	3.03	(2.19 – 3.76)	4.07 ± 0.84	1.44	4
	Large	15	3.65	(2.57 – 4.62)	3.20 ± 0.67	2.20	4
S. invicta × *S. richteri*	Small	15	2.08	(1.36 – 2.58)	5.65 ± 1.69	0.12	4
	Medium	15	3.63	(2.87 – 4.34)	5.02 ± 0.96	1.77	4
	Large	15	5.38	(4.24 – 6.67)	3.50 ± 0.68	3.76	4
S. invicta	Small	15	2.49	(1.44 – 3.29)	3.21 ± 0.76	0.60	4
	Medium	15	4.50	(3.61 – 5.35)	4.81 ± 0.91	0.93	4
	Large	15	8.52	(6.14 – 13.81)	3.38 ± 0.67	4.29	4
S. geminata	Small	15	1.45	(0.27 – 2.22)	3.06 ± 1.03	1.08	4
	Medium	15	4.15	(3.28 – 4.99)	4.44 ± 0.85	2.57	4
	Large	15	7.59	(6.11 – 9.79)	3.35 ± 0.67	2.66	4

N = numbers of individuals; CI = confidence interval.

Table 8. Percentage of total body water (%TBW) lost at median time of death; at lower and upper confidence intervals (CI) for live small, medium, and large workers of *S. richteri*, *S. invicta* × *S. richteri*, *S. invicta*, and *S. geminata* desiccated at 30°C and 0-2% RH.

Species	Size	%TBW lost at LT_{50}	%TBW lost at lower CI	%TBW lost at upper CI	Mean %TBW lost at LT_{50} for species[a]
S. richteri	Small	48.05	39.49	53.53	45.42
	Medium	48.69	39.93	54.77	
	Large	39.53	30.56	46.33	
S. invicta × *S. richteri*	Small	42.64	27.88	48.27	43.31
	Medium	44.57	38.12	49.76	
	Large	42.73	35.82	49.61	
S. invicta	Small	40.97	28.07	48.39	45.79
	Medium	46.59	40.29	51.50	
	Large	49.80	39.13	68.17	
S. geminata	Small	35.74	9.35	46.05	42.47
	Medium	44.91	38.47	50.24	
	Large	46.76	39.90	55.63	

[a] Mean of the three sizes.

Figure Legend

Figure 1: Mean (± SE) percentage of total body water (%TBW) for live and dead small, medium, and large workers of *S. richteri* (Black), *S. invicta* × *S. richteri* (Hybrid), *S. invicta* (Red), and *S. geminata* (Geminata). Means within each worker size-class or species with the same letter are not significantly different (*P* < 0.05).

Figure 2: Mean (± SE) adjusted mass loss for live and dead small, medium, and large workers of *S. richteri* (Black), *S. invicta* × *S. richteri* (Hybrid), *S. invicta* (Red), and *S. geminata* (Geminata). Means within each worker size-class or species with the same letter are not significantly different (*P* < 0.05).

Figure 3: Percentage of initial mass loss over time for live small, medium, and large workers of *S. richteri* (Black), *S. invicta* × *S. richteri* (Hybrid), *S. invicta* (Red), and *S. geminata* (Geminata).

Figure 4: Percentage of total body water (%TBW) lost over time for live small, medium, and large workers of *S. richteri* (Black), *S. invicta* × *S. richteri* (Hybrid), *S. invicta* (Red), and *S. geminata* (Geminata).

Figure 5: Percentage of initial mass loss over time for dead small, medium, and large workers of *S. richteri* (Black), *S. invicta* × *S. richteri* (Hybrid), *S. invicta* (Red), and *S. geminata* (Geminata).

Figure 6: Percentage of total body water (%TBW) lost over time for dead small, medium, and large workers of *S. richteri* (Black), *S. invicta* × *S. richteri* (Hybrid), *S. invicta* (Red), and *S. geminata* (Geminata).

Figure 7: Mean (± SE) calculated cuticular permeability (CP) for live; and absolute CP for dead small, medium, and large workers of *S. richteri* (Black), *S. invicta* × *S. richteri* (Hybrid), *S. invicta* (Red), and *S. geminata* (Geminata). Means within each worker size-class or species with the same letter are not significantly different (*P* < 0.05).

Figure 1

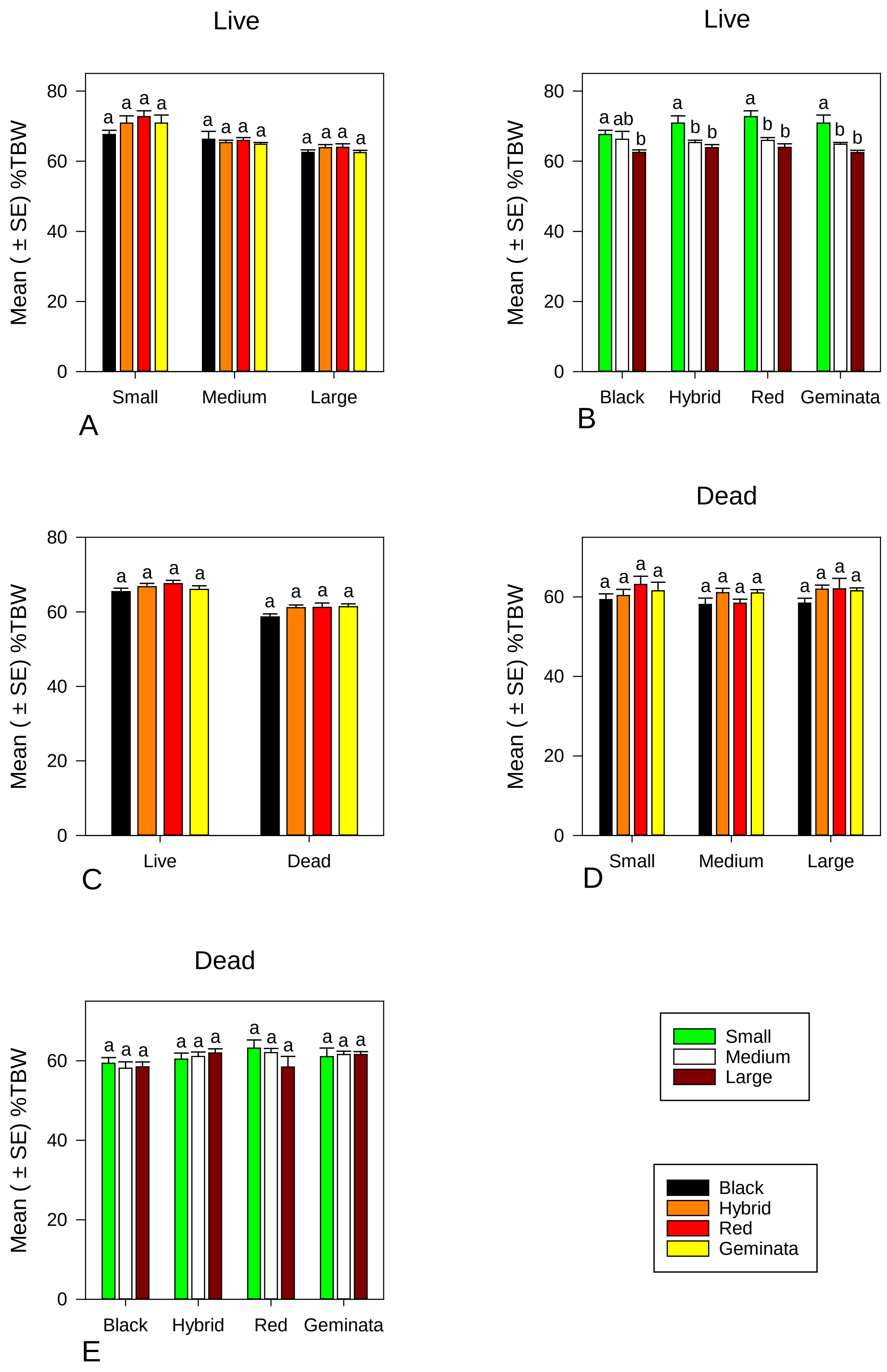

Live
Mean (± SE) %TBW
0
20
40
60
80
a a a a
a a a a
a a a a
Small
Medium
Large
A
Live
Mean (± SE) %TBW
0
20
40
60
80
a ab b
a b b
a b b
a b b
Black
Hybrid
Red
Geminata
B
Mean (± SE) %TBW
0
20
40
60
80
a a a a
a a a a
Live
Dead
C
Dead
Mean (± SE) %TBW
0
20
40
60
a a a a
a a a a
a a a a
Small
Medium
Large
D
Dead
Mean (± SE) %TBW
0
20
40
60
a a a
a a a
a a a
a a a
Black
Hybrid
Red
Geminata
E
Small
Medium
Large
Black
Hybrid
Red
Geminata

Figure 2

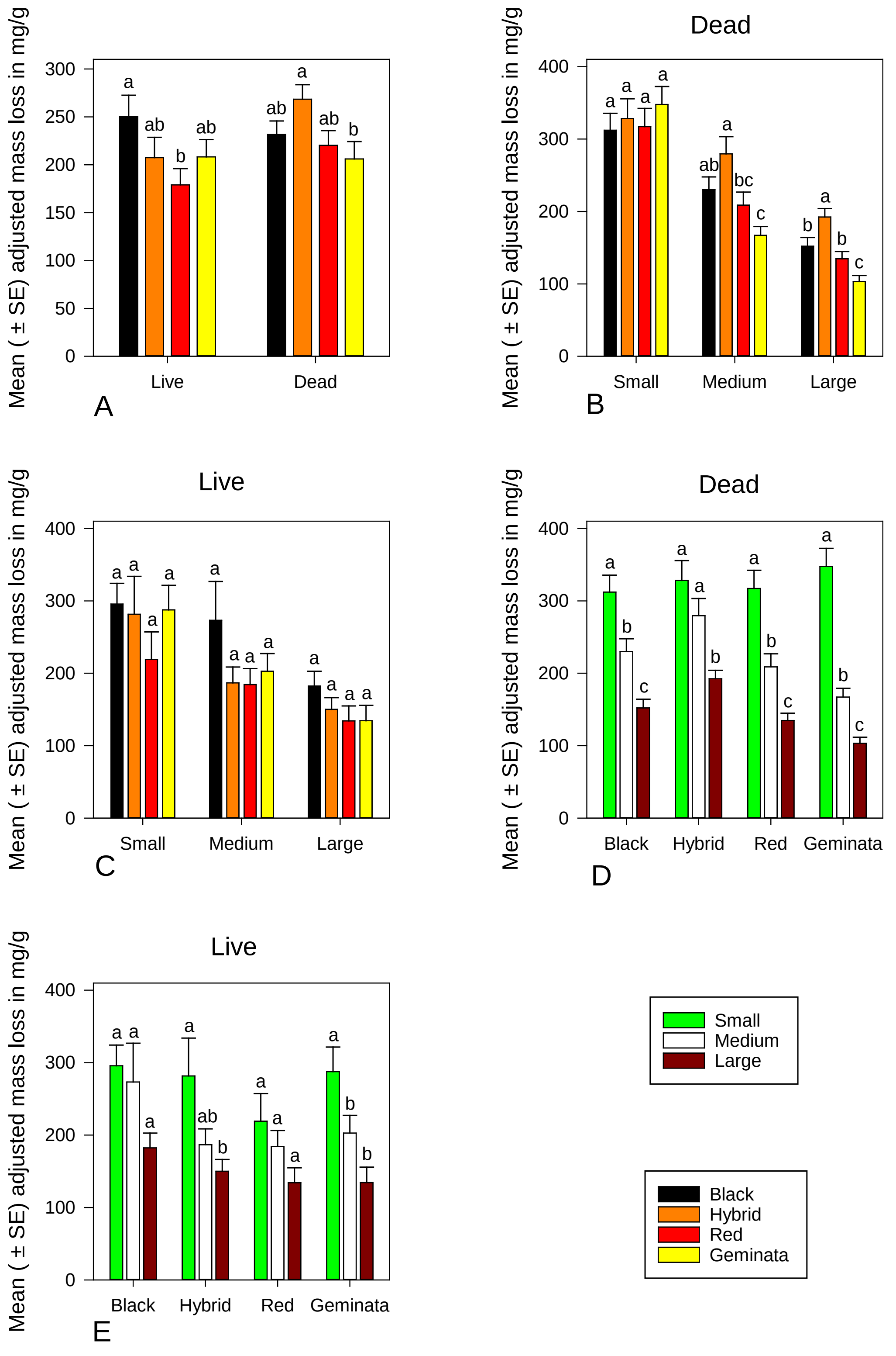

Mean (± SE) adjusted mass loss in mg/g
300
250
200
150
100
50
0
a
ab
b
ab
ab
a
ab
b
Live
Dead
A
Dead
400
300
200
100
0
a
a
a
a
ab
a
bc
c
b
a
b
c
Small
Medium
Large
B
Live
a
a
a
a
a
a
a
a
a
a
a
a
Small
Medium
Large
C
Dead
a
b
c
a
a
b
a
b
c
a
b
c
Black
Hybrid
Red
Geminata
D
Live
a
a
a
a
ab
b
a
a
a
a
b
b
Black
Hybrid
Red
Geminata
E
🟩 Small
☐ Medium
🟥 Large
■ Black
🟧 Hybrid
🟥 Red
🟨 Geminata

Figure 3

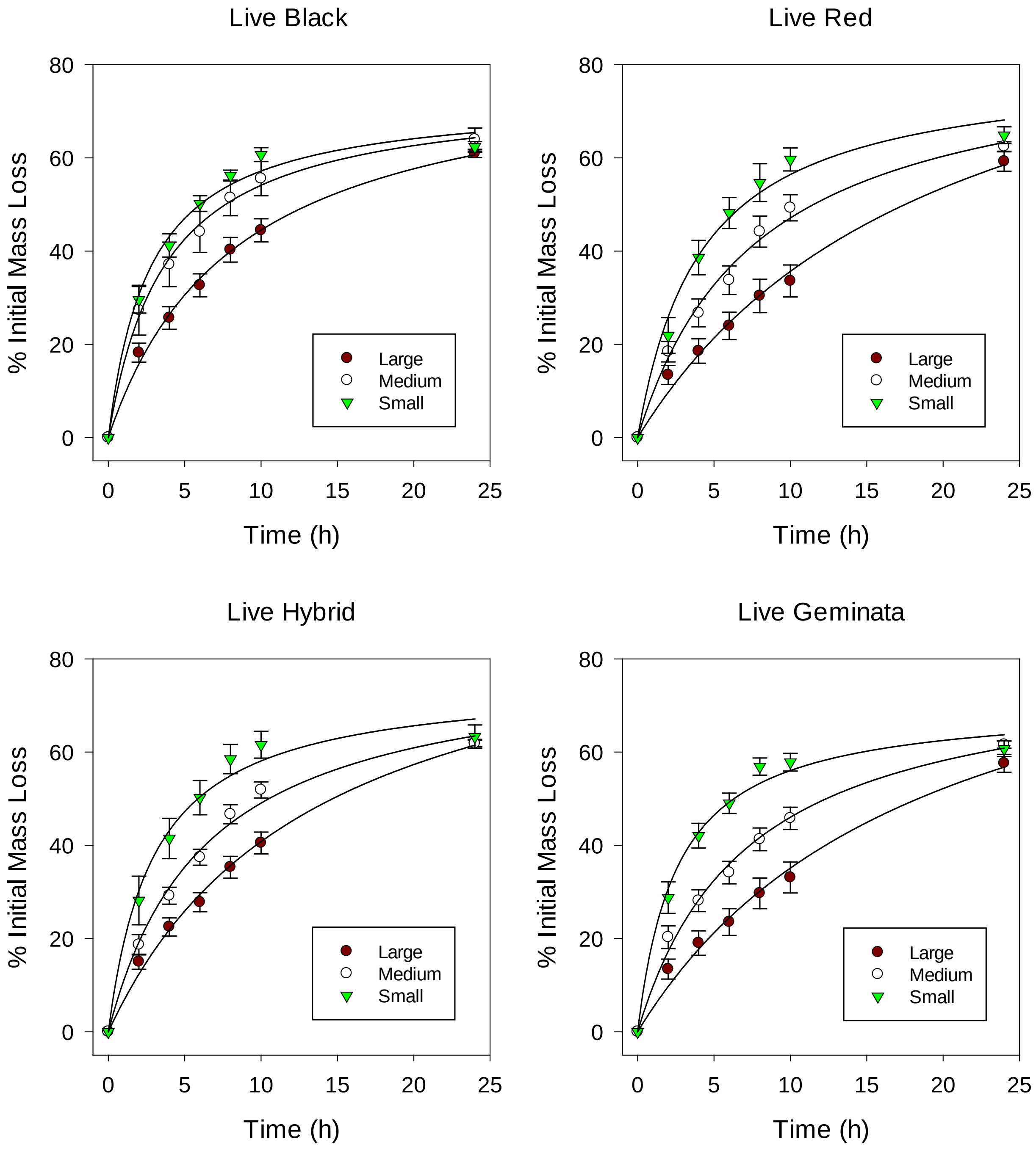

Live Black
Live Red
Live Hybrid
Live Geminata
% Initial Mass Loss
0
20
40
60
80
Time (h)
0
5
10
15
20
25
Large
Medium
Small

Figure 4

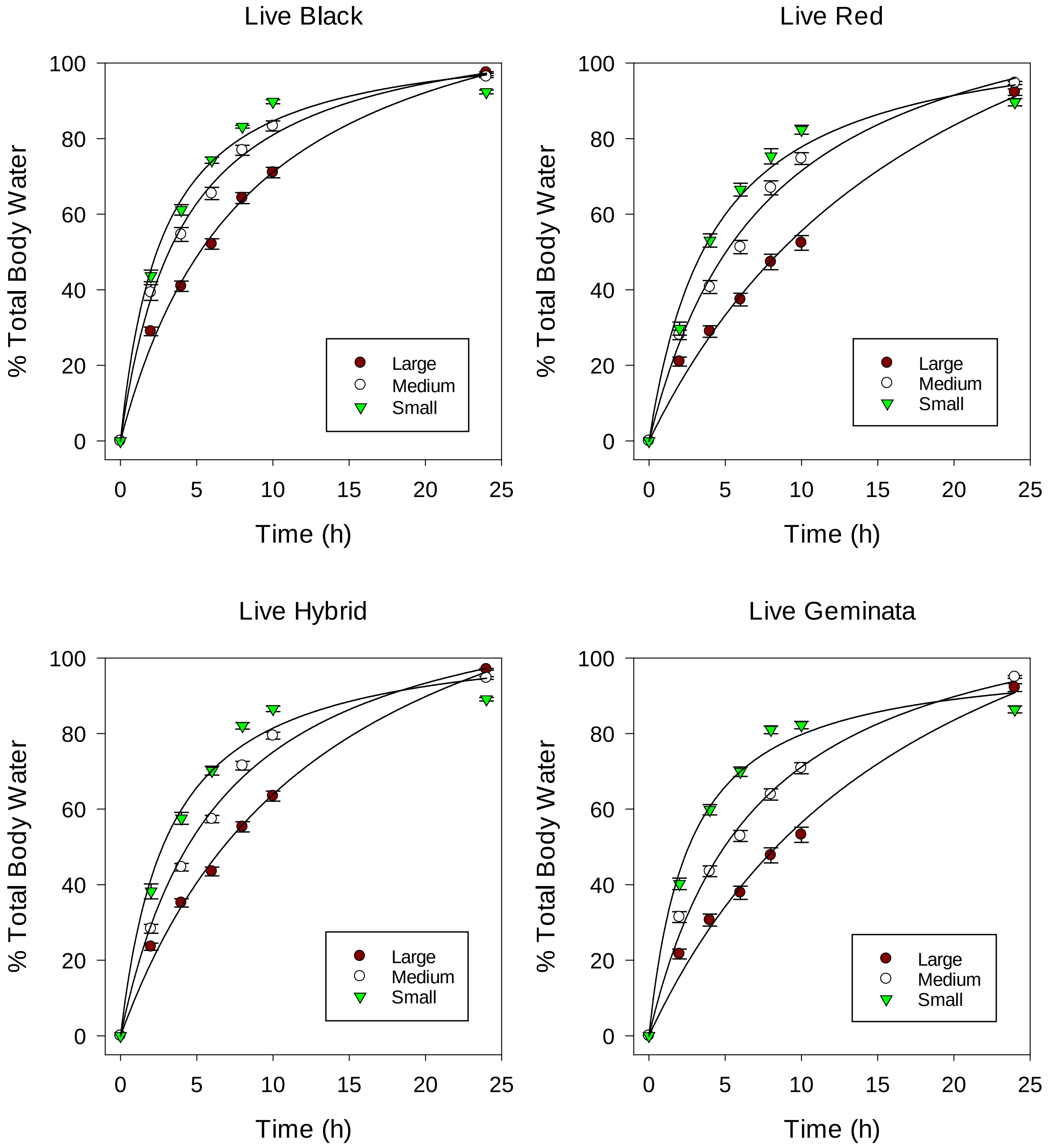

Live Black
Live Red
Live Hybrid
Live Geminata
% Total Body Water
Time (h)
0
20
40
60
80
100
0
5
10
15
20
25
● Large
○ Medium
▼ Small

Figure 5

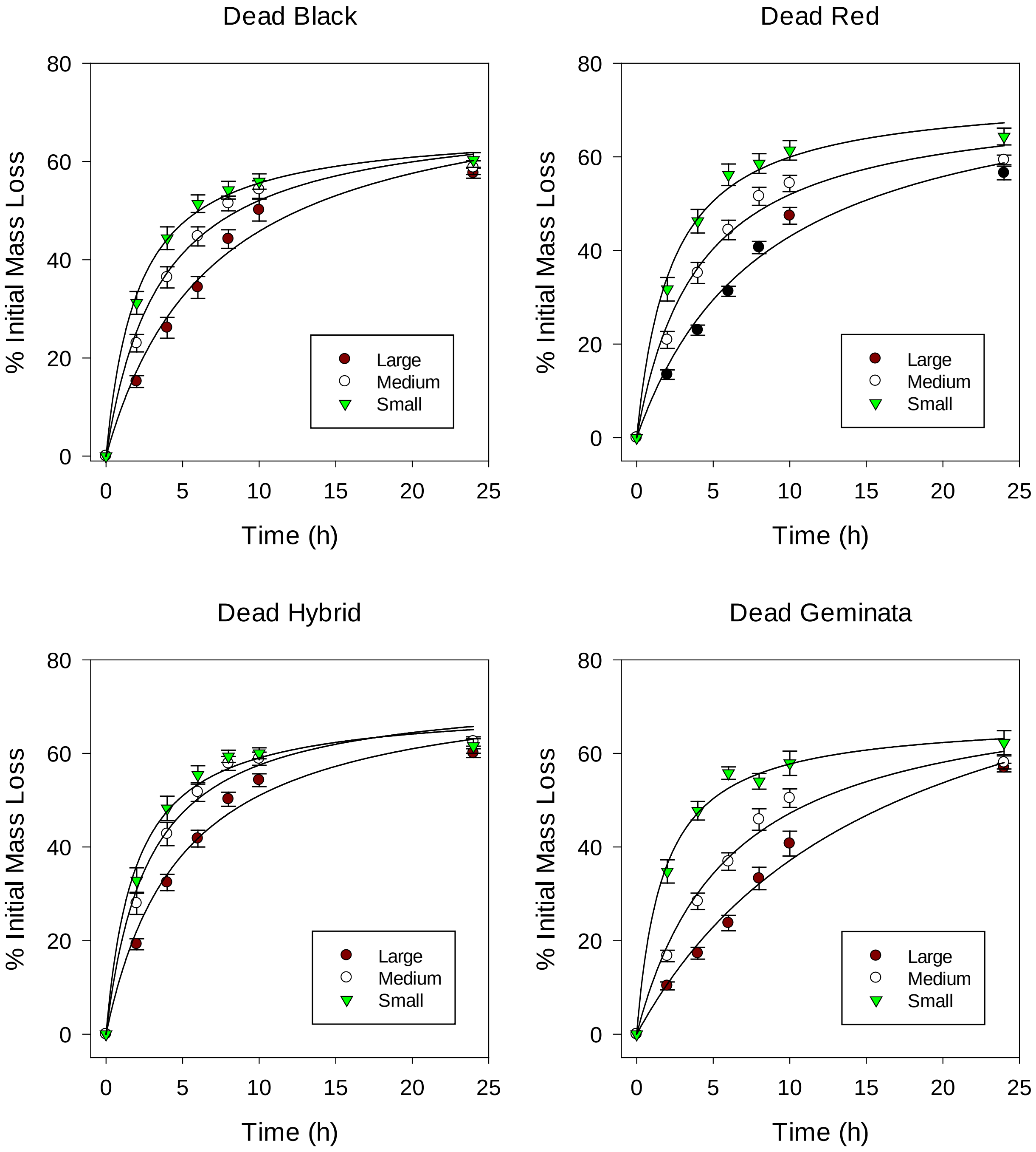

Dead Black
Dead Red
Dead Hybrid
Dead Geminata
% Initial Mass Loss
Time (h)
0
20
40
60
80
0
5
10
15
20
25
● Large
○ Medium
▼ Small

Figure 6

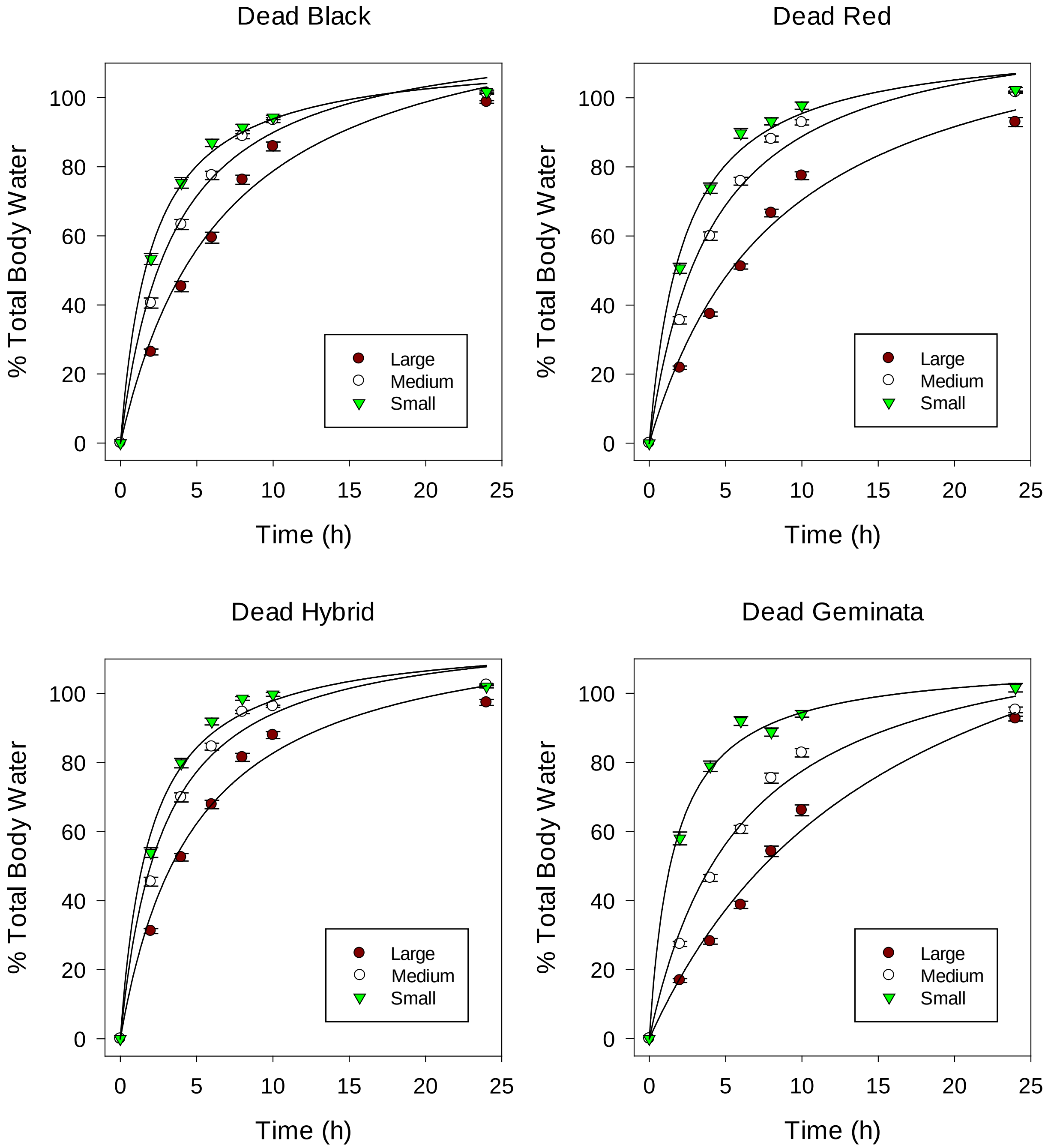
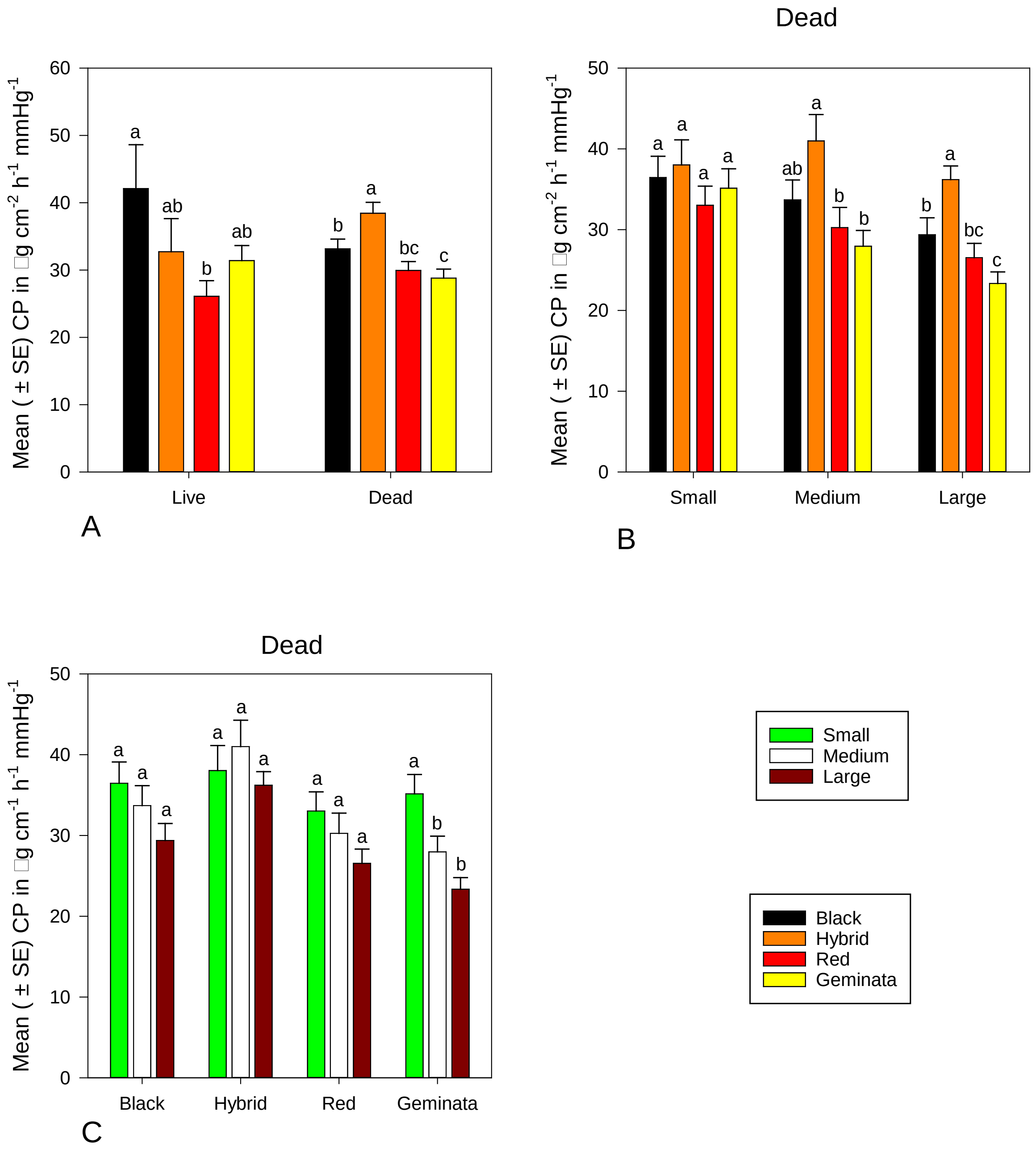

Dead Black
Dead Red
Dead Hybrid
Dead Geminata
% Total Body Water
Time (h)
0
20
40
60
80
100
0
5
10
15
20
25
● Large
○ Medium
▼ Small

Figure 7


Mean (± SE) CP in □g cm-2 h-1 mmHg-1
0
10
20
30
40
50
60
a
ab
b
ab
b
a
bc
c
Live
Dead
A
Dead
Mean (± SE) CP in □g cm-2 h-1 mmHg-1
0
10
20
30
40
50
a
a
a
a
ab
a
b
b
b
a
bc
c
Small
Medium
Large
B
Dead
Mean (± SE) CP in □g cm-1 h-1 mmHg-1
0
10
20
30
40
50
a
a
a
a
a
a
a
a
a
a
b
b
Black
Hybrid
Red
Geminata
C
Small
Medium
Large
Black
Hybrid
Red
Geminata

CHAPTER 3

Discontinuous Gas Exchange in Invasive and Native *Solenopsis* Fire Ants in the Southeastern United States: Effects of Differences in Species, Caste, and Temperature

3.1 Introduction

The discontinuous gas exchange cycle (DGC) in insects is the cyclic intake of oxygen and emission of carbon dioxide (Lighton 1994; Vogt and Appel 2000). The DGC has been demonstrated in adults of many ant species, including *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Vogt and Appel 2000). The adaptive significance of the DGC is unclear. The DGC is widely hypothesized to be driven by the need for the insect to control respiratory water loss (see reviews by Miller 1981; Kestler 1985; Slama 1988; Lighton 1994, 1996; Wasserthal 1996). However, some insects that are xeric-adapted fail to exhibit a DGC (Lighton 1994), and the DGC can be eliminated in desiccated insects [e.g. *Romalea guttata* (Houttyun); Hadley and Quinlan 1993]. The relative importance of respiratory water loss in the overall water economy of an insect may be very low in some cases, due to high cuticular permeability (e.g., Hadley and Quinlan 1993). It has also been hypothesized that the DGC serves as an adaptation for dealing with hypercapnia and/or hypoxia, as in insects that live in the soil such as fire ants (Lighton and Berrigan, 1995). Other hypotheses include avoiding oxygen poisoning and predator/parasite avoidance (Hetz and Bradley 2005).

Two distinct periods in the DGC are the burst and interburst periods. During the burst period, insect spiracles open fully, resulting in escape of CO_2. The burst period is also known as the open (O) period. The interburst period is composed of the flutter (F) and closed (C) periods. During C the spiracles are closed tightly and very little or no gas exchange occurs. During C the insect is utilizing O_2 from the endotracheal space and producing CO_2 which dissolves into the hemolymph. This is usually followed in most insects by the F period, during which the spiracles open and close rapidly and O_2 enters the tracheal system, by bulk flow, diffusion, or a combination of the two (Vogt and Appel 2000).

Temperature is a key abiotic factor impacting terrestrial insects such as fire ants as it directly affects survival (Angilletta 2009). Most terrestrial insects experience substantial variation in body temperature which can drive changes in metabolic rate and the need for oxygen (Gillooly et al. 2001, Irlich et al. 2009). For some insects, higher body temperatures are associated with higher ventilatory frequencies or burst frequencies during discontinuous gas exchange (Lighton 1994, Davis et al. 1999, Chappell and Rogowitz 2000, Vogt and Appel 2000, Harrison et al. 2012). Many studies have demonstrated that the respiratory burst volume decreases with increased temperature in ants (Lighton 1988; Quinlan and Lighton 1999; Vogt and Appel 2000). Temperature plays a crucial role as the essential modifier of the intensity of metabolic processes in poikilothermic organisms, including ants (Vogt and Appel 2000), also the degree of the organism's acclimatization to environmental conditions affects the intensity of its metabolic processes (Nielsen et al. 1999).

Interspecific and intraspecific variation of gas exchange recordings between individuals may be caused by several factors such as body size, caste, and temperature (Chown 2001; Jõgar et al. 2014). The existence and the precise pattern of DGC depends on the species (Lighton 1994, 1996; Sláma 1999; Chown et al. 2006; Chown 2011), individual characteristics (Marais and Chown 2003; Gibbs and Johnson 2004; Karise et al. 2010), life stage of the individuals (Beekman and van Stratum 1999; Mänd et al. 2005, 2006), metabolic rate (Moerbitz and Hetz 2010) and environmental conditions like temperature (Lighton and Lovegrove 1990; Lighton 1998; Vogt and Appel 2000; Kovac et al. 2007; Karise et al. 2010), relative humidity (Duncan et al. 2002b; Lighton 2007; Sláma et al. 2007; Schimpf et al. 2009) and the amount of oxygen or carbon dioxide in the air (Lighton 1998; Vogt and Appel 2000).

The black imported fire ant, *Solenopsis richteri* Forel, and the red imported fire ant, *S. invicta* Buren, were accidentally introduced into the United States of America from South America in the 1920s and 1930s, respectively, through Mobile, Alabama (Lofgren et al. 1975; Patterson 1994). Since their introduction, they have spread over much of the Southeast U.S. from the Carolinas to Texas. *S. invicta* has displaced native fire ants (Vinson 1997), including *S. geminata* (Fabricius). Since their introduction, both *S. richteri* and *S. invicta* have formed an extensive zone of hybridization in their invaded region, producing hybrid *S. invicta* × *S. richteri* species (Diffie et al. 1988; Vander Meer et al. 1985). However, hybridization has not been found in their native South America (Vander Meer et al. 1985; Cohen and Privman 2019). They are pests in agricultural, urban, and natural habitats; and are also medically important pests (Lowe et al. 2000; Chan and Guénard 2020; Tschinkel 2006). Eusociality is observed in *Solenopsis* fire ants, which is typically characterized by a division of labor into groups called castes (Wilson and Hölldobler 2005). Worker caste is non-reproductive, while the reproductive castes are male alates and female alates, and each colony contains at least a reproductive queen (Tschinkel 2006).

This study was designed to quantify and compare the salient characteristics of the DGC in *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata*. The goal was to make comparative DGC for ants occupying different habitats. *Solenopsis invicta* and *S. geminata* seem to be xeric while *S. richteri* and *S. invicta* × *S. richteri* are more of mesic ants (Ajayi et al. unpublished data). We also compared the DGCs of castes subject to hypercapnic and hypoxic conditions, as well as possible water stress, with the DGCs of those more likely to be subject to water stress alone. If, as has been hypothesized, a major driving force for development of a DGC is hypercapnic and/or hypoxic conditions (Lighton and Berrigan, 1995), we would expect alates of *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata* (which presumably encounter the most extreme conditions during claustral colony founding) to exhibit a longer C period than other castes, which would result in greater PCO_2 within and greater PO_2 outside of the four ant species, facilitating respiration.

3.2 Materials and Methods

3.2.1 Insects. *Solenopsis invicta* workers were obtained from mounds on the Auburn University campus, Lee County, Alabama in March 2017. *Solenopsis richteri* workers were obtained from mounds in Hohenwald, Lewis County, and Mount Pleasant, Maury County, Tennessee in March 2017.

Workers of *S. invicta* × *S. richteri* were obtained from mounds in Cullman, Cullman County, and Hollywood, Jackson County, Alabama in March 2017. Workers of *S. geminata* were obtained from mounds in Gainesville, Alachua County, Florida in March 2017. The identity of each species was confirmed by gas chromatography of hexane extracts of *ca*. 50 workers using both venom alkaloid and CHC characters (Vander Meer et al. 1985; Ross et al. 1987; Chen et al. 2010; Hu et al. 2018). Based on large variation in body sizes and behavioral differences among size classes of *Solenopsis* spp. workers, each species was categorized, using the range of their head width, as small (0.72 mm or less), medium (0.73 – 0.92 mm), or large (0.93 mm or more) (Wilson 1978; Hölldobler and Wilson 1990). These colony fragments were provided crickets, a sugar solution, and a test tube nest (25 × 200 mm) filled partially with water and plugged with cotton. Ants were maintained in the laboratory at 25 ± 2 °C with an approximate 12:12 (L:D) photoperiod.

3.2.2 Respirometry. The following protocol was applied to all castes in all four *Solenopsis* species. Prior to being placed in a respirometry chamber each ant was weighed to the nearest 0.01 mg on a digital balance. Care was taken to avoid injury or undue stress to the ants, by allowing them to crawl onto a dissecting probe and transferring them to weighing vials and respirometry chambers. Ants were transferred in this fashion rather than grasping them with forceps. Following weighing, each ant was placed in glass chambers with the barbed metal stoppers which was then placed in a Sable Systems (Henderson, NV, USA) PT-1 Peltier-effect temperature- controlled cabinet. Ants were allowed < 15 min to acclimatize to conditions in the respirometer prior to beginning a recording. Recordings were aborted if ants did not exhibit a DGC within approximately 15 – 20 min. Ants were provided with a small (about 1 × 2 cm) piece of window screening as a substrate they could easily cling to.

Compressed air was forced at about 13 L min^{-1} through a Whatman purge-gas generator (Whatman Inc., Haverhill, MA, USA) to remove CO_2 and H_2O, forced into a large (> 100 L) mixing tank, then into an open manifold to equalize pressure. The resulting dry, CO_2-free air was drawn from the manifold through the respirometer chamber containing the experimental animal, then drawn through a LI-

7000, LI-COR CO_2 and H_2O analyzer (LI-COR Inc., Lincoln, NE, USA) (CO_2 resolution 0.001 ppm) and was set to allow air to flow through the system at a known rate (100 ml/min). Prior to conducting respirometry, the baseline of the CO_2 analyzer was adjusted to zero and the unit was spanned using pressurized air of known CO_2 concentration (BOC Gases, Murray Hill, NJ, USA) drawn through a drying column.

3.2.3 Data Analyses. Data were recorded and analyzed using ExpeData software. The data was baseline corrected to zero baseline. Significant differences in the DGC parameters among the four *Solenopsis* species in each caste/stage was tested using ANOVA followed by Tukey HSD test for mean separation. Correlation between variables were also analyzed using ANCOVA.

3.3 Results

3.3.1 DGC Parameters and Species Effects. We investigated the effects of species and temperature on DGC frequency (in mHz), Burst period volume (in ml), and Burst period rate of CO_2 emission (in ml/min), for all the five castes (small worker, medium worker, large worker, male alate, and female alate) using ANCOVA. There was no caste by temperature interaction ($P > 0.05$) for female alates, small workers, and large workers. Typical, representative real-time CO_2 traces, illustrating the DGC in small workers, medium workers, large workers, male alates, and female alates of *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata* are presented in Figs. 1 – 5.

3.3.2 Small Workers. Temperature had a significant effect on DGC (ANCOVA, $F = 35.41$, $df = 1, 19$, $P < 0.0001$), but species was not significant ($P = 0.79$). Temperature had a significant effect on DGC frequency (ANCOVA, $F = 27.19$, $df = 1, 19$, $P = 0.0002$), but species was not significant ($P = 0.62$). Temperature had a significant effect on Burst volume (ANCOVA, $F = 5.03$, $df = 1, 19$, $P = 0.04$), but species was not significant ($P = 0.05$). Temperature had a significant effect on Burst duration (ANCOVA, $F = 16.59$, $df = 1, 19$, $P = 0.001$), but species was not significant ($P = 0.16$). Temperature had a significant effect on mean burst rate (ANCOVA, $F = 7.00$, $df = 1, 19$, $P = 0.02$), but species was not significant (*P* = 0.13). Temperature had a significant effect on Interburst duration (ANCOVA, *F* = 8.41, *df* = 1, 19, *P* = 0.01), but species was not significant (*P* = 0.11). Temperature had a significant effect on Interburst volume (ANCOVA, *F* = 6.53, *df* = 1, 19, *P* = 0.02), but species was not significant (*P* = 0.11). Temperature had a significant effect on mean Interburst rate (ANCOVA, *F* = 7.18, *df* = 1, 19, *P* = 0.02), but species was not significant (*P* = 0.13). Temperature had no significant effect on rate of mass loss in mg/min (*P* = 0.10), and species was not significant (*P* = 0.37) (Table 1).

3.3.3 Medium Workers. Temperature had no significant effect on burst volume (*P* = 0.54), and species was not significant (*P* = 0.96). Temperature had a significant effect on burst duration (ANCOVA, *F* = 96.85, *df* = 1, 19, *P* < 0.0001), species was significant (ANCOVA, *F* = 5.83, *df* = 3, 19, *P* = 0.01)), also there was significant temperature by species interaction (ANCOVA, *F* = 3.58, *df* = 3, 19, *P* = 0.047). Temperature had no significant effect on mean burst rate (*P* = 0.53), and species was not significant (*P* = 0.49). Temperature had a significant effect on interburst duration (ANCOVA, *F* = 14.20, *df* = 1, 19, *P* = 0.0027), species was significant (ANCOVA, *F* = 5.95, *df* = 3, 19, *P* = 0.01), also there was significant temperature by species interaction (ANCOVA, *F* = 4.06, *df* = 3, 19, *P* = 0.03). Temperature had no significant effect on interburst volume (*P* = 0.54), and species was not significant (*P* = 0.96). Temperature had no significant effect on mean interburst rate (*P* = 0.53), and species was not significant (*P* = 0.96). Temperature had a significant effect on rate of mass loss in mg/min (ANCOVA, *F* = 172.28, *df* = 1, 19, *P* < 0.0001), species was significant (ANCOVA, *F* = 5.26, *df* = 3, 19, *P* = 0.02), also there was significant temperature by species interaction (ANCOVA, *F* = 9.01, *df* = 3, 19, *P* = 0.002). Temperature had a significant effect on DGC frequency (ANCOVA, *F* = 235.32, *df* = 1, 19, *P* < 0.0001), species was significant (ANCOVA, *F* = 9.24, *df* = 3, 19, *P* = 0.002), also there was significant temperature by species interaction (ANCOVA, *F* = 8.21, *df* = 3, 19, *P* = 0.003) (Table 2).

3.3.4 Large Workers. Temperature had a significant effect on burst volume (ANCOVA, *F* = 5.26, *df* = 1, 19, *P* = 0.04), but species was not significant (*P* = 0.07). Temperature had a significant effect on burst duration (ANCOVA, *F* = 22.91, *df* = 1, 19, *P* = 0.0004), but species was not significant (*P* =

0.08). Temperature had a significant effect on mean burst rate (ANCOVA, *F* = 5.68, *df* = 1, 19, *P* = 0.0345), but species was not significant (*P* = 0.08). Temperature had a significant effect on interburst duration (ANCOVA, *F* = 25.94, *df* = 1, 19, *P* = 0.0003), also species was significant (ANCOVA, *F* = 4.25, *df* = 3, 19, *P* = 0.0292). Temperature had no significant effect on interburst volume (*P* = 0.0680), but species was significant (ANCOVA, *F* = 3.69, *df* = 3, 19, *P* = 0.0431). Temperature had a significant effect on mean interburst rate (ANCOVA, *F* = 6.02, *df* = 1, 19, *P* = 0.0304), but species was not significant (*P* = 0.0946). Temperature had a significant effect on rate of mass loss (ANCOVA, *F* = 47.49, *df* = 1, 19, *P* < 0.0001), but species was not significant (*P* = 0.58). Temperature had a significant effect on DGC frequency (ANCOVA, *F* = 77.45, *df* = 1, 19, *P* < 0.0001), also species was significant (ANCOVA, *F* = 6.41, *df* = 1, 19, *P* = 0.0077) (Table 3).

3.3.5 Male Alates. Temperature had no significant effect on burst volume (*P* = 0.05), but species was significant (ANCOVA, *F* = 5.71, *df* = 3, 19, *P* < 0.01). Temperature had a significant effect on burst duration (ANCOVA, *F* = 184.28, *df* = 1, 19, *P* < 0.0001), species was significant (ANCOVA, *F* = 5.89, *df* = 3, 19, *P* = 0.01), also there was significant temperature by species interaction (ANCOVA, *F* = 3.56, *df* = 3, 19, *P* = 0.047). Temperature had no significant effect on mean burst rate (*P* = 0.94), species was significant (ANCOVA, *F* = 5.48, *df* = 3, 19, *P* = 0.01), also there was significant temperature by species interaction (ANCOVA, *F* = 4.29, *df* = 3, 19, *P* = 0.03). Temperature had a significant effect on interburst duration (ANCOVA, *F* = 44.93, *df* = 1, 19, *P* < 0.0001), but species was not significant (*P* = 0.15). Temperature had no significant effect on interburst volume (*P* = 0.07), but species was significant (ANCOVA, *F* = 4.75, *df* = 3, 19, *P* = 0.02). Temperature had no significant effect on mean interburst rate (*P* = 0.07), but species was significant (ANCOVA, *F* = 4.97, *df* = 3, 19, *P* = 0.02). Temperature had a significant effect on Rate of mass loss (ANCOVA, *F* = 33.24, *df* = 1, 19, *P* < 0.0001), but species was not significant (*P* = 0.27). Temperature had a significant effect on DGC frequency (ANCOVA, *F* = 27.95, *df* = 1, 19, *P* = 0.0002), but species was not significant (*P* = 0.47) (Table 4).

3.3.6 Female Alates. Temperature had no significant effect on Burst volume (*P* = 0.0719), but species was significant (ANCOVA, *F* = 4.67, *df* = 3, 19, *P* = 0.022). Temperature had a significant effect on burst duration (ANCOVA, *F* = 93.62, *df* = 1, 19, *P* < 0.0001), but species was not significant (*P* = 0.42). Temperature had no significant effect on mean burst rate (*P* = 0.10762), but species was significant (ANCOVA, *F* = 4.88, *df* = 3, 19, *P* = 0.0191). Temperature had a significant effect on interburst duration (ANCOVA, *F* = 45.32, *df* = 1, 19, *P* < 0.0001), but species was not significant (*P* = 0.0751). Temperature had no significant effect on interburst volume (*P* = 0.09), but species was significant (ANCOVA, *F* = 4.39, *df* = 3, 19, *P* = 0.0264). Temperature had no significant effect on mean interburst rate (*P* = 0.08), but species was significant (ANCOVA, *F* = 4.75, *df* = 3, 19, *P* = 0.0209). Temperature had a significant effect on rate of mass loss (ANCOVA, *F* = 6.89, *df* = 1, 19, *P* = 0.0222), but species was not significant (*P* = 0.90). Temperature had a significant effect on DGC frequency (ANCOVA, *F* = 57.50, *df* = 1, 19, *P* < 0.0001), but species was not significant (*P* = 0.74) (Table 5).

The relationship between the five temperatures tested and each of mass, rate of CO_2 production, volume of or burst CO_2, frequency, burst duration, and interburst duration for each caste of the four ant species are shown in Tables 1 to 5.

3.**4** **Discussion**

One way to investigate changes in burst characteristics with changing rate of CO_2 emission ($\dot{V}CO_2$) is to examine burst volume of CO_2 at different temperatures. Burst volume of CO_2 decreased with increasing temperature (and increasing $\dot{V}CO_2$) in male and female alates in all species. This result is similar to that in Vogt and Appel (2000) for male and female alates of *S. invicta*. These relationships indicate lowered storage capacity for CO_2 with increasing metabolic rate, as rising metabolic CO_2 competes for storage space by means of its increasing partial pressure (e.g., Kestler 1971; Vogt and Appel 2000).

The DGC frequency in workers and alates of *S. invicta* in our results and those of Vogt and Appel (2000) are similar. The DGC values in alate *S. richteri* and *S. geminata* are similar. The DGC values in alate *S. invicta* and *S. invicta* × *S. richteri* are about 3-fold and 4-fold lower than that in *S. geminata*, respectively. The DGC frequency of *S. invicta* and *S. invicta* × *S. richteri* are rapid compared with arid-adapted ant species for which data have been reported, but offers no significant deviation in form or occurrence from DGCs reported in the literature (Vogt and Appel 2000). Furthermore, the DGC frequency values in *S. richteri* and *S. geminata* are higher compared with those in *S. invicta* and *S. invicta* × *S. richteri*. Medium workers of *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata*, (mean masses in this study 1.87, 2.19, 2.10, 2.26 mg, respectively) are part of the smallest ants for which a DGC has been reported to date. Medium workers of these *Solenopsis* species exhibit DGC frequencies of 28.47, 34.16, 24.97, and 25.54 mHz at 25 °C; and 37.88, 37.66, 31.45, and 30.78 mHz, respectively, at 30 °C. DGC frequency in *S. invicta*, *S. invicta* × *S. richteri* and *S. geminata* are therefore comparable to that of somewhat more xeric species, at 25 °C, while those in and *S. richteri* are comparable with more mesic species. Cuticular permeability of H_2O for medium *S. invicta* and *S. geminata* are 30.26 and 27.96 µg cm^{-2} h^{-1} $mmHg^{-1}$, respectively (Ajayi et al. unpublished data), at the range of Edney’s (1977) proposed cuticular permeability values for xerically-classified species. Cuticular permeability of H_2O at 30 °C for medium *S. richteri* and *S. invicta* × *S. richteri* are 33.69, 40.98 µg cm^{-2} h^{-1} $mmHg^{-1}$, respectively, at the range of Edney’s (1977) proposed cuticular permeability values for mesically-classified species. Insects with high cuticular permeabilities, such as grasshoppers (Hadley and Quinlan 1993), may exhibit lower relative respiratory water-loss rates than insects with lower cuticular permeabilities (Lighton 1996). These relative rate differences may explain the well-developed DGCs in more xeric ants with lower cuticular permeabilities (Lighton 1990, Lighton 1996) such as *S. invicta*.

Comparative data on DGC characteristics of alate ants is scarce (Vogt and Appel 2000). In our study, the DGC frequencies of female alate *S. invicta, S. richteri*, *S. invicta* × *S. richteri* and *S. geminata* were 16.65, 21.01, 13.32, and 26.92 mHz, respectively, at 25 °C. DGC frequency of female alate *S.*

invicta at 25 °C in our study (16.65 mHz) is similar to that reported by Vogt and Appel (2000) (14 mHz). Cuticular permeability values in female *S. invicta* alates at 30 °C is 11.7 ± 1.0 µg cm^{-2} h^{-1} $mmHg^{-1}$ (see Vogt and Appel 2000), indicating xeric adaptation (Edney 1977). Male alate *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri* and *S. geminata* were 12.90, 19.93, 18.26, and 12.59 mHz, respectively, at 25 °C. Male *S. invicta* and *S. richteri* alates exhibited lower DGC frequencies than their female alates, at 25 °C. However, male *S. invicta* × *S. richteri* and *S. geminata* alates exhibited higher DGC frequencies than their female alates, at 25 °C.

Our data shows that the DGC was pronouncedly expressed in the alate caste (irrespective of temperature) in all tested *Solenopsis* species. This may be explained by the hygric genesis hypothesis that if the DGC was primarily a mechanism for conserving respiratory water, it would be preferentially expressed in the species and castes subject to more desiccating conditions (Lighton 1996), and alates are exposed to desiccating environments, especially during season of fire ant mating flights. Our findings were in accord with the chthonic genesis, that the DGC in *Solenopsis* species tested acts primarily to facilitate gas exchange in hypoxic and hypercapnic environments. And this is in accord with findings in Lighton and Berrigan (1995). Alates of all *Solenopsis* species tested showed a pronounced DGC; however, the DGC is not as pronounced in workers of these species. Thus, our data shows that the chtonic genesis looks more and more attractive (Lighton 1996), and the DGC could influence the adaptation of these *Solenopsis* species of fire ants in the southeastern USA. Further studies are needed to investigate and compare the DGC in reproductive queens of these *Solenopsis* species.

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Table 1. Discontinuous gas exchange parameters for small workers of *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata* at several temperatures (mean ± SE).

		Small workers				
		10°C	15°C	20°C	25°C	30°C
Mass (mg)	*S. invicta*	0.71 ± 0.05	0.73 ± 0.15	0.69 ± 0.13	0.98 ± 0.19	0.89 ± 0.11
	S. richteri	0.79 ± 0.08	0.83 ± 0.06	0.82 ± 0.05	0.91 ± 0.04	0.81 ± 0.04
	S. invicta × *S. richteri*	0.86 ± 0.02	0.92 ± 0.06	0.78 ± 0.05	0.85 ± 0.03	0.74 ± 0.06
	S. geminata	0.62 ± 0.02	0.51 ± 0.03	0.51 ± 0.03	0.67 ± 0.05	0.64 ± 0.11
$\dot{V}CO_2$ (µl h^{-1})	*S. invicta*	0.00008 ± 0.00008	0.00001 ± 0.00000	0.00000 ± 0.00000	0.00000 ± 0.00000	0.00001 ± 0.00000
	S. richteri	0.00027 ± 0.00011	0.00006 ± 0.00002	0.00001 ± 0.00000	0.00002 ± 0.00000	0.00002 ± 0.00000
	S. invicta × *S. richteri*	0.00117 ± 0.00065	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00002 ± 0.00001	0.00002 ± 0.00001
	S. geminata	0.00170 ± 0.00117	0.00003 ± 0.00001	0.00000 ± 0.00000	0.00001 ± 0.00000	0.00001 ± 0.00000
Burst CO_2 (µl)	*S. invicta*	0.000060 ± 0.000048	0.000014 ± 0.000005	0.000005 ± 0.000002	0.000007 ± 0.000001	0.000019 ± 0.000007
	S. richteri	0.000268 ± 0.000146	0.000055 ± 0.000019	0.000006 ± 0.000001	0.000008 ± 0.000001	0.000013 ± 0.000004
	S. invicta × *S. richteri*	0.000507 ± 0.000237	0.000010 ± 0.000002	0.000006 ± 0.000001	0.000023 ± 0.000007	0.000015 ± 0.000005
	S. geminata	0.002632 ± 0.002291	0.000033 ± 0.000014	0.000003 ± 0.000001	0.000012 ± 0.000006	0.000007 ± 0.000001

Frequency (mHz)	*S. invicta*	11.7451 ± 2.80893	13.2737 ± 2.68963	13.8326 ± 2.36692	18.8615 ± 2.33394	18.0807 ± 1.64327
	S. richteri	15.3162 ± 4.08570	16.1805 ± 5.55646	23.5547 ± 2.76570	36.2251 ± 3.19229	32.0938 ± 2.84991
	S. invicta × *S. richteri*	11.7566 ± 2.04800	14.9631 ± 2.52818	23.5461 ± 1.31360	22.2161 ± 3.68601	21.6651 ± 3.22820
	S. geminata	19.6438 ± 5.79651	15.8131 ± 6.28529	26.9193 ± 4.08473	30.9230 ± 4.60991	25.4578 ± 1.62722
Burst duration (s)	*S. invicta*	85.806 ± 27.8106	76.167 ± 17.5895	44.694 ± 9.1941	38.028 ± 5.2739	48.361 ± 4.0280
	S. richteri	56.361 ± 14.5622	58.694 ± 12.9898	28.500 ± 3.5606	17.361 ± 1.1843	22.357 ± 3.8857
	S. invicta × *S. richteri*	32.000 ± 6.4155	58.972 ± 9.1076	33.143 ± 3.2752	49.167 ± 14.7214	32.611 ± 4.1750
	S. geminata	60.208 ± 16.7491	76.278 ± 20.0341	32.583 ± 6.3989	29.611 ± 9.5769	29.444 ± 1.7066
Interburst duration (s)	*S. invicta*	87.569 ± 47.838	26.417 ± 4.145	44.694 ± 17.743	28.056 ± 6.636	20.722 ± 4.345
	S. richteri	36.750 ± 5.323	44.889 ± 16.088	22.083 ± 3.422	13.028 ± 1.490	13.786 ± 1.708
	S. invicta × *S. richteri*	197.000 ± 64.346	25.472 ± 5.362	15.905 ± 0.750	19.389 ± 7.321	20.972 ± 6.183
	S. geminata	28.694 ± 7.955	30.611 ± 6.103	14.417 ± 3.613	12.806 ± 2.193	12.111 ± 1.084

Table 2. Discontinuous gas exchange parameters for medium workers of *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata* at several temperatures (mean ± SE).

		Medium workers				
		10°C	15°C	20°C	25°C	30°C
Mass (mg)	*S. invicta*	2.25 ± 0.19	1.69 ± 0.18	2.27 ± 0.19	1.52 ± 0.19	1.65 ± 0.13
	S. richteri	2.70 ± 0.22	2.32 ± 0.21	1.89 ± 0.41	2.16 ± 0.13	1.89 ± 0.13
	S. invicta × *S. richteri*	2.19 ± 0.27	2.32 ± 0.22	2.16 ± 0.16	2.11 ± 0.14	1.74 ± 0.25
	S. geminata	1.71 ± 0.17	2.19 ± 0.32	2.76 ± 0.25	1.84 ± 0.26	2.84 ± 0.45
$\dot{V}CO_2$ (μl h^{-1})	*S. invicta*	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00001 ± 0.00000
	S. richteri	0.00016 ± 0.00008	0.00001 ± 0.00000	0.00001 ± 0.00000	0.24434 ± 0.24430	0.00004 ± 0.00001
	S. invicta × *S. richteri*	0.00023 ± 0.00009	0.00001 ± 0.00000	0.00002 ± 0.00001	0.00002 ± 0.00000	0.00002 ± 0.00001
	S. geminata	0.00025 ± 0.00015	0.00002 ± 0.00001	0.00002 ± 0.00000	0.00002 ± 0.00000	0.00002 ± 0.00000
Burst CO_2 (μl)	*S. invicta*	0.000013 ± 0.000004	0.000022 ± 0.000008	0.000018 ± 0.000004	0.000015 ± 0.000002	0.000009 ± 0.000001
	S. richteri	0.000144 ± 0.000089	0.000011 ± 0.000003	0.000015 ± 0.000004	0.043404 ± 0.043382	0.000019 ± 0.000004
	S. invicta × *S. richteri*	0.000326 ± 0.000157	0.000012 ± 0.000007	0.000016 ± 0.000004	0.000022 ± 0.000003	0.000016 ± 0.000003
	S. geminata	0.000252 ± 0.000169	0.000020 ± 0.000006	0.000016 ± 0.000002	0.000022 ± 0.000006	0.000049 ± 0.000035

Frequency (mHz)	*S. invicta*	5.3907 ± 1.82723	9.3679 ± 1.58351	14.0663 ± 1.50712	28.4723 ± 2.44066	37.8859 ± 1.98035
	S. richteri	11.6964 ± 2.28204	18.4915 ± 2.53744	22.8473 ± 3.46878	34.1697 ± 5.64268	37.6605 ± 2.14794
	S. invicta × *S. richteri*	14.1814 ± 4.83826	18.1983 ± 4.21485	21.3076 ± 1.50775	24.9735 ± 3.40600	31.4567 ± 1.23873
	S. geminata	16.2432 ± 2.30782	15.5518 ± 2.20909	22.8602 ± 2.83708	25.5417 ± 3.83609	30.7806 ± 1.74140
Burst duration (s)	*S. invicta*	70.278 ± 11.0759	72.028 ± 26.5652	36.750 ± 2.9198	28.444 ± 3.2196	19.222 ± 1.3975
	S. richteri	43.389 ± 7.9086	38.278 ± 6.1492	37.681 ± 12.0066	19.972 ± 3.1134	17.806 ± 1.4710
	S. invicta × *S. richteri*	79.889 ± 25.2691	61.028 ± 21.0186	33.569 ± 3.5463	32.444 ± 4.3007	22.972 ± 0.7270
	S. geminata	52.250 ± 5.0230	53.694 ± 4.6614	41.139 ± 10.4880	35.444 ± 9.0768	23.917 ± 1.5757
Interburst duration (s)	*S. invicta*	367.694 ± 100.694	82.000 ± 12.882	51.722 ± 10.042	11.292 ± 1.409	8.694 ± 1.027
	S. richteri	78.667 ± 29.794	26.472 ± 3.821	22.319 ± 5.680	19.000 ± 8.278	10.500 ± 0.776
	S. invicta × *S. richteri*	136.250 ± 81.754	38.694 ± 18.210	17.306 ± 1.609	16.000 ± 5.402	10.222 ± 0.567
	S. geminata	23.722 ± 1.906	26.889 ± 5.103	16.833 ± 5.097	15.417 ± 1.949	10.556 ± 0.636

Table 3. Discontinuous gas exchange parameters for large workers of *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata* at several temperatures (mean ± SE).

		Large workers				
		10°C	15°C	20°C	25°C	30°C
Mass (mg)	*S. invicta*	3.76 ± 0.22	3.66 ± 0.21	3.55 ± 0.13	3.09 ± 0.19	3.86 ± 0.11
	S. richteri	3.26 ± 0.12	3.85 ± 0.32	2.79 ± 0.19	3.19 ± 0.14	3.77 ± 0.38
	S. invicta × *S. richteri*	3.65 ± 0.15	4.17 ± 0.29	3.33 ± 0.16	4.08 ± 0.38	3.11 ± 0.17
	S. geminata	5.92 ± 0.45	6.74 ± 0.41	6.55 ± 0.63	5.54 ± 0.67	7.04 ± 0.29
$\dot{V}\text{CO}_2$ (µl h^{-1})	*S. invicta*	0.00001 ± 0.00001	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00003 ± 0.00001
	S. richteri	0.00040 ± 0.00017	0.00002 ± 0.00001	0.00002 ± 0.00000	0.00004 ± 0.00000	0.00005 ± 0.00001
	S. invicta × *S. richteri*	0.00583 ± 0.00415	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00003 ± 0.00001	0.00004 ± 0.00001
	S. geminata	0.00377 ± 0.00218	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00002 ± 0.00001	0.00003 ± 0.00001
Burst CO_2 (µl)	*S. invicta*	0.000022 ± 0.000008	0.000018 ± 0.000006	0.000033 ± 0.000005	0.000023 ± 0.000005	0.000033 ± 0.000006
	S. richteri	0.000212 ± 0.000109	0.000018 ± 0.000004	0.000021 ± 0.000006	0.000034 ± 0.000007	0.000030 ± 0.000003
	S. invicta × *S. richteri*	0.003178 ± 0.001800	0.000037 ± 0.000010	0.000019 ± 0.000003	0.000027 ± 0.000004	0.000044 ± 0.000018
	S. geminata	0.007561 ± 0.004718	0.000023 ± 0.000005	0.000025 ± 0.000006	0.000032 ± 0.000004	0.000030 ± 0.000006

Frequency (mHz)	*S. invicta*	7.9384 ± 3.26806	8.6447 ± 2.30357	12.8229 ± 1.73331	26.1798 ± 2.45773	29.9843 ± 2.06922
	S. richteri	19.6638 ± 5.47656	27.9564 ± 4.59800	22.8112 ± 4.03108	27.5837 ± 2.07826	29.7262 ± 3.03002
	S. invicta × *S. richteri*	10.0047 ± 4.75171	10.7301 ± 3.48647	21.3977 ± 1.33389	26.7291 ± 2.20715	25.7527 ± 2.70454
	S. geminata	8.2330 ± 2.53949	16.0580 ± 2.63795	19.3141 ± 4.67367	24.9532 ± 3.47307	26.4971 ± 3.39133
Burst duration (s)	*S. invicta*	68.167 ± 20.9552	65.417 ± 15.4752	47.278 ± 3.4707	30.111 ± 3.3705	26.917 ± 3.0827
	S. richteri	45.139 ± 19.5293	30.143 ± 6.7158	33.167 ± 6.0625	28.361 ± 5.8134	22.778 ± 2.3835
	S. invicta × *S. richteri*	48.778 ± 14.9970	82.222 ± 17.6714	36.500 ± 2.8626	28.139 ± 1.7477	32.972 ± 7.3104
	S. geminata	111.556 ± 24.5161	50.056 ± 9.8392	47.810 ± 11.1421	31.472 ± 4.7279	29.611 ± 5.0282
Interburst duration (s)	*S. invicta*	334.389 ± 140.019	99.778 ± 22.823	56.194 ± 29.057	13.611 ± 1.414	9.667 ± 0.725
	S. richteri	96.806 ± 55.821	21.786 ± 4.065	21.361 ± 2.968	13.389 ± 0.902	15.444 ± 1.288
	S. invicta × *S. richteri*	156.000 ± 56.510	123.417 ± 60.494	18.139 ± 1.113	11.639 ± 1.689	19.528 ± 6.537
	S. geminata	123.361 ± 81.755	33.556 ± 7.998	44.762 ± 18.328	16.722 ± 3.165	14.667 ± 2.606

Table 4. Discontinuous gas exchange parameters for male alates of *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata* at several temperatures (mean ± SE).

		Male alates				
		10°C	15°C	20°C	25°C	30°C
Mass (mg)	*S. invicta*	7.34 ± 0.12	7.06 ± 0.27	6.63 ± 0.34	6.75 ± 0.28	7.36 ± 0.09
	S. richteri	7.53 ± 0.39	7.07 ± 0.15	6.92 ± 0.41	7.99 ± 0.19	7.69 ± 0.49
	S. invicta × *S. richteri*	7.54 ± 0.19	7.40 ± 0.03	6.98 ± 0.47	7.60 ± 0.21	6.96 ± 0.23
	S. geminata	5.75 ± 0.22	5.38 ± 0.25	5.96 ± 0.16	6.04 ± 0.17	6.05 ± 0.20
$\dot{V}CO_2$ (µl h^{-1})	*S. invicta*	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00002 ± 0.00001
	S. richteri	0.00003 ± 0.00001	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00004 ± 0.00002	0.00003 ± 0.00001
	S. invicta × *S. richteri*	0.00061 ± 0.00034	0.00007 ± 0.00004	0.00001 ± 0.00000	0.00002 ± 0.00001	0.00003 ± 0.00002
	S. geminata	0.00006 ± 0.00001	0.00004 ± 0.00003	0.00000 ± 0.00000	0.00003 ± 0.00001	0.00001 ± 0.00000
Burst CO_2 (µl)	*S. invicta*	0.000122 ± 0.000018	0.000126 ± 0.000013	0.000124 ± 0.000011	0.000105 ± 0.000009	0.000108 ± 0.000006
	S. richteri	0.000113 ± 0.000039	0.000089 ± 0.000018	0.000143 ± 0.000015	0.000125 ± 0.000017	0.000104 ± 0.000006
	S. invicta × *S. richteri*	0.001489 ± 0.000796	0.000202 ± 0.000057	0.000171 ± 0.000026	0.000111 ± 0.000009	0.000061 ± 0.000013
	S. geminata	0.000082 ± 0.000023	0.000198 ± 0.000089	0.000126 ± 0.000009	0.000102 ± 0.000010	0.000090 ± 0.000011

Frequency (mHz)	*S. invicta*	2.3998 ± 0.25278	3.5770 ± 0.46137	5.9018 ± 0.58814	12.9022 ± 1.57451	20.6865 ± 2.02697
	S. richteri	11.2262 ± 3.47692	6.8927 ± 2.24354	4.1523 ± 0.47167	19.9263 ± 3.13722	23.2003 ± 2.57625
	S. invicta × *S. richteri*	3.6668 ± 1.40065	2.3550 ± 0.16687	5.2197 ± 0.66752	18.2624 ± 0.90731	29.0210 ± 4.52603
	S. geminata	11.0679 ± 3.59620	5.6933 ± 1.57765	6.3745 ± 1.26592	12.5850 ± 1.59220	22.6698 ± 1.13482
Burst duration (s)	*S. invicta*	120.306 ± 8.4468	92.944 ± 5.2173	65.306 ± 5.6985	43.944 ± 3.0758	30.750 ± 0.8858
	S. richteri	72.389 ± 19.3837	72.306 ± 10.4449	69.083 ± 2.3663	31.500 ± 3.8195	29.972 ± 2.2238
	S. invicta × *S. richteri*	121.806 ± 11.2801	101.639 ± 2.9591	75.139 ± 3.0814	37.500 ± 1.4561	29.111 ± 7.0995
	S. geminata	91.000 ± 20.8753	98.222 ± 5.8553	65.778 ± 4.4162	43.750 ± 2.2525	26.194 ± 1.1606
Interburst duration (s)	*S. invicta*	401.722 ± 60.946	258.917 ± 63.071	129.722 ± 20.684	51.694 ± 18.317	25.667 ± 6.784
	S. richteri	240.583 ± 124.707	145.722 ± 38.646	226.083 ± 32.315	29.833 ± 9.114	17.333 ± 2.665
	S. invicta × *S. richteri*	424.944 ± 113.942	381.778 ± 41.533	146.806 ± 27.225	24.429 ± 5.625	15.278 ± 4.831
	S. geminata	147.722 ± 60.930	361.722 ± 193.126	150.778 ± 30.832	48.611 ± 9.708	19.472 ± 1.472

Table 5. Discontinuous gas exchange parameters for female alates of *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata* at several temperatures (mean ± SE).

		Female alates				
		10°C	15°C	20°C	25°C	30°C
Mass (mg)	*S. invicta*	12.44 ± 0.56	12.76 ± 0.38	11.96 ± 0.31	12.13 ± 0.45	11.57 ± 0.29
	S. richteri	12.77 ± 0.85	13.29 ± 0.41	10.95 ± 0.31	13.91 ± 0.99	9.35 ± 0.47
	S. invicta × *S. richteri*	13.95 ± 0.25	12.79 ± 0.18	9.16 ± 0.74	14.65 ± 0.91	9.71 ± 0.34
	S. geminata	16.77 ± 1.05	13.28 ± 0.79	14.58 ± 0.15	16.29 ± 1.09	16.70 ± 0.46
$\dot{V}\mathrm{CO}_2$ (μl h^{-1})	*S. invicta*	0.00000 ± 0.00000	0.00001 ± 0.00000	0.00000 ± 0.00000	0.00002 ± 0.00001	0.00001 ± 0.00001
	S. richteri	0.00222 ± 0.00210	0.00014 ± 0.00007	0.00001 ± 0.00000	0.00005 ± 0.00002	0.00002 ± 0.00000
	S. invicta × *S. richteri*	0.00014 ± 0.00011	0.00000 ± 0.00000	0.00001 ± 0.00000	0.00001 ± 0.00000	0.00002 ± 0.00001
	S. geminata	0.00001 ± 0.00000	0.00007 ± 0.00006	0.00003 ± 0.00001	0.00005 ± 0.00001	0.00004 ± 0.00001
Burst CO_2 (μl)	*S. invicta*	0.000104 ± 0.000015	0.000141 ± 0.000014	0.000084 ± 0.000004	0.000075 ± 0.000011	0.000056 ± 0.000006
	S. richteri	0.005247 ± 0.005104	0.000310 ± 0.000091	0.000096 ± 0.000011	0.000084 ± 0.000012	0.000044 ± 0.000006
	S. invicta ×	0.000174 ±	0.000265 ±	0.000104 ±	0.000119 ±	0.000097 ±

	S. richteri	0.000049	0.000022	0.000012	0.000013	0.000008
	S. geminata	0.000071 ± 0.000012	0.000258 ± 0.000166	0.000083 ± 0.000010	0.000076 ± 0.000010	0.000100 ± 0.000007
Frequency (mHz)	*S. invicta*	2.8714 ± 0.67996	2.8958 ± 0.62516	6.1981 ± 0.63912	16.6506 ± 3.54438	28.1359 ± 1.35168
	S. richteri	7.6434 ± 3.71599	3.1289 ± 0.43659	8.0732 ± 1.16623	21.0108 ± 3.97018	29.6893 ± 3.64523
	S. invicta × *S. richteri*	3.9489 ± 1.54471	1.8117 ± 0.19752	6.7857 ± 0.87455	13.3212 ± 2.01768	21.7174 ± 1.29429
	S. geminata	8.2973 ± 1.24171	7.2169 ± 2.76470	15.5719 ± 2.16559	26.9221 ± 3.67668	24.6635 ± 1.63924
Burst duration (s)	*S. invicta*	132.139 ± 19.7218	96.278 ± 9.6268	61.750 ± 4.9010	42.722 ± 3.4404	28.611 ± 0.9105
	S. richteri	86.750 ± 22.7538	100.306 ± 5.7915	59.694 ± 1.5254	30.238 ± 2.8963	23.583 ± 2.2786
	S. invicta × *S. richteri*	120.306 ± 18.6456	125.167 ± 7.7265	72.222 ± 3.5774	48.333 ± 4.6001	33.639 ± 1.6355
	S. geminata	96.167 ± 12.3328	109.595 ± 19.2781	50.556 ± 8.6450	27.389 ± 3.0486	29.056 ± 1.8103
Interburst duration (s)	*S. invicta*	568.167 ± 161.700	393.500 ± 55.407	116.750 ± 18.359	35.750 ± 10.072	8.639 ± 1.032
	S. richteri	362.806 ± 134.031	297.639 ± 48.000	85.056 ± 17.398	27.833 ± 5.465	13.611 ± 1.749
	S. invicta × *S. richteri*	475.722 ± 170.803	564.889 ± 67.127	99.528 ± 17.571	41.306 ± 12.011	15.667 ± 1.586

S. geminata	139.833 ± 53.222	308.476 ± 120.750	31.111 ± 8.441	18.444 ± 5.579	14.000 ± 0.953

Figure Legend

Figure 1: Representative real-time CO_2 traces illustrating the presence of the DGC in small workers of *S. invicta* (RIFA), *S. richteri* (BIFA), *S. invicta* × *S. richteri* (Hybrid), and *S. geminata* (Geminata).

Figure 2: Representative real-time CO_2 traces illustrating the presence of the DGC in medium workers of *S. invicta* (RIFA), *S. richteri* (BIFA), *S. invicta* × *S. richteri* (Hybrid), and *S. geminata* (Geminata).

Figure 3: Representative real-time CO_2 traces illustrating the presence of the DGC in large workers of *S. invicta* (RIFA), *S. richteri* (BIFA), *S. invicta* × *S. richteri* (Hybrid), and *S. geminata* (Geminata).

Figure 4: Representative real-time CO_2 traces illustrating the presence of the DGC in male alates of *S. invicta* (RIFA), *S. richteri* (BIFA), *S. invicta* × *S. richteri* (Hybrid), and *S. geminata* (Geminata).

Figure 5: Representative real-time CO_2 traces illustrating the presence of the DGC in female alates of *S. invicta* (RIFA), *S. richteri* (BIFA), *S. invicta* × *S. richteri* (Hybrid), and *S. geminata* (Geminata).

Figure 1

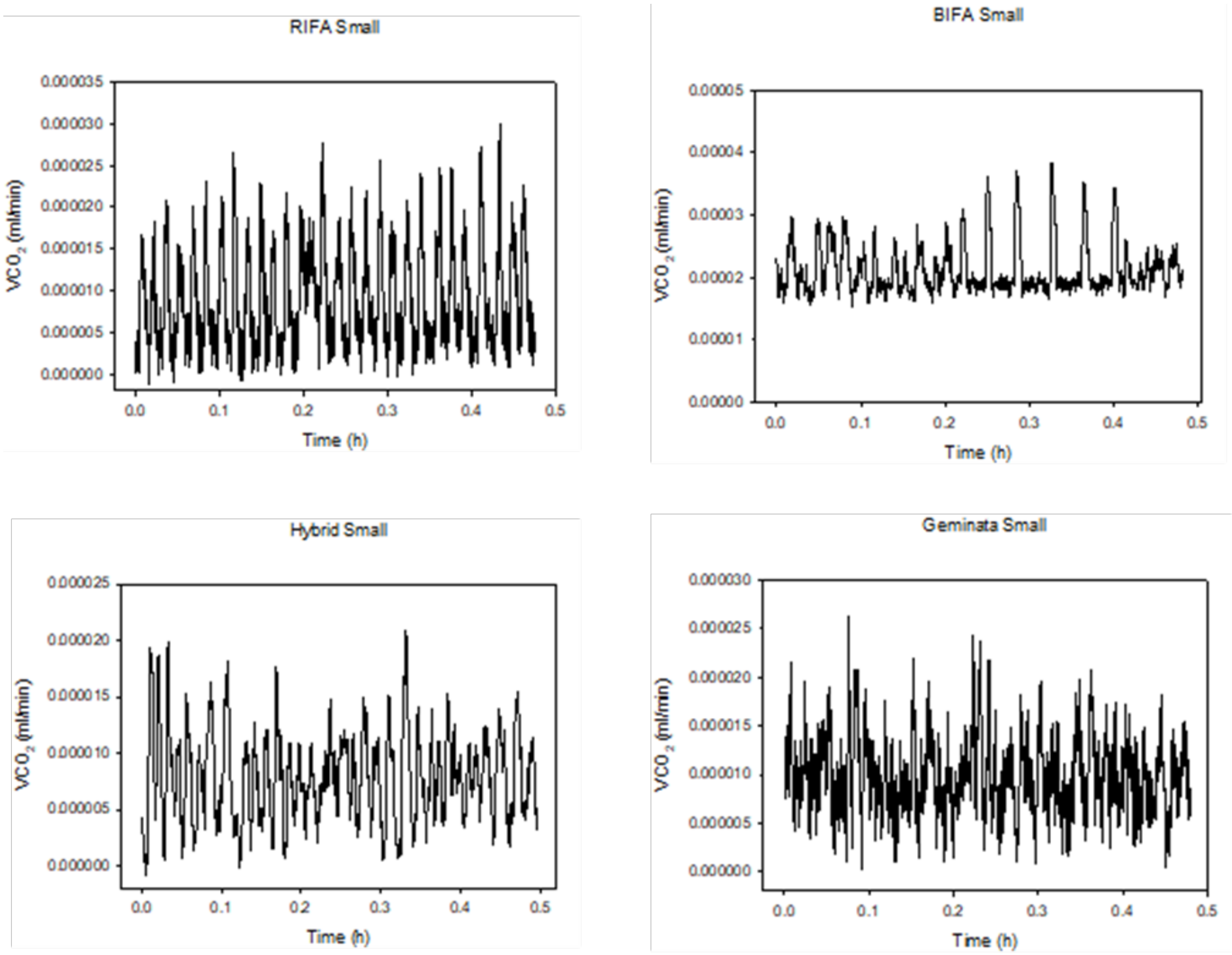

RIFA Small
VCO$_2$ (ml/min)
0.000035
0.000030
0.000025
0.000020
0.000015
0.000010
0.000005
0.000000
0.0
0.1
0.2
0.3
0.4
0.5
Time (h)
BIFA Small
VCO$_2$ (ml/min)
0.00005
0.00004
0.00003
0.00002
0.00001
0.00000
0.0
0.1
0.2
0.3
0.4
0.5
Time (h)
Hybrid Small
VCO$_2$ (ml/min)
0.000025
0.000020
0.000015
0.000010
0.000005
0.000000
0.0
0.1
0.2
0.3
0.4
0.5
Time (h)
Geminata Small
VCO$_2$ (ml/min)
0.000030
0.000025
0.000020
0.000015
0.000010
0.000005
0.000000
0.0
0.1
0.2
0.3
0.4
0.5
Time (h)

Figure 2

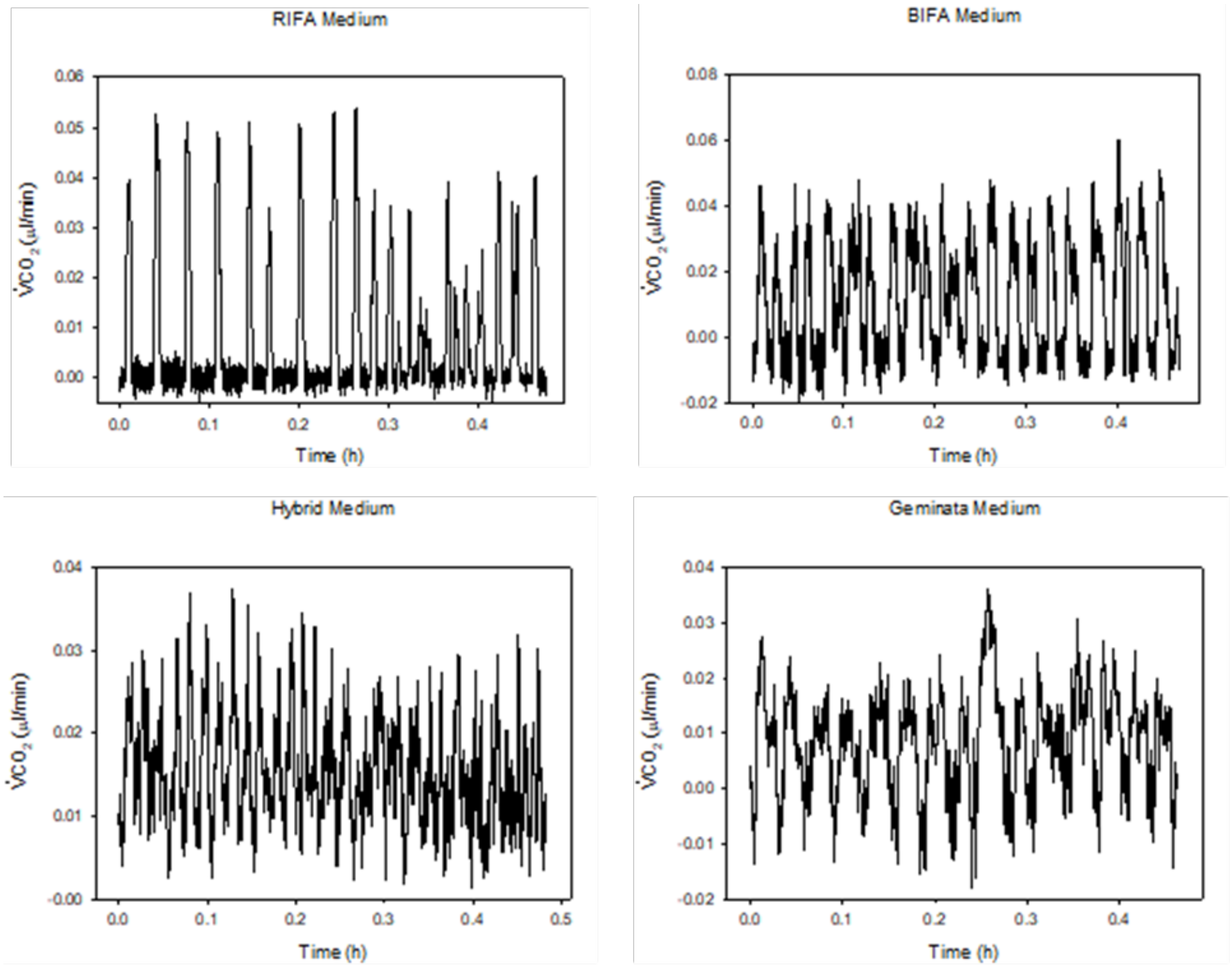

RIFA Medium
0.06
0.05
0.04
0.03
0.02
0.01
0.00
$\dot{V}CO_2$ (μl/min)
0.0
0.1
0.2
0.3
0.4
Time (h)
BIFA Medium
0.08
0.06
0.04
0.02
0.00
-0.02
$\dot{V}CO_2$ (μl/min)
0.0
0.1
0.2
0.3
0.4
Time (h)
Hybrid Medium
0.04
0.03
0.02
0.01
-0.00
$\dot{V}CO_2$ (μl/min)
0.0
0.1
0.2
0.3
0.4
0.5
Time (h)
Geminata Medium
0.04
0.03
0.02
0.01
0.00
-0.01
-0.02
$\dot{V}CO_2$ (μl/min)
0.0
0.1
0.2
0.3
0.4
Time (h)

Figure 3

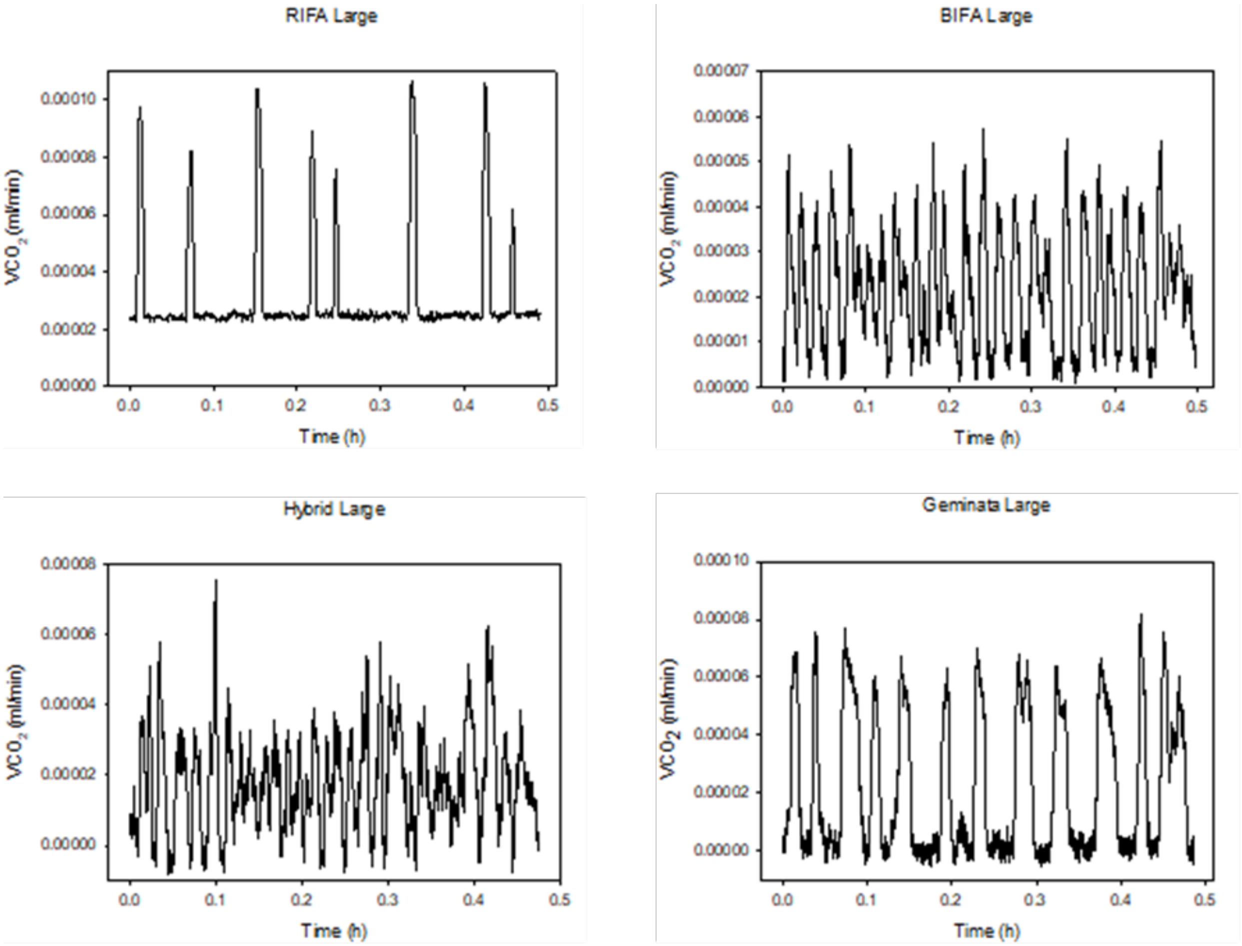
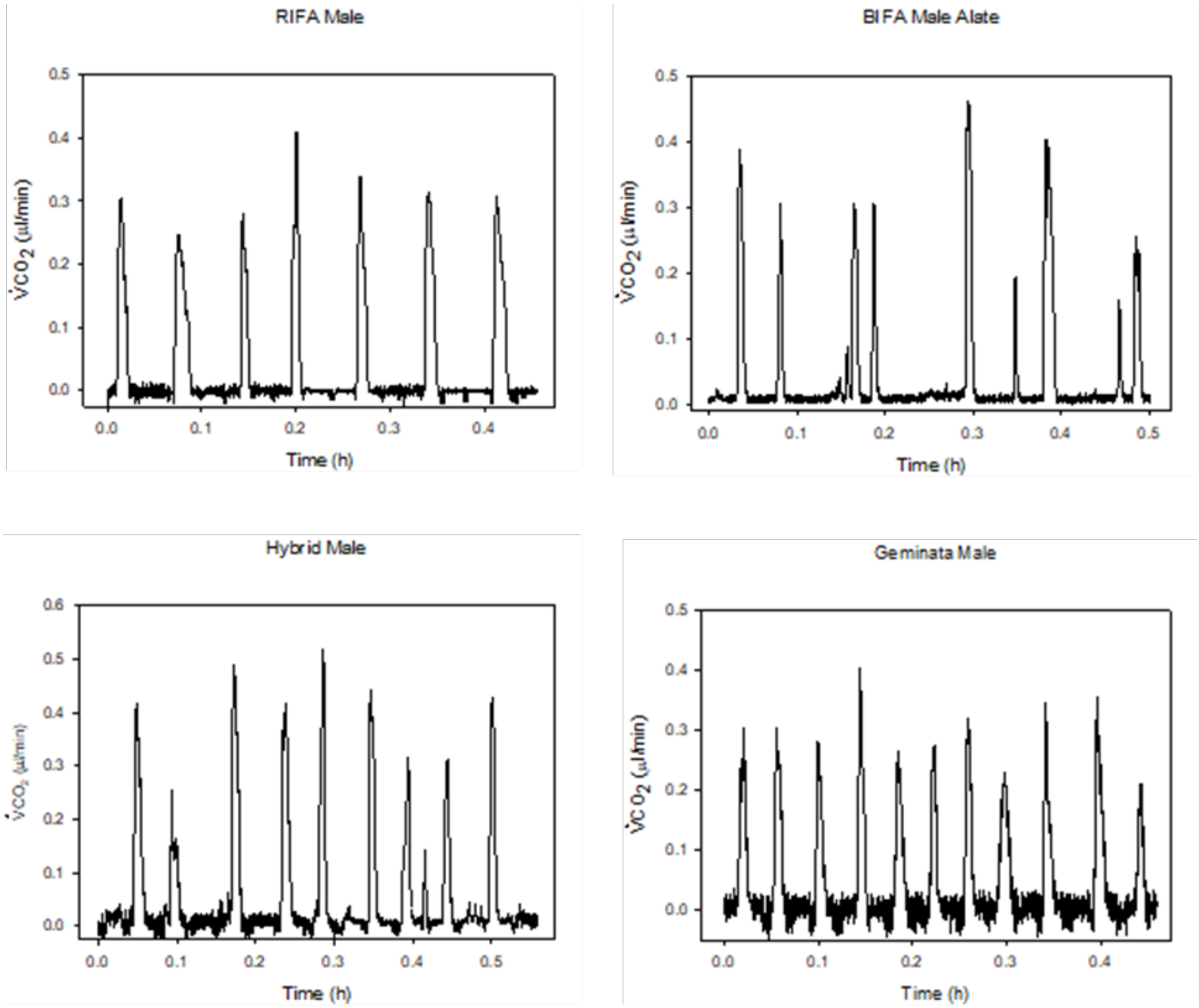
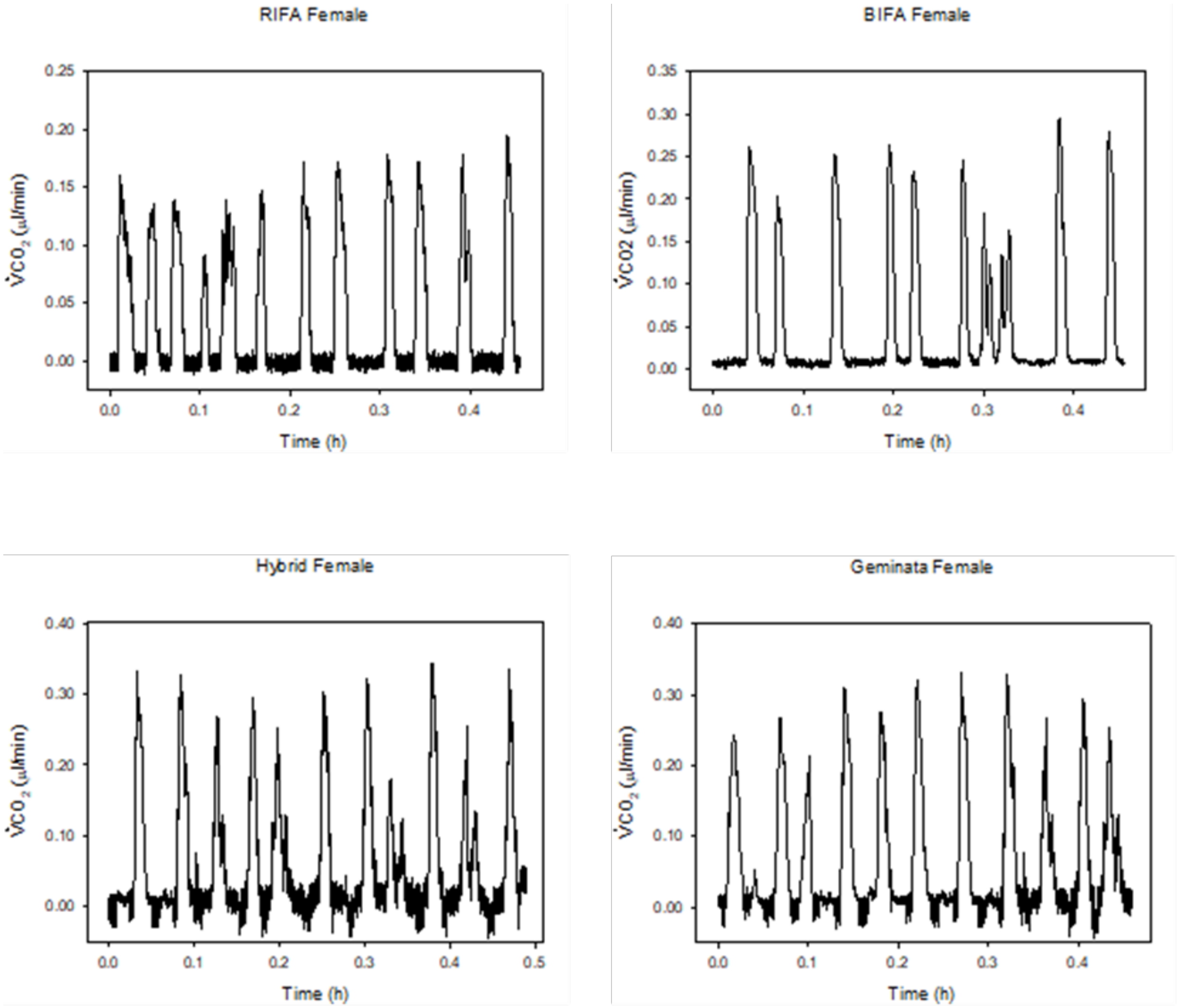

RIFA Large
VCO2 (ml/min)
0.00000
0.00002
0.00004
0.00006
0.00008
0.00010
Time (h)
0.0
0.1
0.2
0.3
0.4
0.5
BIFA Large
VCO2 (ml/min)
0.00000
0.00001
0.00002
0.00003
0.00004
0.00005
0.00006
0.00007
Time (h)
Hybrid Large
VCO2 (ml/min)
0.00000
0.00002
0.00004
0.00006
0.00008
Time (h)
Geminata Large
VCO2 (ml/min)
0.00000
0.00002
0.00004
0.00006
0.00008
0.00010
Time (h)

Figure 4



RIFA Male
$\dot{V}CO_2$ (µl/min)
0.0
0.1
0.2
0.3
0.4
0.5
Time (h)
0.0
0.1
0.2
0.3
0.4
BIFA Male Alate
$\dot{V}CO_2$ (µl/min)
0.0
0.1
0.2
0.3
0.4
0.5
Time (h)
0.0
0.1
0.2
0.3
0.4
0.5
Hybrid Male
$\dot{V}CO_2$ (µl/min)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
Time (h)
0.0
0.1
0.2
0.3
0.4
0.5
Geminata Male
$\dot{V}CO_2$ (µl/min)
0.0
0.1
0.2
0.3
0.4
0.5
Time (h)
0.0
0.1
0.2
0.3
0.4

Figure 5


RIFA Female
0.25
0.20
0.15
0.10
0.05
0.00
V̇CO2 (μl/min)
0.0
0.1
0.2
0.3
0.4
Time (h)
BIFA Female
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
V̇CO2 (μl/min)
0.0
0.1
0.2
0.3
0.4
Time (h)
Hybrid Female
0.40
0.30
0.20
0.10
0.00
V̇CO2 (μl/min)
0.0
0.1
0.2
0.3
0.4
0.5
Time (h)
Geminata Female
0.40
0.30
0.20
0.10
0.00
V̇CO2 (μl/min)
0.0
0.1
0.2
0.3
0.4
Time (h)

CHAPTER 4

HOST PREFERENCE IN PARASITIC PHORID FLIES: RESPONSE OF *Pseudacteon curvatus* AND *P. obtusus* TO VENOM ALKALOIDS OF NATIVE AND IMPORTED *Solenopsis* FIRE ANTS

4.1 Introduction

Host preference is a critical component of parasitoid fitness (Morris and Fellowes 2002). The term, host preference, as used here is a behavioral response mediated by a particular stimulus or stimuli leading to discrimination among suitable hosts of the same species. Host preference has been defined as the hierarchical ordering of different kinds of host by foraging females (Thompson 1988; Mackauer et al. 1996; Storeck et al. 2000). Host preference of a foraging female parasitoid will influence the life-history characteristics of her offspring (Morris and Fellowes 2002). The degree of host preference in a parasitoid species can influence the attack rate on the host, foraging activity of the host, and suppression of host population (Sait et al. 1997). Several studies have reported the benefits and role of host preference in parasitoid fitness (Morris and Fellowes 2002; Sait et al. 1997). However, detailed mechanisms underlying host preference in several parasitoid species are unclear (Sasakawa et al. 2016). Factors influencing adult traits include learning, natal host and genes. These factors play a role in determining host preference (Morris and Fellowes 2002; Hunt et al. 2018).

Successful parasitism in parasitoids involves a number of processes and steps, including host location, host discrimination, and host acceptance (Mathis and Philpott 2012). Parasitoids may utilize semiochemicals to discriminate suitable hosts from less preferred or unsuitable hosts. Semiochemicals can comprise of only a very specific chemical or a blend of major and minor components (Tumlinson 1988). A minor blend component can sometimes have profound effects on parasitoid host preference (Reisenman et al. 2016; Morawo et al. 2017). As the coevolution between hosts and parasitoids get tighter, hosts have developed resistance against parasitization by parasitoids (Kraaijeveld and Godfray 2008). Such resistance includes avoidance of the searching parasitoid, passive defense, and active defense mechanisms (Godfray 1994). Host-parasitoid interactions including host preference and host resistance may have major ecological impacts. For instance, they could contribute to the development of dietary breadth, as host preference may prevent the utilization of potential resources. Little is known of the specific features critical for parasitoid foraging behavior, including host preference (Lewis et al. 1990). Thus, it is critical to seek some insight into the degree of host preference of parasitoids in order to fully appreciate the magnitude of ecosystem services each parasitoid provides. Such studies are central to understanding both the selective impact of hosts on parasitoids as well as the potential for reciprocal selective impact of parasitoids on host traits.

Solenopsis fire ants (Hymenoptera: Formicidae) are a group of about twenty New World species (Trager 1991). Two species, *S. richteri* and *S. invicta*, were accidentally imported from their native region in South America into the United States (US) through Mobile, Alabama around 1918 and 1935, respectively (Lennartz 1973). They are pests of medical and agricultural importance. Their impact as pests is compounded by high fecundity in queens and large colony size. For instance, a *S. invicta* queen in a large colony lays an egg or two every minute, around the clock (Tshinkel 2006). Also, several species of *Solenopsis* including *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri* and *S. geminata* occur in two greatly different social forms, one with a single functional queen in each colony (monogyne), and the other with multiple queens (polygyne), an attribute that impacts their reproductive rates (Ross et al. 1988, 1996a, 1996b). Mature colonies of *Solenopsis* spp. contain hundreds of thousands of workers in the nest. For instance, *S. invicta* colony contains up to 220,000 workers (Markin et al. 1973; Tschinkel 1988b; 1993a).

Pseudacteon phorid flies (Diptera: Phoridae) are parasitoids introduced from their native South American range into the US as classical biological control agents of imported *Solenopsis* fire ants. At least 22 species of *Pseudacteon* flies oviposit in worker fire ants of the *Solenopsis saevissima* complex, which includes *S. invicta* and *S. richteri* (Folgarait et al. 2007b; Chen and Fadamiro 2018). Whereas at least 4 species of *Pseudacteon* flies oviposit in *S. geminata* workers, which belong to the *Solenopsis geminata* complex (Morrison et al. 1999) but none of the four species attack *S. invicta* (Feener 1987;

Disney 1991; Chen and Fadamiro 2018), suggesting host partitioning among *Pseudacteon* flies. *Pseudacteon curvatus* was first released in parts of the southeastern US in 1999 and has since become well established in several parts of the region (Graham et al. 2003). *Pseudacteon obtusus* was first released in 2006 in Texas, and subsequently in other parts of the southeastern US (Plowes et al. 2011).

Pseudacteon phorid flies are attracted to fire ants when the ants are disturbed and release a mixture of compounds including venom alkaloids used primarily for defense, and alarm pheromone to alert conspecifics of danger (Vander Meer and Porter 2002). Thus, the flies are thought to utilize alarm pheromone as a kairomone to locate the ants (Sharma et al. 2011). A component of the alarm pheromone of *S. invicta* was recently identified as 2-ethyl-3,6-dimethyl pyrazine (Vander Meer et al. 2010), hereafter referred to as alarm pheromone. This alarm pheromone has been reported to play a role in mediating attraction of phorid flies to host fire ant workers (Sharma et al. 2011). Similarly, two (*cis* and *trans*) venom alkaloid fractions of *S. invicta* have been reported to act as key attractants for the phorid fly, *P. tricuspis* (Chen et al. 2009). The venom consists of a complex mixture of 2-methyl-6-alkylpiperidines (Brand et al. 1973; MacConnell et al. 1976; Blum et al. 1992), and several other compounds such as 2,6-dialkyl-$\Delta^{1,2}$-piperideines and 2,6-dialkyl-$\Delta^{1,6}$-piperideines (Chen and Fadamiro 2009a,b). The relative proportions of these alkaloids in the venom may differ qualitatively between *Solenopsis* fire ant species (Brand et al. 1973; Deslippe and Guo 2000; Yu et al. 2014), suggesting that venom alkaloids may provide host-specific cues to *Pseudacteon* phorid flies (Chen et al. 2009).

Solenopsis fire ants use a distinctive defense posture to defend themselves against phorid parasitoids hovering over recruitment trails or nest sites (Feener 1987). When it finds an appropriate ant, the female fly injects an egg in mid-flight into the thorax of the worker ant (Folgarait et al. 2005). Thus, visual and short-range olfactory chemical cues are used by phorid flies to locate and inspect potential hosts (Mathis et al. 2011; Mathis and Philpott 2012) and for host preference. It is obvious that the visual and chemical cues can only be successfully utilized under the right and appropriate conditions including enough content of chemical blend components and sufficient lighting to detect appropriate size of host. The choice of the

Pseudacteon parasitoids is expected to be dependent on the quality and quantity of the host semiochemicals. While the volatile alarm pheromone of *Solenopsis* spp. is thought to be the long-range stimulus that attracts *Pseudacteon* parasitoids, other host semiochemicals may be used for short-range attraction. These additional cues could play a role in enabling some parasitoid species to discriminate among hosts or locate specific oviposition sites. The innate tendency to oviposit on particular host taxa is thought to be based on the differential responses of *Pseudacteon* parasitoids to host semiochemicals. Venom alkaloids and alarm pheromone (2-ethyl-3,5 (or 6)-dimethyl pyrazine) of *S. invicta* were demonstrated to act in concert to attract *P. tricuspis* (Sharma and Fadamiro 2013). As alarm pheromone component is shared by both native and imported fire ants (Hu et al. 2018), species-specific venom alkaloids are expected to be used for host preference. Nevertheless, little information is available on the influence of the differences among venom alkaloid profiles of fire ants in mediating host preference in phorid flies.

The objective of this study was to determine the possible role of venom alkaloids of imported fire ant species *S. invicta* and *S. richteri* (both in *S. saevissima* complex), and the native species *S. geminata* (*S. geminata* complex) in mediating host preference in *P. curvatus* and *P. obtusus*. It is hypothesized that differences in the venom alkaloid profiles of fire ant species mediate host preference in phorid flies for imported versus native fire ant hosts.

4.2 Materials and Methods

4.2.1 Insects. Female *P. curvatus* (Formosa biotype from Argentina) and *P. obtusus* (Formosa biotype from Argentina) flies used in this study were reared at USDA-APHIS rearing facility in Gainesville, FL on imported *S. invicta* fire ants. Originally, *P. curvatus* were collected from El Toro Ranch east of Las Flores, Buenos Aires Province, Argentina in 1997 (Porter 2000; Graham et al 2003). *Solenopsis richteri* fire ants used for extraction of venom alkaloids were collected from southwest Tennessee and north Mississippi. *Solenopsis invicta* and *S. geminata* fire ants were collected from Alabama and Florida, respectively.

4.2.2 Test Chemicals. Two types of chemical stimuli were evaluated in this study: alarm pheromone of *S. invicta*, and venom alkaloids of *S. invicta*, *S. richteri* and *S. geminata*. The alarm pheromone component of *S. invicta*, 2-ethyl-3,6-dimethyl pyrazine is commercially available as 2-ethyl-3,6(or 5)-dimethyl pyrazine, which is a mixture of 2-ethyl-3,6-dimethyl pyrazine (40%) and its isomer, 2-ethyl-3,5-dimethyl pyrazine (60%). The alarm pheromone isomer, 2-ethyl-3,6(or 5)-dimethyl pyrazine (<95%) was previously shown to be attractive to *P. tricuspis* (Sharma et al. 2011). The isomer was purchased from Sigma® Chemical Co. (St. Louis, MO) and tested as the fire ant alarm pheromone. Solutions of this compound were prepared in HPLC grade hexanes and stored at -20 °C until use. Venom alkaloid fractions (*cis* and *trans*) of *S. invicta*, *S. richteri* and *S. geminata* workers were extracted, isolated and identified as described by Chen and Fadamiro (2009a, b) and Chen et al. (2009). One worker ant equivalent (WE) of the venom alkaloids (*cis*, *trans*, or a 1:1 mixture of *cis* + *trans*) was evaluated, based on previous studies (Chen et al. 2009; Sharma and Fadamiro 2013).

4.2.3 Behavioral Bioassays. The responses of *P. obtusus* and *P. curvatus* females to *S. geminata*, *S. invicta*, and *S. richteri* venom alkaloid fractions (5 µl *cis* + 5 µl *trans*) were evaluated in a series of four-choice olfactometer bioassays. The olfactometer apparatus and procedures were as previously described by Chen et al. (2009) with minor modifications. Briefly, the apparatus consisted of a central chamber (20 cm long × 20 cm wide × 20 cm high) connected to four cylindrical glass jars or "arms" (19 cm long × 11 cm wide). The orifices of the olfactometer were connected through Teflon-glass tube connectors to four pumps on an air delivery system equipped with a vacuum pump (ARS, Inc., Gainesville, FL). Purified air was pushed at a constant rate of 300 ml/min through each of the four arms and removed by suction via the vacuum pump through the central orifice at the rate of 1,300 ml/min. The apparatus was positioned under a fluorescent light source (~100 lux) for uniform lighting.

The two venom alkaloid fractions were compared with a negative control (hexane) and positive control (alarm pheromone component) in four separate experiments at 10 µg dosage. This dosage was chosen because it was found to trigger significant response to alarm pheromone component in *P. tricuspis*

(Sharma et al. 2011). Each treatment was delivered as 10-µl sample impregnated on filter paper strips (1 × 1 cm, Whatman$^{®}$ no, 1). The venom alkaloids were tested at a dose equivalent to one fire ant worker equivalent (1 WE) (as determined by Sharma and Fadamiro 2013) and compared with a solvent control (hexane) and a standard or positive control (alarm pheromone component). After allowing for solvent evaporation, (~ 15 s), the filter paper strip was inserted into its designated olfactometer arm. Groups of female phorid flies (1-day old), each group comprised of 20 individual flies of the same species, were released into the central chamber from the top. The flies were observed continuously for 15 min, and those found in each arm were counted and removed. Flies that did not choose one of the four arms within 15 min were considered as "non-responders" and not included in the analysis. After each test, the olfactometer was cleaned with hexane and acetone and the arms were rotated (90°) to minimize positional effect. All experiments were conducted at 25 ± 1 °C, 50-60% RH, and between 12:00 h and 16:00 h, the time of day for high phorid fly activity (Pesquero et al. 1996). For each experiment, 20 phorid flies were released per replicate. The experiments were replicated seven times using new flies (i.e. an individual fly was tested only once).

The responses of female *P. curvatus* and *P. obtusus* to the venom alkaloid fractions (*cis* or *trans*) of *S. geminata*, *S. invicta*, and *S. richteri* and a 1:1 mixture of both fractions were evaluated in a series of four-choice olfactometer bioassays. Each *Pseudacteon* species was evaluated in separate tests for a total of 4 experiments. The first experiment tested the response of *P. obtusus* to *cis* + *trans* alkaloids of *S. invicta* and *S. geminata*. The second experiment tested the response of *P. obtusus* to *cis* + *trans* alkaloids of *S. richteri* and *S. geminata*. The third experiment tested the response of *P. curvatus* to *cis* + *trans* alkaloids of *S. invicta* and *S. geminata*. The fourth experiment tested the response of *P. curvatus* to *cis* + *trans* alkaloids of *S. richteri* and *S. geminata*. The aim was to determine the preference of each phorid fly species for native versus imported worker fire ant using their venom alkaloids.

4.2.4 Data Analyses. Olfactometer data were analyzed using analysis of variance (ANOVA) followed by Tukey–Kramer HSD comparison test ($P < 0.05$; JMP$^{®}$ Pro 12.0.1, SAS Institute Inc., Cary, NC) to establish significant differences among the different treatments evaluated in each experiment.

4.3 Results

Significantly more ($P < 0.001$) female *P. obtusus* chose the venom alkaloids (*cis* + *trans* alkaloids) of imported *S. invicta* fire ant than the venom alkaloids of native *S. geminata* fire ant in the four-choice olfactometer (F= 212.16, df= 3) (Fig. 1). Similarly, significantly more ($P < 0.001$) female *P. obtusus* chose the venom alkaloids of imported *S. richteri* than the venom alkaloids of native *S. geminata* (F= 77.46, df= 3) (Fig. 2). Female *P. obtusus* that had emerged from decapitated *S. invicta* fire ant heads showed a significant preference for *S. invicta* as well as for *S. richteri* fire ants on which they were not reared, compared to native *S. geminata*

Similar results were obtained for female *P. curvatus*, which showed significant a significant behavioral response to the venom alkaloids (*cis* + *trans* alkaloid) of imported *S. invicta* fire ants than that of native *S. geminata* fire ant (F= 57.67, df= 3, $P < 0.001$) (Fig. 3). Also, a significantly higher number of female *P. curvatus* were attracted to the venom alkaloids of imported *S. richteri* compared to native *S. geminata* (F= 40.03, df= 3, P< 0.001) (Fig. 4). Female *P. curvatus* parasitoids that had emerged from decapitated *S. invicta* fire ant heads showed a significant preference for *S. invicta*, as well as for *S. richteri* fire ants on which they were not reared, compared to native *S. geminata*.

Only in one of the four experiments (Fig. 3) did the parasitoid show a significantly higher attraction to the alarm pheromone component than to the venom alkaloids (*cis* + *trans*) of the *Solenopsis* species. In that experiment, significantly more female *P. curvatus* were attracted to the alarm pheromone component than to the venom alkaloids of *S. invicta* (Fig. 3). In the other three experiments, there were no significant differences in parasitoid attraction to the alarm pheromone component versus the venom alkaloids, although the alarm pheromone component attracted numerically high numbers of parasitoids than the venom alkaloids (Figs. 1, 2 and 4).

4.4 Discussion

Our results from the behavioral experiments provide evidence for the involvement of venom alkaloids in mediating host preference of *Pseudacteon* species between native and imported *Solenopsis* fire ants. In the olfactometer bioassays, both *P. curvatus* and *P. obtusus* exhibited stronger attraction to *cis* + *trans* venom alkaloids of imported *S. invicta* and *S. richteri* fire ants over those of native fire ants. This is in agreement with predictions by Chen et al. (2009) that the venom alkaloids of native *S. geminata* will not elicit significant behavioral response in *Pseudacteon* spp. This probably explains how *Pseudacteon* species associated with either the *S. geminata* or *S. saevissima* complexes have not switched to the other group despite host and parasitoid overlap in some areas of their distributions (Fowler et al. 1995, Porter et al. 1995a, Patrock et al. 2009). Altogether, the results indicate that fire ants venom alkaloids are involved in host preference in parasitic phorid flies. However, host preference and performance of a parasitoid are not always tightly linked (Kaser et al. 2018).

Female *P. curvatus* and *P. obtusus* tested in this study had no adult foraging experience and these species seems to possess an innate response to fire ant alarm pheromone. It has been demonstrated that some ant parasitoids show attraction based on host-body semiochemical cues (Chen and Fadamiro 2007; Chen et al. 2009; Sharma et al. 2011). Preference for host *Solenopsis* spp. on which the parasitoids had been reared was expressed in this experiment. This phenomenon can be explained by the 'chemical legacy hypothesis' (Corbet 1985), and has been demonstrated previously for Hymenopteran parasitoids such as *Microplitis demoitor* Wilkinson, developing in *Helicoverpa zea* (Boddie) larvae; the leafminer parasitoid *Opius dissitus* Muersebeck; and for *Aphidius colemani* Viereck (Hérard et al. 1988; Turlings et al. 1993). In addition, Storeck et al. (2000) proposed the term 'emergence conditioning', a process in which emerging parasitoid apparently becomes 'conditioned' to respond to the first chemical information that it encounters after emergence. In case of *Pseudacteon* species, the first chemical encounter is usually that provided by contact with the decapitated fire ant head from which it emerged. He and Fadamiro (2009) investigated the attack rate of *P. tricuspis* and *P. curvatus* on *S. invicta*, *S. richteri*, and *S. invicta* × *S. richteri*, and reported 'chemical legacy hypothesis' as playing a role in the preference of both *P. tricuspis* and *P*. *curvatus* for *S*. *invicta*. The strength of this conditioning process was emphasized in our study when *P*. *curvatus* and *P*. *obtusus* expressed a preference for semiochemicals of *S*. *invicta* on which they were reared, over those of the native fire ant *S*. *geminata* on which they were not reared. However, even though emergence conditioning can establish initial host preferences, these preferences can rapidly change as a result of subsequent foraging experiences (Storeck et al. 2000). In our experiments, when parasitoids that had been reared on *S*. *invicta* were given a choice between semiochemicals of *S*. *richteri* and *S*. *geminata*, they expressed preference for *S*. *richteri* over *S*. *geminata*, even though they were not reared on *S*. *richteri*. This probably demonstrates the influence of host specificity of the parasitoids to a specific subcomplex within the genus *Solenopsis* (Fowler et al. 1995; Porter et al. 1995a; Patrock et al. 2009). Further tests will be necessary to determine if adult learning in these parasitoids would change their preferences for *S*. *invicta*. The strong responses evoked in *Pseudacteon* parasitoids by the venom alkaloids of fires ants in the *S*. *saevissima* complex over those in the *S*. *geminata* complex could be due to the reliability of such host-derived cues as indicators of host presence, thus evoking strong innate responses that are not easily modified by adult experience (Vet et al. 1995; Storeck et al. 2000). Kairomones, which elicit attack responses by *P*. *tricuspis*, have been shown to be present in the thorax of host *S*. *invicta* (Chen and Fadamiro 2007). Extracts of *S*. *invicta* worker body and thorax elicited strong olfactometer and electroantennogram responses in female *P*. *tricuspis* (Chen and Fadamiro 2007), so it is possible that these kairomones could still be detectable on the decapitated host heads at the time of parasitoid emergence and therefore could be involved in the emergence conditioning and host preference.

It is interesting that in our experiments, the alarm pheromone component, derived from *S*. *invicta*, elicited parasitoid attraction as many as those by *cis* + *trans* venom alkaloids of *Solenopsis* species. It is possible that this alarm pheromone component is typical of all *Solenopsis* species tested in this study and is released in greater concentration than venom alkaloids within and across the *Solenopsis* spp. tested in this study. Sharma et al. (2011) demonstrated strong attraction of *P*. *tricuspis* to this alarm pheromone component in both olfactometer and electroantennogram experiments. Our results support previous reports that fire ant alarm pheromone is used for host location by parasitoids of the ants (Vander Meer and Porter 2002, Morrison and King 2004, Morrison and Porter 2006), and are in agreement with previous reports of the role of alarm pheromones of ants a as kairomones for their natural enemies (Feener et al. 1996; Schönrogge et al. 2008; Witte et al. 2010). Sharma et al. (2011) proposed that this alarm pheromone component and venom alkaloids of fire ants act in tandem to attract phorid flies to fire ant workers, and alarm pheromones are utilized as long-range host location cues while venom alkaloids as medium-/short-range host location cues by *Pseudacteon* species. Our results seem to support this proposal as both semiochemicals attract *Pseudacteon* species. However, additional studies are needed to test this proposal regarding the individual and combined roles of fire ant alarm pheromone s and venom alkaloids in mediating attraction in *Pseudacteon* phorid flies.

In conclusion, we demonstrated the attraction of *P. curvatus* and *P. obtusus*, two parasitoids of *Solenopsis* fire ants, to *cis* and *trans* venom alkaloid fractions of imported fire ants over native fire ants. Our results suggest that host preference in *P. curvatus* and *P. obtusus* is mediated, at least in part, by differences in the venom alkaloid profile of various fire ant species. This finding may explain why *Pseudacteon* species associated with either the *S. geminata* or *S. saevissima* complexes have not switched to the other group despite the overlap of native and imported fire ants in some areas of their distributions. We propose that *cis* alkaloids probably play a synergistic role in enhancing the activity of *trans* alkaloids in mediating host preference in *Pseudacteon* phorid flies. To our knowledge, this is the first report which demonstrated that behavioral preference of *P. obtusus* and *P. curvatus* for imported compared to native *Solenopsis* species of fire ants is mediated by differences in the venom alkaloid profiles of imported versus native *Solenopsis* fire ants. Future studies will test this hypothesis in the field.

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Figure Legend

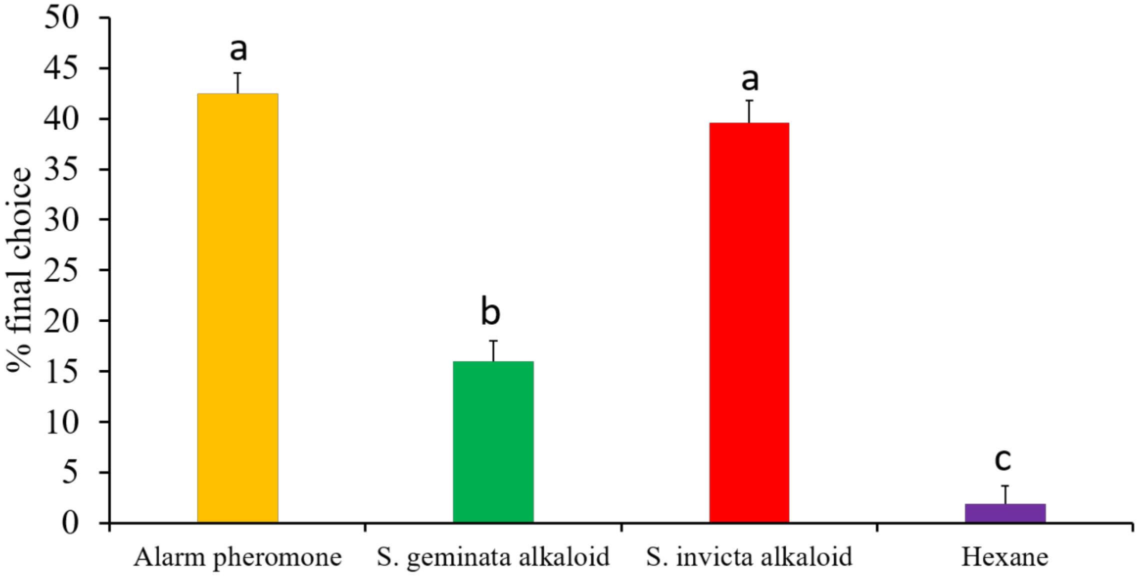
Figure 1: Behavioral response of *Pseudacteon obtusus* to venom alkaloids of imported *S. invicta* and native *S. geminata* fire ants. Means having no letter in common are significantly different ($P < 0.05$).

Figure 2: Behavioral response of *Pseudacteon obtusus* to venom alkaloids of imported *S. richteri* and native *S. geminata* fire ants. Means having no letter in common are significantly different ($P < 0.05$).

Figure 3: Behavioral response of *Pseudacteon curvatus* to venom alkaloids of imported *S. invicta* and native *S. geminata* fire ants. Means having no letter in common are significantly different ($P < 0.05$).

Figure 4: Behavioral response of *Pseudacteon curvatus* to venom alkaloids of imported *S. richteri* and native *S. geminata* fire ants. Means having no letter in common are significantly different ($P < 0.05$).

Figure 1



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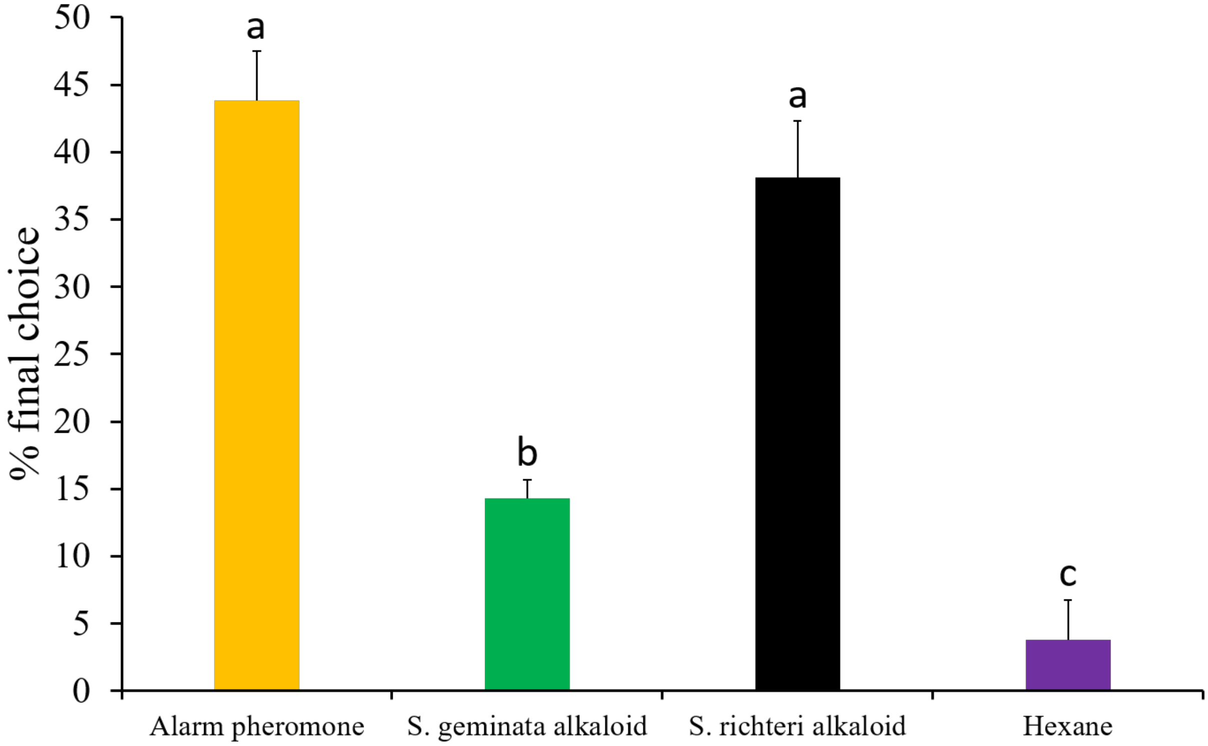
S. geminata alkaloid
S. invicta alkaloid
Hexane

Figure 2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Hexane

Figure 3

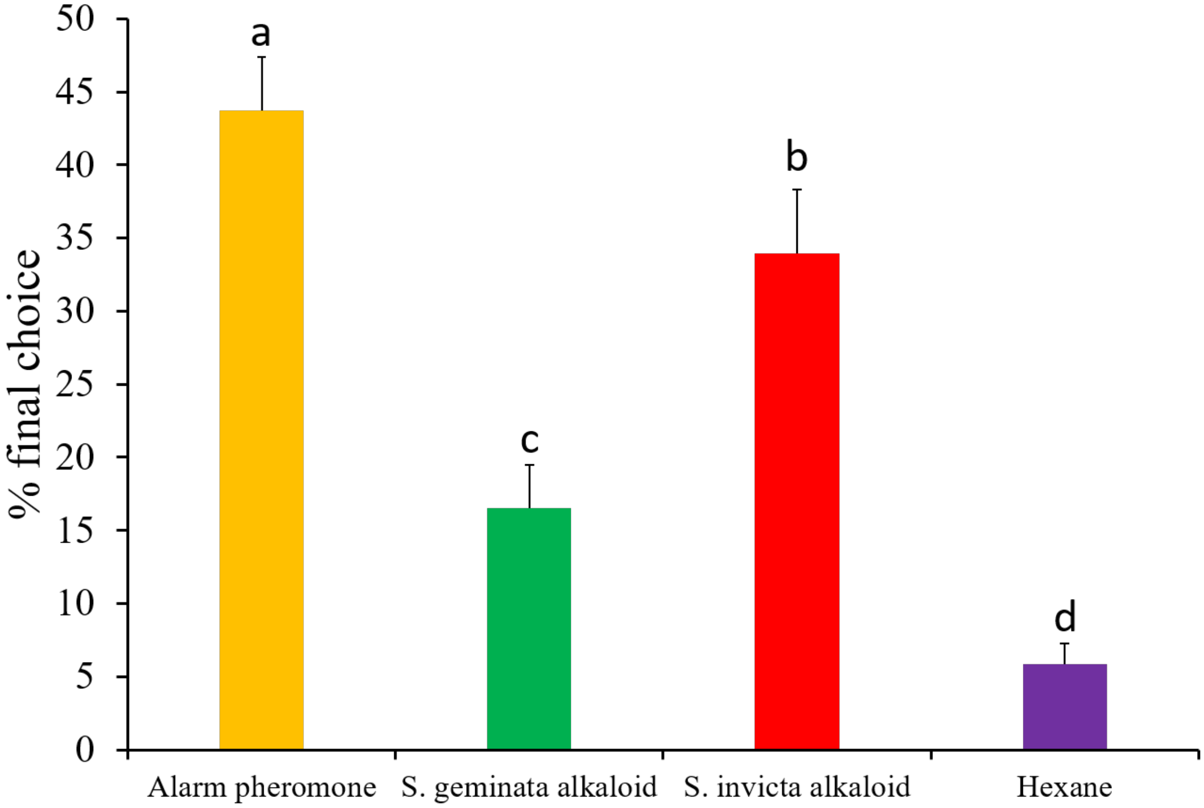

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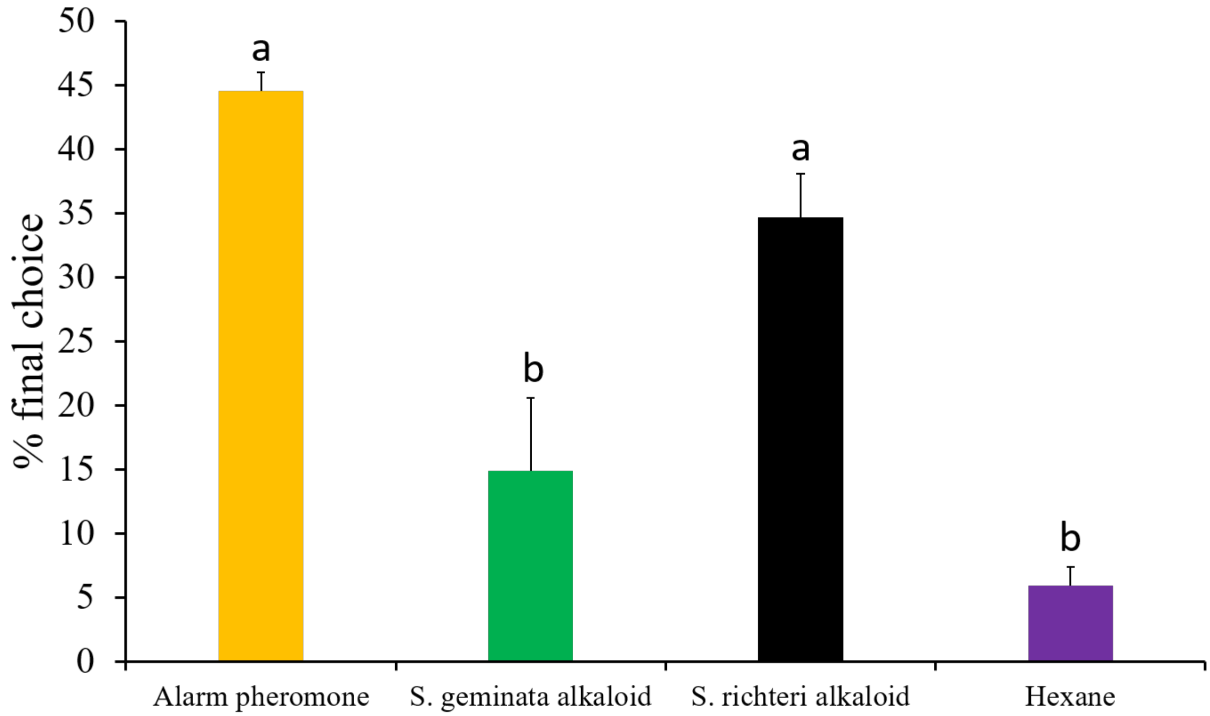
Figure 4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S. richteri alkaloid
Hexane

Chapter 5

Queen venom alkaloids in *Solenopsis* species: Interspecific variation and association with fertility

5.1 Introduction

Ant venoms contain a diversity of proteins, alkaloids and other classes of compounds (Fry et al. 2009; Hoffman 2010). There is significant interspecific and intraspecific diversity in ant venom components leading to variation in venom use (Brand et al. 1973a). Utilization of venoms by ants include prey capture, self- and kin-defense, and antimicrobial purposes (Blum et al. 1985). Venoms have also been suggested as a potential tool in chemotaxonomy Touchard et al. 2014). Furthermore, venom has been suggested to serve as a means of biotic resistance against invaders (Sorrells et al. 2011); thus, the structure of ant communities has been suggested to be shaped by venom (Andersen et al. 1991; Westermann et al. 2015). Venom may also give invasive species a substantial advantage against native species. For example, venoms of some native ant species are simpler (consisting of fewer components) and more primitive than those of their invasive ant competitors (Greenberg et al. 2008). Also, some native ants are greatly susceptible to venoms of their invasive ant competitors which may be a possible contributing factor to the displacement of such native ants by invasive ants (Tshinkel 2006). Analysis of venom chemical compositions could prove to be useful in elucidating the mechanisms underlying evolution of ant venoms (Brand et al. 1973b; Brand 1978).

The ant genus *Solenopsis* (Hymenoptera: Formicidae) contains ~20 stinging and venomous fire ant species (Brand et al. 1973a). This genus is well represented in the southeastern United States (US), where some species are considered to be invasive pests. Among *Solenopsis* fire ant species in the region, the pre-dominant *S. invicta* Buren, which was inadvertently introduced, is considered to be one of the world's most invasive alien species (Lowe et al. 2000). Other species in the southeastern US include the introduced *S. richteri* Forel, and native *S. geminata* (Fabricius) and *S. xyloni* (MacCook). *Solenopsis invicta* and *S. richteri* have formed an extensive zone of hybridization in their invaded region, producing the viable hybrid species *S. invicta* × *S. richteri* Vander Meer et al. 1985). The introduced *S. invicta* and *S. richteri* and their hybrid all belong to a group of ants taxonomically known as the *Solenopsis saevissima* complex and are distantly related to *S. geminata* which belong to the *S. geminata* complex (Trager 1991; Ross and Trager 1990). The introduced species are more aggressive and deliver more painful stings than the native species (MacConnell et al. 1976); thus, *S. geminata* are found to be highly susceptible to venom from *S. invicta* Lai et al. 2015).

Solenopsis richteri appeared in the US about two decades earlier than *S. invicta* in the early 20th century from their native South America through the Port of Mobile, AL (Buren et al. 1974). Since introduction, *S. invicta* has displaced several ant species including *S. geminata* and *S. richteri* (Vinson 1997) and has spread beyond the southeastern US establishing in California. It has also established in West Indies, New Zealand, Australia, and parts of Asia (Callcott and Collins 1996; Davis et al. 2001; Mescher et al. 2003; Ascunce et al. 2011). The pattern of spread of *S. invicta* and *S. richteri* in the invaded southeastern US, has been predicted to take similar shape to that in their native South America (Morrison et al. 2005). In their native origin, *S. invicta* and *S. richteri* have not been observed to co-occur on a large scale, with only slight overlaps at the borders of their occupied ranges (Tschinkel 2006).

Eusociality is observed in these ants, which is typically characterized by a division of labor into reproductive and non-reproductive groups called castes (Wilson and Hölldobler 2005). Queen *S. invicta* fire ants communicate their reproductive potential and status to their nestmate workers through semiochemicals, but the compounds mediating these interactions are poorly understood (Eliyahu et al. 2011). Studies have shown that fully reproductive *S. invicta* queens produce a pheromone that inhibits winged/ alate non-reproductive virgin queens from shedding wings (dealating) and developing ovaries (Fletcher and Blum 1981; Vargo and Hulsey 2000). The inhibitory pheromone(s) apparently originates from the venom sac and the postpharyngeal glands (Vargo and Hulsey 2000), but the components are not yet identified. The venom sac of *S. invicta* has been shown to be the source of large amounts of venom alkaloids (Fry et al. 2009; Brand et al. 1972). Queen fire ants apply venom over eggs as they are laid, and it has been suggested that this behavior protects eggs from entomopathogenic fungi (Tschinkel 2006; Vander Meer and Morel 1995). This behavior raises the possibility that venom alkaloids on the surface of eggs may play a role in advertising the presence and reproductive status of queens, in a similar method as cuticular hydrocarbons in other social insects Eliyahu et al. 2011; Smith et al. 2008; Peeters and Liebig 2009). A fundamental question regarding mechanisms underlying organization of fire ant societies is what differences exist in the abundance of venom alkaloid components between reproductive and non-reproductive alate queens of invasive and native fire ant species in *Solenopsis geminata* and *S. saevissima* complexes.

Brand et al. (1973b) investigated the chemistry of venom alkaloids in alate queens of *S. xyloni*, *S. geminata*, *S. richteri*, and *S. invicta*. The venom of alate queens of these four species produce primarily 2,6-dialkylpiperidine alkaloids with short side C-chain, i.e., (2R,6S)-2-methyl-6-undecylpiperidine (*cis*-C_{11}) and (2R,6R)-2-methyl-6-undecylpiperidine (*trans*-C_{11}). Furthermore, *cis*-C_{11} is at least twice as abundant as *trans*-C_{11} in all the four *Solenopsis* species (Brand et al. 1973a). Minor venom alkaloid components in the introduced species and their hybrid are 2,6-dialkyl-$\Delta^{1,2}$-piperideines and 2,6-dialkyl-$\Delta^{1,6}$-piperideines (Chen and Fadamiro 2009a; Chen and Fadamiro 2009b; Chen et al. 2010; Yu et al. 2014). Chen et al. (2012) found that alate queens of hybrid *S. richteri* × *S. invicta* share similar *cis*- and *trans* alkaloids to those of the two parental species *S. invicta* and *S. richteri*. Eliyahu et al. (2011) found that the composition of venom alkaloids differed between reproductive and non-reproductive alate queens of *S. invicta*. Social form (monogyny or polygyny) and *Gp-9* genotype are part of factors that influence the proportions of *cis* piperidine in non-reproductive alate *S. invicta* queens (Eliyahu et al. 2011). It has not been reported if similar differences exist in other *Solenopsis* species that belong to the *S. saevissima* and *S. geminata* complexes that are found in the southeastern US.

Here, we quantified and compared venom alkaloid profiles of queens of different reproductive status among invasive and native species in both *Solenopsis saevissima* and *S. geminata* complexes. We hypothesized that there is difference in quantity of venom alkaloid components among reproductive and non-reproductive alate *Solenopsis* fire ant queens. Our goal was to detect associations between chemical profiles and reproductive state of queens of different *Solenopsis* species. Such associations may indicate a role for specific compounds as releaser or primer pheromones involved in communicating fertility status of queens to workers, roles essential to the regulation of colony social organization in fire ants Eliyahu et al. 2011).

5.2 Materials and Methods

5.2.1 Insects. Fire ant colonies were excavated from the field in June 2019 and transported to the laboratory in buckets that were painted on the inside with Fluon® (Daikin, Decatur, AL) to prevent escape of fire ants. *Solenopsis invicta* colonies were collected from the Auburn University campus, Lee County, Alabama. *Solenopsis richteri* colonies were obtained from Hohenwald, Lewis County, and Mount Pleasant, Maury County, Tennessee. *S. richteri* queens used in this experiment were only the non-reproductives. Colonies of *S. invicta* × *S. richteri* were collected from Cullman, Cullman County, and Hollywood, Jackson County, Alabama. *Solenopsis geminata* colonies were obtained from Gainesville, Alachua County, Florida. All colonies were maintained in the laboratory until used and were fed 10% sugar-water and crickets.

The identities of *S. invicta*, *S. richteri*, and *S. invicta* × *S. richteri* were confirmed by gas chromatography of hexane extracts of *ca*. 50 workers using both alkaloid and CHC characters (Vander Meer et al. 1985; Chen et al. 2010; Ross et al. 1987) following methodology in Hu et al. (Hu et al. 2018). The identity of *S. geminata* was confirmed by keying out reference *S. geminata* worker specimens (Trager 1991), and geographic distribution in Gainesville, FL (Porter SD and Vander Meer RK, personal communication). Reproductive and non-reproductive alate queens were differentiated by the absence or presence of wings. Dealation is a behavior associated with onset of oogenesis (Fletcher and Blum 1981; Brent and Vargo 2003). The mating status of reproductive queens in our experiment was unknown. Reproductive and non-reproductive alate queens were removed for chemical analysis within from each colony using soft forceps the same week they were collected.

5.2.2 Sample Preparation. Individual reproductive and non-reproductive alate queens were soaked separately in HPLC-grade *n*-hexane for alkaloid extraction as previously described (Chen et al. 2012; Yu et al. 2014; Shi et al. 2015). For each colony, an individual alate or dealate queen was placed into a 2 mL glass vial containing 1 mL hexane at room temperature for 2 hours. This time was determined to be long enough for alkaloid extraction (Shi et al. 2015). Solvent from the extract was evaporated under N_2, and the residue reconstituted with 1 mL hexane.

5.2.3 GC/MS Analyses. GC/MS analyses of alkaloid samples were performed on an Agilent 7890A GC coupled to a 5975C mass selective detector, with an HP-5ms capillary column as previously described (Chen et al. 2012; Shi et al. 2015). The GC oven temperature was programmed from 90 °C (isothermal for 1 min) to 160 °C at 10 °C/min, then to 250 °C at 3 °C/min, and held for 2 min. Total run time was 40 min. The transfer line temperature was set at 250 °C. Mass spectra were obtained using electron impact (EI, 70eV).

Racemic *cis*-2-methyl-6-undecylpiperidine (*cis*-C_{11}) was synthesized by reduction of corresponding pyridine with hydrogen and palladium/carbon catalyst (Pianaro et al. 2012) and used as external standard for quantitative analysis. A stock solution of synthetic racemic *cis*-C_{11} (2000 ng/µL), was prepared and then diluted to a series of concentrations ranging from 1.95 to 1000 ng/µL. All dilutions were transferred to the GC autosampler, and 1 µL of each dilution was used for GC-MS analysis under same conditions as described above. A standard curve was calculated by linear regression analysis. The concentrations of the identified venom alkaloids in the samples were calculated against the standard curve. The obtained concentration for a given alkaloid component was multiplied by the final volume of the extract to give exact amount of the alkaloid component per individual.

5.2.4 Data Analyses. The absolute abundances of the alkaloid components were analyzed using *t*-test and ANOVA as applicable ($P < 0.05$), and Tukey HSD test was used to establish significant differences between mean quantities of venom alkaloid components of reproductive and non-reproductive alate queens within and across the *Solenopsis* species (JMP Inc. 2019). *t*-Test analysis was used to complete the means of reproductive and non-reproductive alate queens within a species, while ANOVA was used for means across all *Solenopsis* species.

5.3 Results

Typical GC-MS chromatograms of the *cis*- and *trans*-alkaloid fractions of reproductive and non-reproductive alate queens of *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata* are shown in Figure 1. About 90 % of the venom alkaloids from reproductive and non-reproductive alate queens in all species are composed of *cis*-C_{11} and *trans*-C_{11} (Table 1). In non-reproductive alate queens, the total percentages of these two major components were 85.69, 90.38, 83.96 and 86.99 % for *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata*, respectively. In reproductive queens, the total percentages of these two major components were 88.53, 85.86, and 76.76 % for *S. invicta*, *S. invicta* × *S. richteri*, and *S. geminata*, respectively. *cis*-C_{11} was the only dominant component of the *cis* alkaloids, while *trans*-C_{11} was the only dominant component of the *trans* alkaloids, irrespective of queen reproductive status and *Solenopsis* species (Table 1). Among the *Solenopsis* species, only in *S. invicta* × *S. richteri* was the amount of *cis*-C_{11} from non-reproductive alate queens (72.30 ± 10.87 μg/g ant) significantly higher than that from reproductive queens (40.99 ± 6.98 μg/g ant) (Table 1). Although the amount of *trans*-C_{11} from *S. invicta* × *S. richteri* non-reproductive alate queens (37.47 ± 1.89 μg/g ant) was relatively higher than that from reproductive queens (27.38 ± 5.34 μg/g ant), a significant difference was not observed. The amounts of the two piperideines, $\Delta^{1,2}$-C_{11} and $\Delta^{1,6}$-C_{11}, were quite low ranging from 0.94 ± 0.11 to 9.62 ± 0.92 μg/g ant in all *Solenopsis* species. The amounts of all other minor components, *cis*-C_{13}, *cis*-$C_{13:1}$, *cis*-$C_{13:1}'$, *trans*-C_{13}, and *trans*-$C_{13:1}$, were all under 11.74 μg per ant. Among all species, reproductive and non-reproductive alate queens of *S. invicta* had more *cis* and *trans* alkaloids components and piperideines than those of other species, except *cis*-$C_{13:1}'$, *trans*-$C_{13:1}$, even though the differences were not always statistically significant.

We further calculated the total amount of alkaloids depending on structure of piperidine ring (Table 2). The trend in the differences in total amount of different alkaloid types in reproductive and non-reproductive alate queens among species was similar to the trend concluded from data in Table 1. Reproductive and non-reproductive *S. invicta* alates had higher amounts of *cis* alkaloids, *trans* alkaloids and piperideines than other species. The total amount of alkaloids produced by a reproductive and non-reproductive alate queen ant ranged from about 80 to 344 µg and 97 to 225 µg, respectively.

Moreover, we calculated the ratio of *cis*-C_{11} to *trans*-C_{11} for reproductive and non-reproductive alate queens across species (Table 3). Areas of GC peaks were used to calculate the *cis*-C_{11} to *trans*-C_{11} ratio. Across species, the ratio of *cis*-C_{11} to *trans*-C_{11} in non-reproductive alate queens were significantly higher in *S. geminata* than in *S. invicta* × *S. richteri* and *S. invicta* ($df = 3$, $F = 12.50$, $P < 0.0001$), but similar to that in *S. richteri*. There was no significant difference in the ratios of *cis*-C_{11} to *trans*-C_{11} between *S. invicta* and *S. invicta* × *S. richteri*. Though not significantly different, ratios of *cis*-C_{11} to *trans*-C_{11} were more in reproductive than non-reproductive alate queens of *S. invicta* ($df = 1$, $F = 0.13$, $P = 0.72$) and *S. invicta* × *S. richteri* ($df = 1$, $F = 0.02$, $P = 0.88$), but lower in reproductive queens of *S. geminata* ($df = 1$, $F = 3.98$, $P = 0.06$) than non-reproductive alate queens.

5.4 Discussion

The results of this study confirm that reproductive and non-reproductive alate queens of invasive and native species in both *Solenopsis geminata* and *S. saevissima* complexes prominently contain *cis*-C_{11} and *trans*-C_{11} in their venom alkaloids as previously reported (Brand et al. 1973a; Chen et al. 2012; Shi et al. 2015). We also quantified some minor components, *cis*-C_{13}, *cis*-$C_{13:1}$, *cis*-$C_{13:1}'$, *trans*-C_{13}, *trans*-$C_{13:1}$, $\Delta^{1,2}$-C_{11}, and $\Delta^{1,6}$-$C_{11,}$ across all four *Solenopsis* species. An earlier report (Shi et al. 2015) only quantified these components in the venom of non-reproductive alate *S. geminata* queens; whereas the present study includes the relative quantities of these components in reproductive and non-reproductive alate queens of *S. invicta*, *S. invicta* × *S. richteri*, and *S. geminata*.

The total amount of alkaloids produced by individual non-reproductive alate queen of native *S. geminata* fire ant was 97 µg. However, non-reproductive alate queens of imported fire ants had more than

2-fold higher amount of alkaloids in *S. invicta*, and more than 1-fold higher amount of alkaloids in *S. invicta* × *S. richteri* and *S. richteri* than that in *S. geminata*. This indicates that the imported non-reproductive alate fire ant queens can produce more alkaloids than the native non-reproductive alate queens. It suggests that the production of more total alkaloids in imported fire ants could render them greater aggressiveness than native fire ants. This increased aggressiveness attributed to higher venom composition has been hypothesized in the snake *Calloselasma rhodostoma* to favor natural selection for greater efficiency in killing prey (Daltry et al. 1996a; Daltry et al. 1996b; Daltry et al. 1997). Thus, the lower production of alkaloids in *S. geminata* queens may be a factor in the displacement of this species in its native range by imported *S. invicta*.

Among the four *Solenopsis* species, ratios of *cis*-C_{11} to *trans*-C_{11} in reproductive and non-reproductive alate queens varied from 1.88 to 3.97, indicating that *cis*-C_{11} was more dominant than *trans*-C_{11} in queens of these species. A similar trend was reported by Brand et al. (1973a), Chen et al. (2012) and Shi et al. (2015). The venom in non-reproductive alate queens of another *Solenopsis* species native to the US, *S. xyloni*, consisted mainly of *cis*-C_{11} and *trans*-C_{11} as well Brand et al. 1973a). The ratio of *cis*-C_{11} to *trans*-C_{11} in *S. xyloni* non-reproductive alate queens was also at least 2:1 (Brand et al. 1973a). The ratios of *cis*-C_{11} to *trans*-C_{11} in reproductive and non-reproductive alate queens of *S. geminata* in our study (2.74 and 3.97, respectively) are similar to that reported by Brand et al. (1973a) for non-reproductive alate *S. geminata* queens (2.53 – 3.90). Though not significantly different, the ratio of *cis*-C_{11} to *trans*-C_{11} is relatively higher in reproductive queens than non-reproductive alate queens in the invasive *S. invicta*, and its hybrid offspring *S. invicta* × *S. richteri*, whereas the ratio is lower in the native *S. geminata*. Further studies with increased sample size are needed to test for significant associations in the ratio between reproductive and non-reproductive alate queens.

Differences in total alkaloids and ratios of *cis*-C_{11} to *trans*-C_{11} between species in *S. saevissima* and *S. geminata* complexes could be a characteristic factor useful in chemotaxonomy of these species (Brand 1978). In our study, ratio of *cis*-C_{11} to *trans*-C_{11} was higher in *S. geminata* than other species in reproductive and non-reproductive alate queens. Number of total alkaloids was more in *S. invicta* than other species in reproductive and non-reproductive alate queens. Chemotaxonomy could be a useful tool in addition to other taxonomic tools in identifying species, especially cryptic species (Touchard et al. 2014). Presence of characteristic piperidine alkaloids has been used as an indicator of fire ant evolution (Shi et al. 2015). The primitive venom alkaloid profile would have a much higher ratio of *cis*-C_{11} to *trans*-C_{11} (Brand et al. 1973a; Shi et al. 2015). It is possible that this arrangement has been conserved across reproductive and non-reproductive alate queens as in workers of the four *Solenopsis* species tested in this study.

This study supports previous studies that suggests that total alkaloids and ratio of *cis*-C_{11} to *trans*-C_{11} in queen *Solenopsis* fire ants are associated with reproductive status and taxonomic classification into species complexes (Brand 1978; Chen et al. 2012; Shi et al. 2015). In *S. invicta*, the proportion of *cis* piperidines (the less abundant geometric configuration in workers) was 1.5-fold more abundant in reproductive than non-reproductive alate queens. This supports previous study by Eliyahu et al. (2011) which showed that the proportion of *cis* piperidines gradually increases in the venom of *S. invicta* queens as they become fully reproductive. A suggested explanation of such differences in profile of *cis* piperidines between reproductive and non-reproductive alate *S. invicta* queens has been that this chemical signal can be used by workers to recognize and discriminate between queens of the two reproductive states (Eliyahu et al. 2011). Such behavioral discrimination can be evident only when the queen becomes sexually mature (Ross and Keller 1998; Gotzek and Ross 2007). This has been suggested as an association between ontogeny of these presumptive queen chemical signals and discrimination behavior directed toward them (Eliyahu et al. 2011). Furthermore, the proportion of *cis* piperidines in queen *S. invicta* from polygyne colonies is found to be higher in individuals of the *Gp-9* BB genotype than in those of the Bb genotype (Eliyahu et al. 2011). The impact of this gene becomes more pronounced as queens age, accelerate oogenesis, and become fully competent reproductively (Eliyahu et al. 2011). In our study, it is noteworthy that the proportion of *cis* piperidines was lower in reproductive than in non-reproductive alate queens of *S. geminata*. This suggests that a decrease rather than an increase of *cis* piperidine signals reproductive fertility in *S. geminata*, compared to *S. invicta*.

Previous studies that have investigated fertility signaling in social insects have assigned this mechanism to cuticular and egg surface hydrocarbons (Eliyahu et al. 2011; Peeters and Liebig 2009; Monnin 2006; Le Conte and Hefetz 2008; Liebig 2010). In *Solenopsis* species, it appears that venom alkaloid components rather than cuticular and egg hydrocarbons may play roles in providing information relevant to queen fertility status, as described in this and previous studies (Eliyahu et al. 2011). Piperidines with long side carbon chains and hydrocarbons are derived from acetate Leclercq et al. 1996), implying the possibility of similar metabolic processes needed to regulate the synthesis of carbon chains of hydrocarbons and piperidines (Eliyahu et al. 2011).

It has been suggested that venom alkaloids act as antimicrobial agents (Blum et al. 1958; Jouvenaz et al. 1972). Workers of *S. invicta* spray the contents of their poison gland onto the nest and their body when displaying gaster-flagging, a behavior in which fire ants disperses venom through the air by raising and vibrating their gaster (Obin and Vander Meer 1985). Venom alkaloids are nonvolatile, high molecular weight compounds (MacConnell et al. 1971), and potentially can remain on the cuticle for long periods. Thus, it is not surprising that piperidines are found in abundance throughout the nest (Chen 2007). It is possible that this abundance of venom alkaloids produced for a possible role in protecting ants from microbial pathogens may have influenced their use in fertility signaling within colonies, especially if a queen's venom becomes distributed on her cuticle Eliyahu et al. 2011). Thus, the present study suggests that venom alkaloids are the primary the communication method in *Solenopsis* ants as opposed to cuticular hydrocarbons that are used in many other social insects (Eliyahu et al. 2011; Peeters and Liebig 2009; Monnin 2006; Le Conte and Hefetz 2008; Liebig 2010). However, cuticular hydrocarbons may still play a role in regulating social organization within fire ant colonies, such as by signaling queen fertility status (Eliyahu et al. 2011). Future behavioral studies should be conducted to examine if other compounds, especially those found on the queen’s cuticle, are involved in maintaining the structure of fire ant societies.

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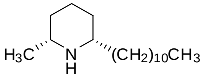
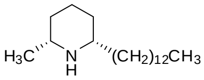
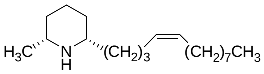
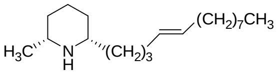
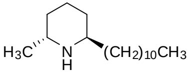
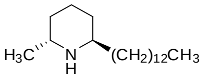
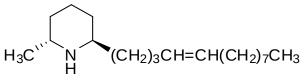
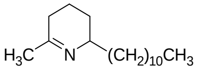
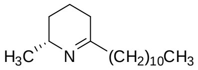
Table 1. Quantitation of alkaloids from *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata* fire ants (μg/ant ± SE).

Structure	**Name**	**Queen status**	***S. invicta***	***S. richteri***	***S. invicta* × *S. richteri***	***S. geminata***
***cis* alkaloids**						
	*cis*-C_{11}	Non-reproductive	138.79 ± 23.58a (61.66)	52.51 ± 16.65b (52.02)	72.30 ± 10.87*b (55.30)	68.29 ± 9.40b (69.89)
		Reproductive	217.91 ± 16.86A (64.50)	–	40.99 ± 6.98B (51.48)	50.62 ± 33.11B (57.19)
	*cis*-C_{13}	Non-reproductive	8.99 ± 1.52a (3.99)	1.85 ± 0.42b (1.83)	4.87 ± 0.68*b (3.72)	2.32 ± 0.59b (2.37)
		Reproductive	11.74 ± 0.87A (3.48)	–	2.76 ± 0.64B (3.47)	5.29 ± 1.25B (5.98)
	*cis*-$C_{13:1}$	Non-reproductive	6.17 ± 1.54a (2.74)	1.28 ± 0.38bc (1.27)	5.26 ± 0.69*ab (4.02)	0.20 ± 0.02c (0.20)
		Reproductive	5.63 ± 0.33A (1.67)	–	2.16 ± 0.53B (2.71)	0.28 ± 0.02B (0.32)
	*cis*-$C_{13:1}$’	Non-reproductive	0.29 ± 0.05b (0.13)	0.25 ± 0.04b (0.25)	0.19 ± 0.01b (0.15)	1.28 ± 0.37a (1.31)
		Reproductive	0.15 ± 0.01B (0.04)	–	0.38 ± 0.07*B (0.48)	2.40 ± 0.82A (2.71)
***trans* alkaloids**						
	*trans*-C_{11}	Non-reproductive	54.10 ± 9.55a (24.03)	38.72 ± 15.11ab	37.47 ± 1.89ab (28.66)	17.30 ± 2.37b (17.71)

				(38.36)		
		Reproductive	81.19 ± 9.76A (24.03)	–	27.38 ± 5.34B (34.38)	17.32 ± 1.99B (19.57)
	*trans*-C_{13}	Non-reproductive	4.98 ± 1.15a (2.21)	0.75 ± 0.22bc (0.74)	3.08 ± 0.32*ab (2.36)	0.43 ± 0.08c (0.44)
		Reproductive	6.27 ± 0.83A (1.86)	–	1.20 ± 0.24B (1.51)	0.38 ± 0.02B (0.43)
	*trans*-$C_{13:1}$	Non-reproductive	2.63 ± 0.69 (1.17)	1.18 ± 0.37 (1.17)	2.34 ± 0.32 (1.79)	1.66 ± 0.49 (1.69)
		Reproductive	1.74 ± 0.07 (0.52)	–	1.90 ± 0.53 (2.39)	4.21 ± 0.43 (4.76)
Piperideines						
	$\Delta^{1,2}$-C_{11}	Non-reproductive	2.66 ± 0.49a (1.18)	1.22 ± 0.42b (1.21)	1.50 ± 0.13*ab (1.15)	1.63 ± 0.18ab (1.67)
		Reproductive	3.57 ± 0.28A (1.06)	–	0.94 ± 0.11C (1.18)	1.96 ± 0.08B (2.21)
	$\Delta^{1,6}$-C_{11}	Non-reproductive	6.49 ± 1.09a (2.88)	3.19 ± 1.05b (3.16)	3.73 ± 0.36*b (2.85)	4.59 ± 0.43ab (4.69)
		Reproductive	9.62 ± 0.92A (2.85)	–	1.92 ± 0.43C (2.41)	6.05 ± 0.64B (6.84)

Data in parentheses are percentage of each component (%). An * indicates a statistical difference between reproductive and non-reproductive alate queens from the same species. Different uppercase letters indicate statistical difference among reproductive queens in all species. Different lowercase letters indicate statistical difference among non-reproductive alate queens in all species.

Table 2. Total amounts of alkaloids (µg/ant ± SE).

Compound	**Queen status**	***S. invicta***	***S. richteri***	***S. invicta* × *S. richteri***	***S. geminata***
***cis* alkaloids**	Non-reproductive	154.25 ± 25.75a	55.89 ± 17.47b	82.62 ± 12.05b	72.09 ± 10.14b
	Reproductive	235.44 ± 17.64A	–	46.29 ± 7.81B	58.60 ± 31.02B
***trans* alkaloids**	Non-reproductive	61.72 ±10.58a	40.65 ± 15.68ab	42.89 ± 2.18ab	19.39 ± 2.62b
	Reproductive	89.19 ± 10.64A	–	30.49 ± 5.94B	21.91 ± 1.55B
Piperideines	Non-reproductive	9.15 ± 1.57a	4.41 ± 1.47b	5.23 ± 0.49b	6.23 ± 0.60ab
	Reproductive	13.19 ± 1.19A	–	2.86 ± 0.48C	8.01 ± 0.72B
Total alkaloids	Non-reproductive	225.08 ± 37.51a	100.95 ± 34.57b	130.74 ± 14.08*b	97.31 ± 13.33b
	Reproductive	343.93 ± 29.23A	–	79.64 ± 11.41B	88.24 ± 31.87B

An * indicates a statistical difference between reproductive and non-reproductive alate queens from the same species. Different uppercase letters indicate statistical difference among reproductive queens in all species. Different lowercase letters indicate statistical difference among non-reproductive alate queens in all species.

Table 3. Ratio of *cis*-C_{11} to *trans*-C_{11} (Mean ± SE)

Queen status	***S. invicta***	***S. richteri***	***S. invicta* × *S. richteri***	***S. geminata***
Non-reproductive	2.60 ± 0.18b	2.92 ± 0.69ab	1.88 ± 0.21b	3.97 ± 0.15a
Reproductive	2.73 ± 0.19	–	1.94 ± 0.38	2.74 ± 1.59

Different lowercase letters indicate statistical difference among non-reproductive alate queens in all species.

CHAPTER 6

SUMMARY AND FUTURE STUDIES

This project investigated some aspects of the chemical ecology and physiology of *Solenopsis* fire ants' interactions with biotic and abiotic factors in the southeastern USA that were considered underexplored. The first study compared the water relations of temperate and tropical/sub-tropical fire ant workers across different latitudes in southern USA and is the first report of cuticular permeability (CP) and desiccation tolerance of *S. invicta* × *S. richteri* hybrid workers. The results demonstrate differences in the water relations of species adapted to temperate compared with tropical latitudes. Temperate *S. invicta* × *S. richteri* had significantly greater cuticular permeability than the tropical *S. invicta* and *S. geminata*. Live temperate *S. richteri* lost significantly more %TBW than tropical *S. invicta*. These results provide insights into differences in the water relations of these four *Solenopsis* species and help to explain the relative distribution of these species in the southeastern US. Capability of these four *Solenopsis* species to survive, limit cuticular water loss, and tolerate desiccation influences their distribution both in their native South America and in their introduced North American range. Extremes of hot and cold temperature and low relative humidity will limit the range of *S. invicta* and *S. richteri*. However, during global warming, the ranges of *S. invicta* and *S. richteri* are predicted to increase to the north of their present range, and *S. invicta* to the west and east of its present. Further studies including physiological and genetic analyses of desiccation tolerance in *Solenopsis* spp. are needed to determine what physiological and genetic attributes enable the tropical *S. invicta* to tolerate desiccation better than the temperate *S. richteri*.

The second study quantified and compared the salient characteristics of the DGC in *S. invicta*, *S. richteri*, *S. invicta* × *S. richteri*, and *S. geminata* fire ants occupying different habitats. The results indicate that the DGC was pronouncedly expressed in the alate caste (irrespective of temperature) in all tested *Solenopsis* species. This may be explained by the hygric genesis hypothesis that if the DGC was primarily a mechanism for conserving respiratory water, it would be preferentially expressed in the species and castes subject to more desiccating conditions, and alates are exposed to desiccating environments, especially during season of fire ant mating flights. This result is in accord with the chthonic genesis, that the DGC in *Solenopsis* species tested acts primarily to facilitate gas exchange in hypoxic and hypercapnic environments. Alates of all *Solenopsis* species tested showed a pronounced DGC; however, the DGC is not as pronounced in workers of these species. Thus, the results indicate that the DGC could influence the adaptation of these *Solenopsis* species of fire ants in the southeastern USA. Further studies are needed to investigate and compare the DGC in reproductive queens of these *Solenopsis* species.

The hypothesis that certain compounds in the venom alkaloid profiles of fire ant species mediate preference in their hosts, *Pseudacteon* phorid flies for imported versus native fire ants was tested in the third study. The results demonstrated the attraction of *P*. *curvatus* and *P*. *obtusus* to *cis* and *trans* venom alkaloid fractions of imported fire ants over native fire ants. It also suggests that host preference in *P*. *curvatus* and *P*. *obtusus* is mediated, at least in part, by differences in the venom alkaloid profile of various fire ant species. This finding may explain why *Pseudacteon* species associated with either the *S*. *geminata* or *S*. *saevissima* complexes have not switched to the other group despite the overlap of native and imported fire ants in some areas of their distributions. We propose that *cis* alkaloids probably play a synergistic role in enhancing the activity of *trans* alkaloids in mediating host preference in *Pseudacteon* phorid flies. To our knowledge, this is the first report which demonstrated that behavioral preference of *P*. *obtusus* and *P*. *curvatus* for imported compared to native *Solenopsis* species of fire ants is mediated by differences in the venom alkaloid profiles of imported versus native *Solenopsis* fire ants. Future studies will test this hypothesis in the field.

In the final study, I compared venom alkaloid profiles of queens of different reproductive status among invasive and native species in both *S*. *saevissima* and *S*. *geminata* complexes. The study suggests that venom alkaloids are the primary the communication method in *Solenopsis* ants as opposed to cuticular hydrocarbons that are used in many other social insects. However, cuticular hydrocarbons may still play a role in regulating social organization within fire ant colonies, such as by signaling queen fertility status. Thus, future behavioral studies should be conducted to examine if other compounds, especially those found on the queen’s cuticle, are involved in maintaining the structure of fire ant societies. Furthermore, future studies should investigate the effect of combination of other venom alkaloid components of *Solenopsis* fire ants on host preference in *Pseudacteon* phorid flies, in order to better understand the mechanisms underlying host preference in these parasitoids. In addition, the effect of genes that determine monogyny or polygyny in *Solenopsis* fire ants (such as *Gp-9* genes) should be investigated on fertility signaling in several species of Solenopsis fire ants in both the *S. saevissima* and *S. geminata* complexes.