

**PERCEPTION AND HANDLING OF INANIMATE OBJECTS BY THE RED
IMPORTED FIRE ANT *Solenopsis invicta* BUREN WITH AN EMPHASIS ON
POLYMORPHISM AND RECOGNITION CHEMICALS**

A Dissertation

by

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ABSTRACT

Invasive ant species can cause serious impacts ecologically and economically. One of the most documented invasive ant species is the red imported fire ant *Solenopsis invicta*. This invasive ant species has an impact on people, agriculture, and wildlife. This dissertation addresses how *S. invicta* perceives and handles objects in their environment. More specifically, studies investigated if workers can discriminate colors, how well polymorphic workers handle different sizes of objects, and behavioral responses by workers on recognition chemicals. The results of these studies can be used to make more efficient baits to reduce their populations.

The color discrimination study consisted of five colored glass beads: blue, green, yellow, orange, and red. Workers of *S. invicta* dug into colored glass beads, which consisted of equal proportions for two colors. Any color glass beads brought to the surface by the workers were counted. Workers can discriminate colors and have preferences for green, orange, and red.

Polymorphic workers of *S. invicta* were observed in how well they handled different sizes of glass beads when digging and foraging. For digging, the glass bead sizes consisted of 0.25, 0.50, 1.0, and 2.0 mm, while for foraging, the glass bead sizes consisted of 0.50, 1.0, and 2.0 mm. Results indicated that smaller workers can handle the smaller beads, while the larger workers can handle the larger beads. When digging and foraging, the most preferred bead size was 0.50 mm.

The behavioral response by *S. invicta* workers to recognition chemicals was determined when the chemicals were placed on glass beads. Recognition chemicals consisted of cuticular hydrocarbons and polar cuticular lipids. Glass beads treated with the recognition chemicals, along with control glass beads, were placed in three locations of the colony's environment: (1) near the food sources, (2) near the nest entrance, and (3) inside the nest. Workers were more attracted to the glass beads treated with recognition chemicals than the control glass beads. Workers of monogyne colonies were more attracted to cuticular hydrocarbons compared to polar cuticular lipids, while there was no difference between the recognition chemicals and the responses from workers of polygyne colonies.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Invasive Ant Species

Biological invasions of organisms can have serious impacts both ecologically and economically (Allen et al. 2004). This is especially true of ant species (Williams 1994, Allen et al. 2004). At least one hundred forty-seven ant species are recorded outside of their native areas, which often were originally Neotropical or Oriental regions (McGlynn 1999). When invasive ant species are introduced in a new area, they may cause local ant diversity to decrease and affect other organisms (Holway et al. 2002). Once a new ant species is established, it is very difficult to control their populations, and eradication is nearly impossible. Many invasive ant species share similar characteristics: (1) multiple-queen colonies, (2) interconnected nests, and (3) highly active foraging behavior (McGlynn 1999). The most important difference between native and non-native ant species is their colony sizes, with non-native ant species having larger colonies (Jeschke and Wittenborn 2011). These qualities are true of the highly researched red imported fire ant *Solenopsis invicta* Buren (Tschinkel 2006). A better understanding of the causes and consequences of ant biological invasion, may inform new methods to control invasive ants once they are established, and can help prevent other additional ant species from establishing populations outside their native areas (Holway et al. 2002).

Biology of the Red Imported Fire *Solenopsis invicta*

Solenopsis invicta is originally from the grasslands of South America located in the countries of Argentina, Brazil, and Paraguay (Lofgren et al. 1975). It is believed that they arrived in the United States at Mobile, Alabama during the 1930s (Hölldobler and Wilson 1990, Tschinkel 2006). Since then, the invasive ant has spread throughout the southern United States, and also invaded Mexico, some Caribbean Islands, Australia, New Zealand, and eastern Asia (Davis Jr et al. 2001, Tschinkel 2006).

Solenopsis invicta usually make nests in open, sunny areas, which is important for brood thermoregulation (Porter and Tschinkel 1993). They are normally not found in wooded areas, and instead, they are adapted to disturbed areas (Tschinkel 1993). In general, the impacts of humans on the environment has helped their invasion (King and Tschinkel 2008).

Solenopsis invicta are omnivorous, feeding on different arthropods, obtaining honeydew from aphids and scales, along with feeding on small vertebrates (Wilson 1978, Taber 2000). They also consume their eggs, larvae, and dead adults and queens (Taber 2000). Workers share food with other workers, larvae, and queens by the process of trophallaxis, in which regurgitated liquid food is exchanged (Tschinkel 2006). When processing solid foods for the colony, solid foods are first given to the fourth instar larvae, which break it down with their sclerotized mandibles or with the use of digestive enzymes (Petralia and Vinson 1978, 1979, Petralia et al. 1980). Then the food is shared with other larvae, workers, and queens.

Two social forms exist in *S. invicta*: monogyne and polygyne (Hölldobler and Wilson 2009). Monogyne colonies only have one queen, while polygyne colonies have more than one queen. The original colonies in the United States were monogyne, but then polygyne colonies started appearing during the 1970s. Both social forms occur in their native area (Tsutsui and Suarez 2003). Queens in polygyne colonies form a dominance hierarchy that affects their reproductive status (Ross 1988). Polygyne colonies consist of interconnected mounds, in which the density of workers is higher than monogyne colonies (Greenberg et al. 1985). The workers of polygyne colonies are 16% smaller than the workers of monogyne colonies and they are less aggressive towards non-nestmates (Obin et al. 1993). Population densities of polygyne colonies are about two times larger than monogyne colonies (Macom and Porter 1996).

The major difference between monogyne and polygyne colonies comes from one gene: *Gp-9* (Ross and Keller 1998). Workers that carry at least one polygyne allele of that gene will kill any queens that do not carry the polygyne allele (Keller and Ross 1998). It is suggested that the product of *Gp-9* is linked to how workers distinguish recognition chemicals that are used to distinguish nestmates from non-nestmates (Hölldobler and Wilson 2009).

During the first year, a monogyne colony can have about 7000 workers (Markin et al. 1973). In the second year, the colony can have about 25,000 workers and in third year, it can have about 50,000 workers. During the second year, the colony begins to make sexual reproductive adults. The sexual reproductive adults mate in the air during

nuptial flights. Afterwards, the mated queen sheds her wings and encloses herself into a small chamber to produce the first set of workers for the colony.

Solenopsis invicta consists of polymorphic workers, which are more effective in terms of defending the colony and foraging for food, compared to monomorphic workers (Porter and Tschinkel 1985a, b). Workers in one colony, may differ by 3-fold in body length and by 15-fold in weight. The number of larger workers increases as both the colony age and its size increases (Markin et al. 1973, Wood and Tschinkel 1981).

Polymorphic workers in *S. invicta* are classified into three categories: minors, media, and majors (Wilson 1978). Minors take care of the brood, while majors spend time in the nest periphery (Mirenda and Vinson 1981) or forage (Cassill and Tschinkel 1999). Majors live longer than minors (Calabi and Porter 1989). From an energy investment perspective, one major is equivalent to four minors. Media workers are flexible in the tasks they perform; they can easily switch between tending the brood and foraging (Cassill and Tschinkel 1999). It has been observed that media workers groom others more so than minors or majors do (Mirenda and Vinson 1981).

Impacts

Solenopsis invicta have an impact on people, agriculture, and wildlife (Adams 1986, Allen et al. 2004, Vinson 2013). The cost of damage per year by this ant in the United States is around \$1 billion (Pimentel et al. 2000). Workers of *S. invicta* sting people, but most people will not develop any health issues (Kemp et al. 2000, Tschinkel 2006). However, a few people experience anaphylactic shock due to the injected venom

by the ant, which may even cause death. In agriculture, the ants destroy or lower the yield of various crops, including corn, soybeans, okra, potatoes, eggplants, and citrus trees (Adams 1986). In animals, the ants have an effect on different vertebrate species, especially the young, by causing lower weight or even death (Allen et al. 2004). Examples include reptiles, such as the American alligator, various turtle species, and a few snake species. The ant has also affected several bird species, especially water birds, and small mammals, such as rabbits and rodents. *Solenopsis invicta* also can lower the diversity of native ants in an area. After *S. invicta* invaded the Brackenridge Field Laboratory of The University of Texas in Austin in the 1980s, ant species richness decreased by 70% (Porter and Savignano 1990). During that time, it even competitively replaced *Solenopsis geminata* (Porter et al. 1988). *Solenopsis invicta* has been known to cause structural damage, along with damaging roads and electrical equipment (Adams 1986, Vinson 2013).

Solenopsis invicta usually becomes a dominant ant when invading new areas causing serious impacts due to several factors: (1) lack of predators, (2) high reproductive rate, and (3) aggressive foraging behavior (Allen et al. 2004). It is also suggested that the lack of interspecific competition allows this invasive ant to establish new populations (Buren 1983). It is believed that interspecific competition controls populations of *S. invicta* in South America, where ant species are more aggressive and diverse compared to ant species in North America. Populations of *S. invicta* are five to eight times higher in the United States compared to the native populations in South America (Morrison 2002). However, populations of *S. invicta* are limited by arid

conditions and cold temperatures (Morrison et al. 2004) and therefore, it is likely that the ant species can inhabit 25% of the continental United States (Kemp et al. 2000). Models have indicated that *S. invicta* can invade portions of Africa, Asia, Australia, Europe, and many island nations (Morrison et al. 2004).

Population Control

The first method to control populations of *S. invicta* in the United States was in 1937 by using calcium cyanide dust (Williams et al. 2001). Unfortunately, during World War II, there was not any method being used to prevent *S. invicta* from spreading. Then in 1948, chlordane dust was used to control their populations. Eventually, granular heptachlor was used. However, residuals from heptachlor were found in milk and meat. Therefore, research started figuring out how to apply toxic chemicals to baits, which the use of corn grit as bait became the best option (Lofgren et al. 1963). The toxin placed on the corn grit was mirex, along with soybean oil to attract workers. Eventually residues of mirex were found in other organisms and therefore, it stopped being used on baits in 1978 (Williams et al. 2001). In the 1980s, hydramethylnon started being applied as the toxin on baits, which is still used today. In addition, insect growth regulators were developed during the same decade. More toxins were developed in the 1990s and still used today on baits. Overall, among the uses of contact insecticides and baits over the years, baits are the most effective in controlling populations of *S. invicta* in large areas.

Other methods to control the populations of *S. invicta* have also been researched. Two biological control agents have been used for a while to reduce the populations of *S. invicta* (Williams 2004). One of them is the phorid flies from the genus *Pseudacteon*, which lays eggs in the thorax of *S. invicta* (Morrison et al. 1997). Eventually, the egg hatches and larvae consumes the inside of the worker until it releases enzymes to separate the head of the worker from the rest of the body, in which then it will finish developing into an adult. *Pseudacteon tricuspis* was the first phorid fly to be introduced in the United States in Florida in 1997 (Williams 2004). Several species of phorid flies have been released since then. It has been observed to affect the foraging behavior of *S. invicta* (Orr et al. 1995).

The second biological control agent that has been used in controlling the populations of *S. invicta* is the protozoan pathogen *Kneallhazia (Thelohania) solenopsae* (Williams et al. 1998). It was first discovered in the United States in 1996, but it has been common among the fire ant species in South America. The pathogen can easily be transferred among queens, workers, and brood (Williams 2004). Infected queens lose about 50% of their weight and stop laying eggs.

Research Studies

The overall objective of the following studies in this dissertation is to enhance our understanding of how *S. invicta* perceive and handle inanimate objects in their environment. The first study determined whether *S. invicta* can discriminate colors, and if so, whether they have color preferences. The most widely studied social insects

involving color vision are bees (Von Frisch 1914, De Ibarra et al. 2014, Rao and Ostroverkhova 2015). A few ant species can perceive colors (Kretz 1979, Depickère et al. 2004, Aksoy and Camlitepe 2012), but it is not known if the workers of *S. invicta* can discriminate among colors.

Another study observed how well the polymorphic workers of *S. invicta* could handle objects of different sizes when digging and foraging. In particular, the study looked at how well the minors, media, and majors handle different sizes of objects. Based on a previous study with *S. invicta*, there is the general idea that larger workers can handle large objects, while smaller workers handle smaller objects (Wilson 1978), but that study did not look specifically at how well each physical caste handles different sizes of objects. In addition, this same study determined whether media workers could handle different sizes of objects when digging and foraging. Media workers are classified as generalists because they perform different tasks for the colony compared to minors and majors (Cassill and Tschinkel 1999), but little is known about how well media workers perform tasks with differently sized objects.

The final study observed the behavioral responses by *S. invicta* when introduced to objects treated with either cuticular hydrocarbons or lipids. Cuticular hydrocarbons and lipids are known recognition chemicals, and has been suggested that cuticular hydrocarbons are used for recognizing nestmates (Franks et al. 1990, Lenoir et al. 1999, Howard and Blomquist 2005, d'Etorre and Lenoir 2010). The objects were placed in different locations of *S. invicta*'s environment to determine whether their behavioral response was mediated by location. A few studies have studied nestmate recognition in

S. invicta (Obin 1986, Obin and Vander Meer 1988, Vander Meer et al. 1989, Obin et al. 1993, Vander Meer et al. 2008), but little is known about how workers recognize chemicals on objects, and whether they perceive the recognition chemicals differently in different parts of their environment.

Results from these studies may help in making baits more efficient for controlling *S. invicta* in non-native areas, such as Texas. For example, if *S. invicta* workers are found to discriminate among colors and have at least one color preference, such information can be used to improve baits. Currently available baits for *S. invicta* generally consist of defatted corn grit particles, and tend to have a yellowish appearance (Williams et al. 2001, Barr 2005, Reed et al. 2015). Another way to make baits more efficient is to consider workers' preferences for particular object sizes that are easier to handle and move. Making bait particles of the size that workers prefer may make enhance the attractiveness of baits. Finally, if workers are found to be differently attracted to objects treated with cuticular hydrocarbons or lipids in different locations of their environment, such information could be used to tailor baits for particular environments. Studies have already shown that *S. invicta* workers find baits with recognition chemicals sooner than they do baits without recognition chemicals (Vander Meer 1996, Wiltz et al. 2010).

CHAPTER II

**COLOR DISCRIMINATION AND PREFERENCE IN THE RED IMPORTED
FIRE ANT *Solenopsis invicta* BUREN**

Introduction

A large body of literature describes odor perception in insects (Howard and Blomquist 2005, Hallem et al. 2006, De Bruyne and Baker 2008, Provost et al. 2008). However, odor is not the only important stimuli in insects, which have receptors for perceiving different cues in their habitats, such as sounds (Hoy and Robert 1996, Pollack 2000), substrate-borne vibrations (Cokl and Virant-Doberlet 2003, Cocroft and Rodríguez 2005), polarized light (Dacke et al. 2003, Horváth et al. 2008), electrical (Newland et al. 2008, Clarke et al. 2013), magnetic fields (Prolić et al. 2003, Wajnberg et al. 2010), and color (Aquino et al. 2012, Telles et al. 2014). Color vision is important in several insects, allowing them to find food, mates, oviposition sites, and hosts (Bernard and Remington 1991, Wäckers 1994, Aquino et al. 2012, Torrissi and Hoback 2013, Telles et al. 2014). The ability to perceive environmental cues helps insects navigate, forage, find mates, and avoid predators. Hymenopteran species have sophisticated visual organs that allow them to perceive depth, movement, and color (Von Frisch 1914, Lehrer et al. 1988, Depickère et al. 2004, Srinivasan 2010, Aksoy and Camlitepe 2012). For example, bees and wasps use color discrimination to forage for food (Von Frisch 1914, Shafir 1996, Romeis et al. 1998, Tao et al. 2012, De Ibarra et al. 2014, Rao and Ostroverkhova 2015). Similarly, some ant species perceive color

(Depickère et al. 2004, Aksoy and Camlitepe 2012). However, little is known about the role that color plays in ant biology.

Most studies on signal perception in ants focus on chemical cues, such as pheromones (Hölldobler and Wilson 1990, 2009, d’Ettorre and Lenoir 2010, Sturgis and Gordon 2012). However, ants have the ability to use other cues as well. For example, ants have receptors for detecting sounds and vibrations (Hölldobler 1999, Hickling and Brown 2000, Barbero et al. 2012, Casacci et al. 2013), collectively known as vibroacoustic (Richard and Hunt 2013), along with receptors to detect magnetic fields (Stradling 1995, Banks and Srygley 2003, Sandoval et al. 2012). Interestingly, studies on the roles that vision may play in ant biology are few (McLeman et al. 2002, Gronenberg 2008, Bowens et al. 2013, Larabee and Suarez 2014), and less is known about color vision in ants.

The importance of vision to highly invasive and cosmopolitan species of ants, such as the red imported fire ant *Solenopsis invicta* Buren, is poorly understood. For example, it is unknown if *S. invicta* distinguishes color, while several studies have demonstrated that ant workers of other species can distinguish color (Marak and Wolken 1965, Roth and Menzel 1972, Wehner and Toggweiler 1972, Martinoya et al. 1975, Kretz 1979, Depickère et al. 2004, Aksoy and Camlitepe 2012). Thus, behavioral studies showed that workers of the Sahara desert ant *Cataglyphis bicolor* F., the mining ant *Formica cunicularia* Latreille, and the black garden ant *Lasius niger* L. can all detect different wavelengths within the visual spectrum (Wehner and Toggweiler 1972, Kretz 1979, Depickère et al. 2004, Aksoy and Camlitepe 2012). Other studies, using

electroretinograms have shown that workers of the European red wood ant *Formica polyctena* Foerster and the leaf cutter *Atta sexdens* L. can also discriminate among different wavelengths (Roth and Menzel 1972, Martinoya et al. 1975). There is also evidence that species of *Solenopsis* can detect color. For example, in behavioral studies of *S. saevissima* Smith, workers were found to distinguish among ultraviolet, green and near-red wavelengths (Marak and Wolken 1965). Therefore, it is likely that *S. invicta* also has the ability to distinguish among wavelengths. This supported by the presence of genes for UV-, blue-, and green-sensitive rhodopsins in the *S. invicta* genome (Wurm et al. 2011).

Since the introduction of *S. invicta* from the grasslands of South America into the United States during the 1930s, this invasive ant has spread throughout the southern United States (Hölldobler and Wilson 1990, Tschinkel 2006). This ant has also invaded Mexico, some Caribbean Islands, Australia, New Zealand, and eastern Asia. *Solenopsis invicta* has not invaded Africa or Europe yet (Ascunce et al. 2011, Wetterer 2013). However, climate models indicate that *S. invicta* can invade those continents (Morrison et al. 2004, Sutherst and Maywald 2005). Due to the lack of predators, high reproductive rate, and aggressive foraging behavior, *S. invicta* can quickly become the dominant ant in an invaded area (Allen et al. 2004) causing serious impact on agriculture, wildlife and households (Adams 1986, Lofgren 1986, Vinson 2013).

It is currently unknown whether *S. invicta* workers can discriminate colors. Understanding color perception of *S. invicta* could be used to construct more efficient baits to reduce their populations. If workers show color preferences, then the preferred

colors could be used to stain baits. Currently, baits for *S. invicta* generally consist of defatted corn grit particles, which have a general yellowish appearance (Williams et al. 2001, Barr 2005, Reed et al. 2015). Some studies have indicated that specific colored traps may help reduce insect populations (Al-Ayedh and Al-Doghairi 2004, Natwick et al. 2007, Döring et al. 2012, Shimoda and Honda 2013, Rao and Ostroverkhova 2015). The color of the traps depends on the color preference of the insect of interest. Similar principles could be used in red imported fire ant control.

The present study used behavioral experiments to answer: (1) whether workers of *S. invicta* have the ability to discriminate colors, and if so, (2) whether they have color preferences. I relied on the known behavior of *S. invicta* workers to dig in glass beads to answer these questions. Previous behavioral studies involving color vision in other ant species tested for behavioral changes in workers exposed to light of various wavelengths (Marak and Wolken 1965, Wehner and Toggweiler 1972, Kretz 1979, Depickère et al. 2004, Aksoy and Camlitepe 2012). However, this behavioral study went further by introducing colored objects to *S. invicta*, which provides a method that is more realistic in how they see their surrounding habitat.

Methods

Collection and Rearing

Ten colonies of *S. invicta* were collected around Bryan and College Station, Texas, along roads in farmland areas. I collected four monogyne (i.e., a colony having a

single queen) and six polygyne (i.e., a colony having more than one queen) colonies. Colonies were at least 1.2 km apart from each other.

In the lab, colonies were maintained at 23°C under a 12 light: 12 dark regime. Each colony was placed in a plastic container (27 x 39.5 x 9.5 cm) with Fluon[®] (BioQuip Products, Inc., Rancho Dominguez, California) on the inner edges to prevent queens and workers from escaping. The bottom of the container was layered with paper towels. A nesting area for the queens, brood, and some workers was placed on one side of the container. The nesting area consisted of a Petri dish (14 cm in diameter) with two smaller Petri dishes within it (9 and 5.5 cm in diameter) to form three chambers for the colony (Figure 1). Queens and brood were usually found in the two inner chambers. Each chamber had one access opening. All three chambers were filled with approximately 0.5 cm of cream-colored plaster (Castone[®] Dental Stone, Patterson Logistics, Fort Worth, Texas) that provided humidity for the brood when moistened periodically with de-ionized water. A water source was placed near the nesting area, which consisted of de-ionized water in a test tube (15 x 2 cm) stuffed with a cotton ball. Two Petri dishes (9 cm diameter) for food sources were placed opposite to the nesting area and to the water source. Frozen crickets, *Acheta domesticus* Linnaeus, were added to one of the small Petri dishes, while the other one contained a plastic weighing boat (5 x 1 cm) with a small amount of cotton that was used to absorb honey water (25% honey in de-ionized water).

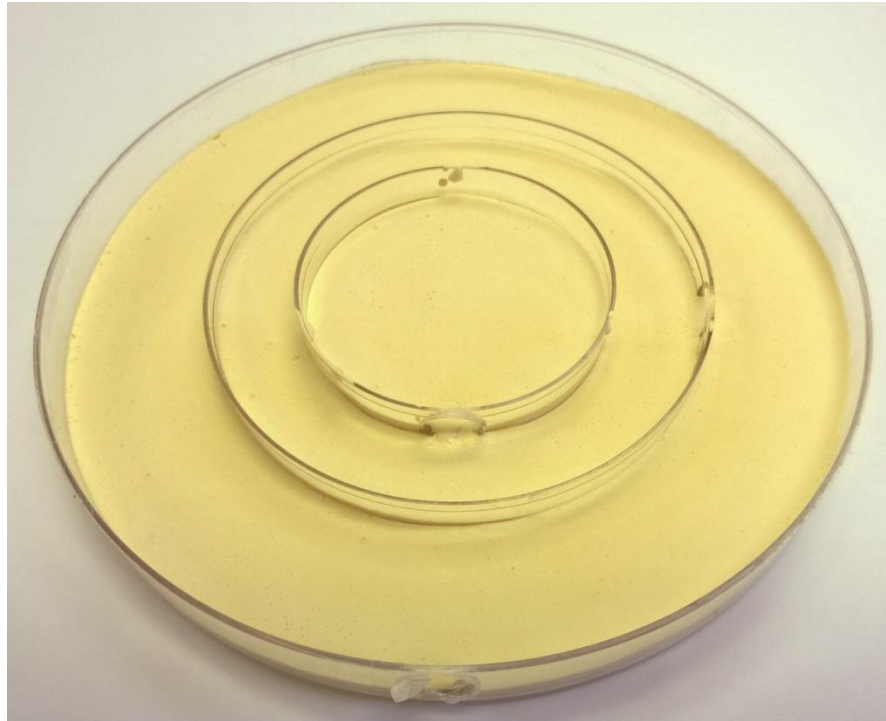


Figure 1. Structure of the nest for the queens, brood, and workers in the color discrimination study. Queens and brood are normally located in the two inner chambers.

Preparing Colored Glass Beads

Blue, green, yellow, orange, and red solvent resistant glass beads (OOAK Artist Emporium, San Bruno, California) measuring 0.9 mm in diameter were used for our experiments. Glass beads were cleaned before each trial by placing them in a sonicator (Cole-Parmer, Chicago, Illinois) with de-ionized water and Alconox[®] detergent powder for about 10 min. Then still within the sonicator, glass beads were rinsed six times with de-ionized water for about 10 min each time to remove any soap residue.

Color Perception Experiment

A plastic cylinder container (2.5 x 2.0 cm) with an attached snap-on lid was glued to the bottom of one side of a Petri dish (9 cm diameter) (Figure 2). The cylinder container was filled with glass beads. A hole was drilled into both the bottom of the Petri dish and the lid of the cylinder container for workers to have access to the glass beads. In addition to the cylinder container, a black rubber stopper was glued to the bottom of the Petri dish opposite of the cylinder container for balance.

Ten separate color pair trials were performed for each colony. For each trial, approximately 50 workers were randomly selected by exploiting their aggressive behavior using a wooden applicator stick to which they climbed. Workers were taken from both inside and outside of the nesting area. After each trial, the workers were returned back to their original colony. Talcum powder (Johnson & Johnson Consumer Companies, Inc., Skillman, New Jersey) was applied to the inner edges of the Petri dish to prevent workers from escaping. The cylinder container was filled with two different

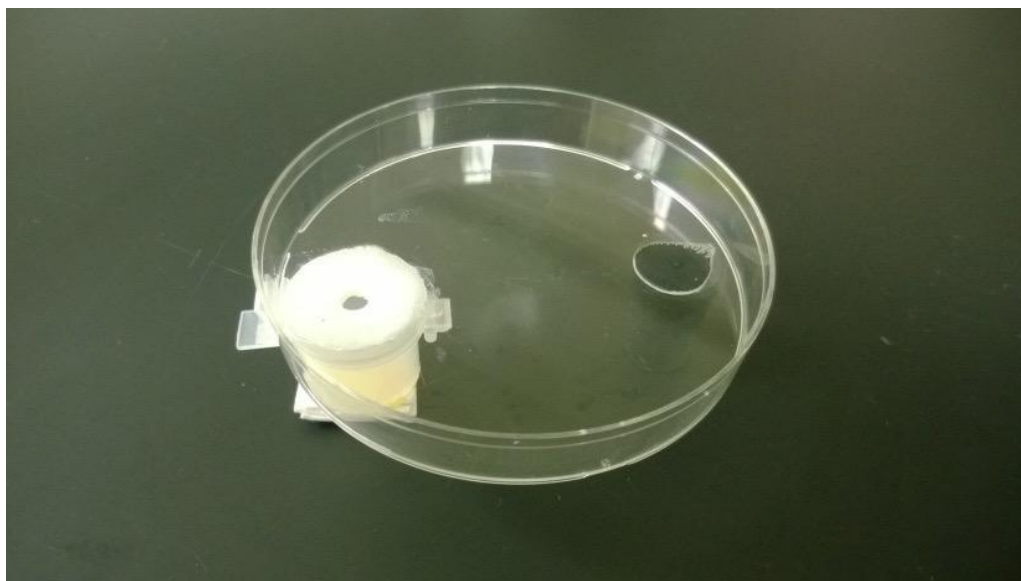


Figure 2. Set-up for color discrimination study. The plastic cylinder container was filled with colored glass beads.

colors of glass beads. Ants were exposed to only two colors because paired choice tests in behavioral studies are generally better than giving animals several choices (Raffa et al. 2002, Hutchinson 2005, Bruzzone and Corley 2011). Beads were thoroughly mixed in a 50:50 ratio with approximately 4,784 beads for each color.

For the experiment, workers were given the opportunity to dig into the colored glass beads for 1 hour. Trials were conducted between 12:00 to 18:00. The experiment was divided into an experimental and a control group. In the experimental group, workers were given the opportunity to dig into the colored glass beads under light conditions. Light was provided by white fluorescent bulbs (GE Ecolux Starcoat, F32T8 SP41ECO, 32 W). The control group was identical, except for workers digging in dark conditions. The control group was necessary to test for the possibility of ants using stimuli other than vision. Dark conditions were attained in a room with no windows and with black painted walls, ceiling, and door. Colonies were moved to the dark room one hour before each trial for the workers to acclimate to the darkness.

Data Analysis

For each color pair trial, the number of each colored glass bead that was removed from the cylinder container by ant workers was counted for each of the ten colonies. Each color pair within the experimental and control groups were analyzed independently using Wilcoxon signed-rank test applied to the difference in the 10 pairs of counts, one for each colony. The Spearman rank correlation test was preformed to determine if there were any correlations among the same colors removed from the cylinder container

between light and dark conditions. Due to multiple testing, the Bonferroni correction was applied, using a p -value less than 0.005 for designating a significant difference in the attraction to a color for each of the 10 pairs of colors. This results in an overall Type I error rate of 0.05 for comparing the ten pairs of color.

A separate variance t -test was also applied to the data to determine if there was a difference between the number of glass beads brought to the surface between the experimental and control group. For this test, the level of significance was set at an alpha of 0.05.

Results

Solenopsis invicta workers had the ability to discriminate color (Figures 3 and 4). Workers could discriminate blue from green, orange, or red and yellow from orange and red (Wilcoxon signed-rank test: $T = 0$, $p = 0.002$). Interestingly, workers could not discriminate blue from yellow, or green from yellow or orange (Wilcoxon signed-rank test: $T = 2$, $p = 0.0059$). In addition, workers could not discriminate green from red (Wilcoxon signed-rank test: $T = 11$, $p = 0.2031$) and orange from red (Wilcoxon signed-rank test: $T = 3$, $p = 0.0098$).

In the dark, workers could not discriminate among colors, with the exception of the yellow-orange pair ($p < 0.005$; Figures 5 and 6) (Wilcoxon signed-rank test: $T = 1$, $p = 0.0039$), in which the workers preferred orange over yellow. No correlation existed for any of the same colors removed from the cylinder container between light and dark conditions (Spearman rank correlation: $p > 0.005$).

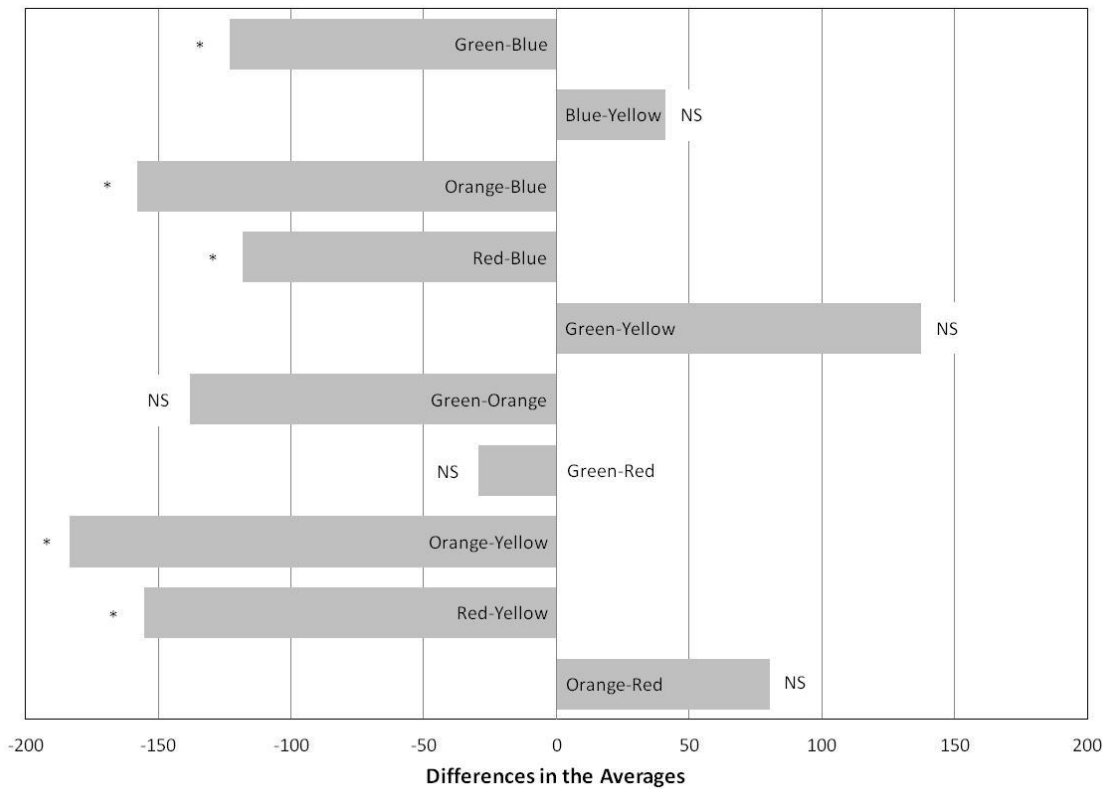


Figure 3. For each color pair, the bar indicates the average difference per colony in the number of beads of each color that was brought to the surface by the workers in the experimental group (NS=not-significant; $*$ = $p < 0.005$). The first color listed in the color pair bars indicates the preferred color when there is a significant difference.

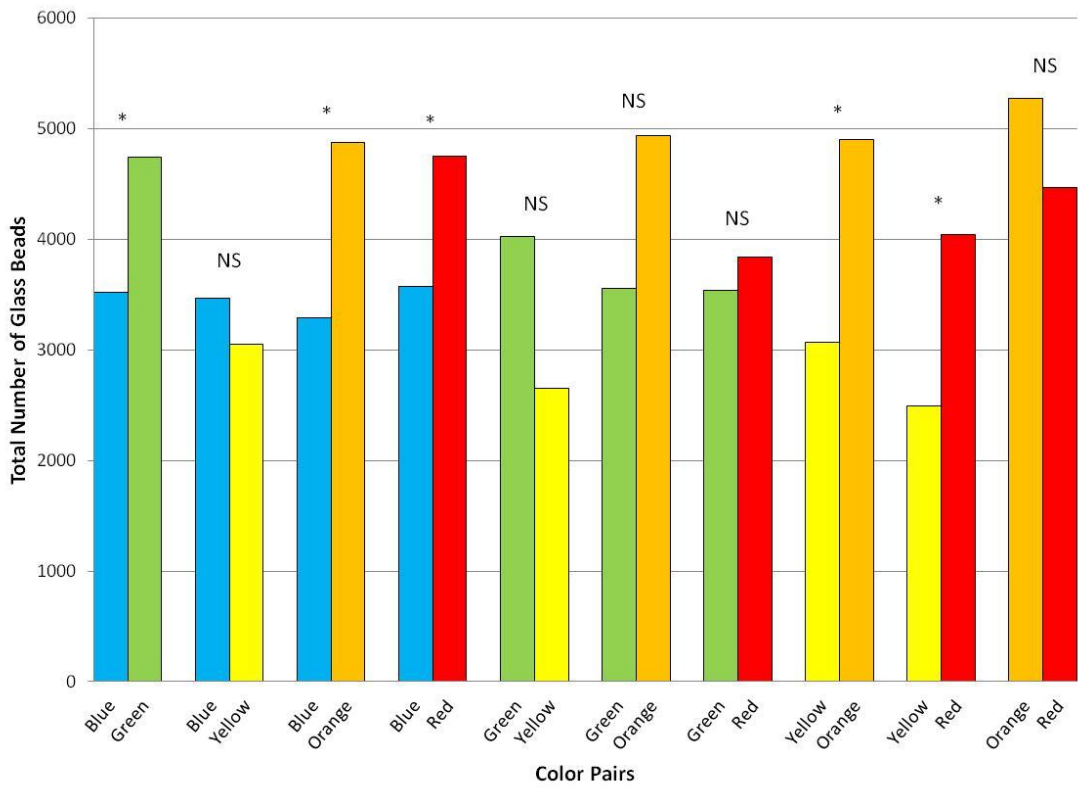


Figure 4. Total number of glass beads removed by workers for each color within each color pair in the experimental group (NS=not significant; *= $p < 0.005$).

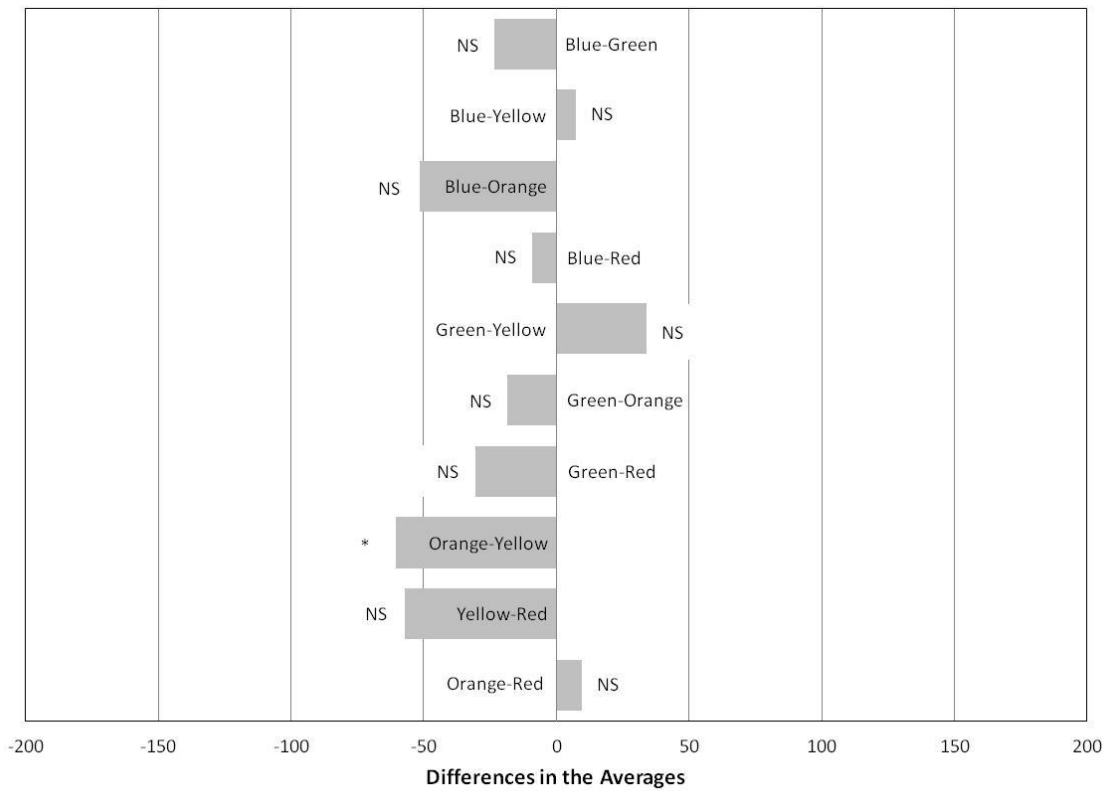


Figure 5. For each color pair, the bar indicates the average difference per colony in the number of beads of each color that was brought to the surface by the workers in the control group (NS=not-significant; $*= p < 0.005$). The first color listed in the color pair bars indicates the preferred color when there is a significant difference.

In addition, it was observed that workers dug more in the light compared to the dark conditions (Figure 7). The workers digging in the light brought more colored glass beads to the surface compared to the workers digging in the dark (separate-variance t test: $t = 5.37, p < 0.0001$).

Discussion

Solenopsis invicta workers were able to discriminate and show preference for some colors in paired tests. Workers show preference for colors with relatively long wavelengths, while colors with shorter wavelengths seemed to be less preferred. Workers preferred colors above 590 nm (i.e. orange and red), while colors below 590 nm (i.e. blue and yellow, with the exception of green) were less preferred. Among the colors used in this study, blue was the least preferred. Similar results have been reported for *S. saevissima*, in which workers perceived green and red light, but not blue or yellow light (Marak and Wolken 1965). The next step would be to pair each color used in this study to either black or white to potentially provide further evidence about the color preferences by *S. invicta* workers as observed in this study and to analyze all those color pairs to determine the strength of the color preferences by the workers of *S. invicta*. There is the misconception that ants and bees cannot see red (Chittka and Waser 1997, Aksoy and Camlitepe 2012, 2014). This idea was first concluded about the honeybee *Apis mellifera* Linnaeus (Von Frisch 1914) and afterwards, there were conflicting conclusions regarding whether bees can perceive red (reviewed in Chittka and Waser 1997). However, the honeybee *A. mellifera* can see wavelengths consisting of red

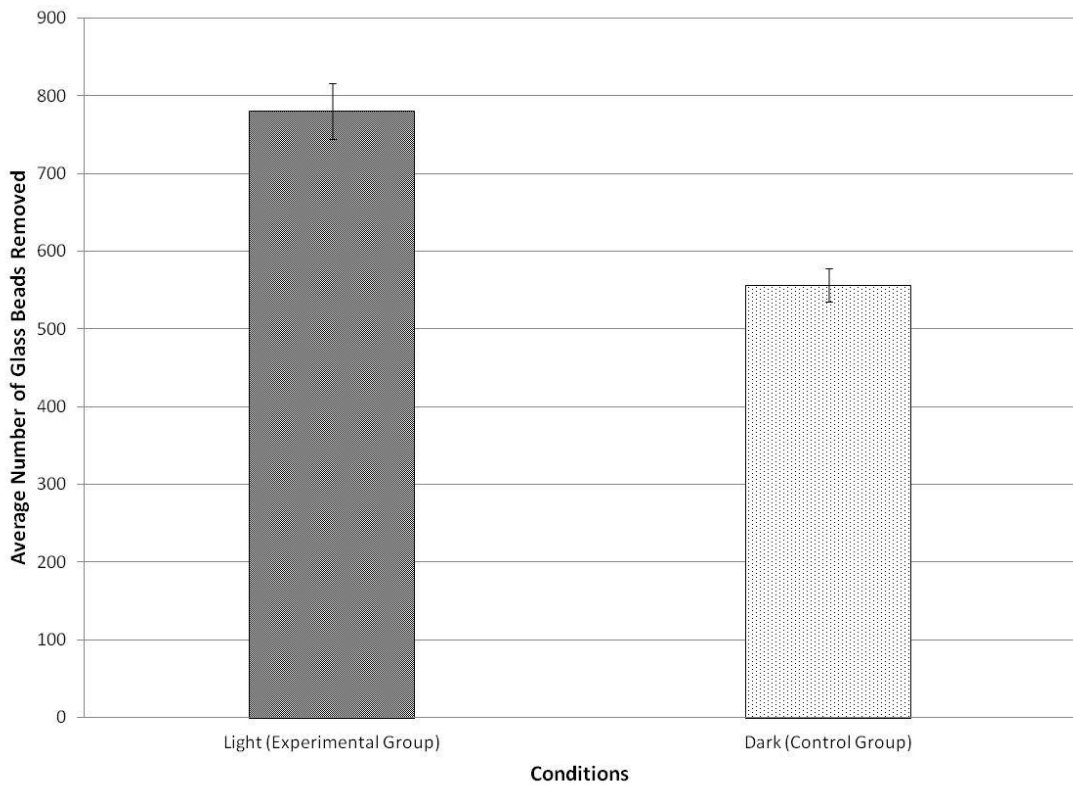


Figure 7. Average number per colony of colored glass beads brought to the surface by workers in experimental and control groups. Workers brought more beads to the surface in light conditions compared to dark conditions ($p < 0.0001$).

(Reisenman and Giurfa 2008) and the bumblebee *Bombus dahlbomii* Guérin-Ménéville can detect red flowers (Martínez-Harms et al. 2010). It was also assumed that ants could not perceive red due to being closely related to bees (Aksoy and Camlitepe 2012). However, our study suggests that *S. invicta* workers can perceive red. Similarly, workers of the closely related species *S. saevissima* and foragers of *Cataglyphis aenescens* Nylander and *F. cunicularia* can also perceive red (Marak and Wolken 1965, Aksoy and Camlitepe 2012, 2014). Although, *S. invicta* lacks red-sensitive photoreceptors, a comparison test might provide a distinction that could be the basis of a choice between different colored objects. In white light, a visual system that is blind to red light may perceive red-transmitting objects as darker than yellow-transmitting objects.

This study involved wavelengths found within the visible spectrum. However, hymenopterans can also see ultraviolet light (Peitsch et al. 1992, Aksoy and Camlitepe 2012, De Ibarra et al. 2014). Colors were limited in this study, but results of this study show that color preference exist in *S. invicta*. Thus, it would be important to consider which colors that can potentially be perceived by *S. invicta*. Future studies should determine the wavelength values that *S. invicta* workers can perceive. It is already known that foragers of the ants *F. cunicularia* and *C. aenescens*, as well as bees, can detect shorter wavelengths of red better than longer wavelengths (Chittka and Waser 1997, Aksoy and Camlitepe 2012, 2014).

Worker behavior in our dark control experiment strengthens the argument that *S. invicta* can discriminate colors. That is, they show significantly less color preference in the color choice tests in the dark. However, it was expected for the workers to show no

preference for any colors and that was not the case with all the color pairs they were exposed to. In the dark, they preferred orange over yellow. Although a few studies have indicated that some ant species are adapted to forage and navigate at night (Narendra et al. 2010, Reid et al. 2011), they use dimmed light produced by the moon and stars (Warrant and Dacke 2011). In this study, the dark control was performed in a completely dark room. Therefore, it is possible that workers must have used other cues, such as odor, to distinguish between the orange and yellow beads. Ants rely heavily on odors for communication (Hölldobler and Wilson 1990, 2009, d’Ettorre and Lenoir 2010, Sturgis and Gordon 2012). Thus, it is likely that their sensitive odor receptors allowed them to discriminate between the dyes used in the orange and yellow beads used in this experiment.

Color discrimination in *S. invicta* may play a role in foraging, in which they may have a preference for food sources appearing green, orange, or red. Overall, little is known about the role of color perception in ants. It is suggested that color vision in animals is an evolutionary adaptation to discriminate objects in the environment (Lythgoe and Partridge 1989), which can be true for ants (Aksoy and Camlitepe 2012). Color perception is important in other hymenopterans. Parasitoid wasps use color vision to find hosts and host habitats (Segura et al. 2007), along with finding food (Wäckers 1994). Similarly, bees and wasps use color vision when foraging (Shafir 1996, Briscoe and Chittka 2001, Kunze and Gumbert 2001, De Ibarra et al. 2014).

Digging is another behavior that may be influenced by ant workers’ color preference. Little is known about how light stimulates digging behavior in ants (Gautrais

et al. 2014). The larger number of glass beads brought to the surface by *S. invicta* during light conditions as compared with dark conditions in our experiments suggests that light may stimulate workers to dig. Light has been shown to stimulate burrowing in the short-tailed cricket *Anurogryllus muticus* De Geer (Lee and Loher 1996). Similarly, bumblebees, *Bombus terrestris* Linnaeus, dig at a higher rate when workers are exposed to sunlight (Godzinska and Korczynska 1997). Also, the highest amount of digging by workers of the Oriental hornet *Vespa orientalis* Linnaeus, occurs at noon, when UV radiation from the sun is the most intense (Ishay and Elly Lior 1990, Ishay and Shira 2000).

Results from this study suggest that perhaps baits used against *S. invicta* can be improved. Several studies have shown that some insects are attracted to specific colored traps. Examples include non-*Apis* bees, western flower thrips *Frankliniella occidentalis* Pergande, onion thrips *Thrips tabaci* Lindeman, and hoverflies which prefer blue traps (Chen et al. 2004, Stephen and Rao 2005, Natwick et al. 2007), while yellow traps are preferred by the false chinch bug *Nysius raphanus* Howard, the pollen beetle *Meligethes aeneus* Fabricius, and aphids (Demirel and Cranshaw 2006, HU et al. 2011, Döring et al. 2012). Thus, the attraction of *S. invicta* to currently used baits could be improved by having them made in the most preferred color. Modern baits generally consist of defatted corn grit particles (Williams et al. 2001, Barr 2005, Reed et al. 2015), which are usually light yellow. Results of this study show that yellow baits may not be as efficient in attracting workers as other colors. Future experiments should stain baits with different odorless colored dyes and present these stained baits to workers in paired-choice tests to

determine if the color preferences observed in this study are translatable to bait choice. Dye odorlessness should be tested with dark control arenas (like in this study) or using physiological response methods (Ozaki et al. 2005, Greene and Gordon 2007, Tanner and Adler 2009).

Previous studies assessing color vision in various ant species involved a mixture of behavioral and electrophysiological studies (Marak and Wolken 1965, Roth and Menzel 1972, Wehner and Toggweiler 1972, Martinoya et al. 1975, Depickère et al. 2004, Aksoy and Camlitepe 2012). Electrophysiological studies can indicate if an animal has the physiological capacity to perceive colors, while behavioral studies can indicate the expression of color perception (Kretz 1979, Aksoy and Camlitepe 2012). Previous behavioral studies with other ant species tested for behavioral changes in workers exposed to light of various wavelengths (Marak and Wolken 1965, Wehner and Toggweiler 1972, Kretz 1979, Depickère et al. 2004, Aksoy and Camlitepe 2012). Our behavioral study went further by introducing colored objects to *S. invicta*, which provides a method that is more realistic and get closer to how they may see their surrounding habitat.

It will be interesting to explore if color perception varies among different castes by observing minors, majors, medias, and majors (Tschinkel 2006). Eye structures evolved to correspond with the behavior and the lifestyle of ants, such as living in certain habitat or whether the ants are more active during the daytime or nighttime (Moser et al. 2004, Cammaerts and Cammaerts 2014). A difference in color perception could exist among castes due to differences in eye structures. It has already been

observed that the size of the compound eyes coincides with the body size, in which majors have wider and more facets than minors (Baker and Ma 2006). In *Formica integroides* Wheeler (Bernstein and Finn 1971) and *Camponotus pennsylvanicus* De Geer (Klotz et al. 1992), the larger workers have more ommatidia than smaller workers. In these ant species, larger workers forage, while smaller workers complete tasks inside the nest. By having larger eyes, it is suggested that foragers have better vision for exploring outside of the nest compared to the smaller workers. In addition, it is suggested that larger workers' eyes of the Australian desert ant *Melophorus bagoti* Lubbock (Schwarz et al. 2011) provide better resolution than smaller workers' eyes due to having more ommatidia. However, any possible differences in color vision among different body sizes in ants, including *S. invicta*, are unknown.

The ability to discriminate colors could also be different among the task groups of *S. invicta*, such as nurses taking care of the brood inside the nest and foragers looking for food outside of the nest. It has been suggested that this could be the case in the nectar-feeding ant *Camponotus rufipes* Fabricius, in which the eye receptors of nurses that perceive color are not as developed as in the foragers (Yilmaz et al. 2016). Age polyethism occurs in minor and media workers, in which younger workers perform tasks inside the dark nest, while older workers forage outside of the nest. Overall, younger workers rely more on odors than vision inside the dark nest (Hölldobler and Wilson 1990). This same phenomenon occurs in the honeybee *A. mellifera* (Sasagawa et al. 2003). Therefore, it could be possible that different life stages of *S. invicta* workers can perceive colors differently. This has already been observed in a behavioral study of the

black garden ant *L. niger*, in which foragers can perceive red light, while nurses cannot (Depickère et al. 2004).

There is incomplete knowledge about color perception in ants probably due to the general assumption that ants rely more on chemicals than vision (Hölldobler and Wilson 1990, 2009). However, it would be informative to keep exploring ants' perception of other cues, such as color, sounds, vibrations, magnetic fields, and touch. This would provide more information about how ants perceive their environment, including food sources and baits against ant pests. This study has indicated that *S. invicta* workers have color preferences for green, orange, and red. These colors can be applied to baits to test whether they are more attractive than the general yellow appearance of current baits. Color perception is now known in two species in the genus *Solenopsis*: *S. invicta* and *S. saevissima* (Marak and Wolken 1965). Color perception studies can be performed on other species in the genus to determine if results are similar to *S. invicta* and *S. saevissima* and expand our knowledge about color perception in ants. If preferred colors were identified in other ant species, it would then be interesting to investigate why those colors are preferred or to hypothesize how those preferences may have evolved.

CHAPTER III

**PARTICLE SIZE PREFERENCES IN POLYMORPHIC WORKERS OF THE
RED IMPORTED FIRE ANT *Solenopsis invicta* BUREN**

Introduction

Division of labor is common in social insects (Robinson 1992, Beshers and Fewell 2001, Hölldobler and Wilson 2009, Duarte et al. 2011). Colonies generally consist of reproductive and non-reproductive individuals. Non-reproductive individuals can be further divided by their performing of different tasks, such as tending to the brood, nest building, defending, and foraging. It has been suggested that the ecological success of social insects is due in part to their division of labor (Wilson 1985, Wilson and Hölldobler 2005).

Different forms of division of labor occur in social insect colonies, such as temporal polyethism, and worker polymorphism (Robinson 1992). Temporal polyethism involves workers changing tasks as they become older (Oster and Wilson 1978, Wilson 1985, Hölldobler and Wilson 2009). Generally, younger workers are inside the nest maintaining it and tending to the brood, while older workers are outside the nest foraging or defending the colony. Temporal polyethism occurs in wasps (Shorter and Tibbetts 2009, Kim et al. 2012), bees (Johnson 2008, Tobbyack et al. 2011), termites (Badertscher et al. 1983, Hinze and Leuthold 1999), and ants (Wilson 1976, Camargo et al. 2007), including *Solenopsis invicta* Buren (Mirenda and Vinson 1981). It has been suggested that genes regulate temporal polyethism (Robinson et al. 1994). Polyethism has been

observed in workers of different matriline in the desert leaf-cutter ant *Acromyrmex versicolor* Pergande when switching tasks from inside to outside of the nest (Julian and Fewell 2004). Similarly, different patriline influence the timing of honey bee workers when they leave the nest to start foraging or defending the colony (Kolmes et al. 1989) and it was discovered that genotypic differences in the behavioral development of honey bee workers can exist (Giray and Robinson 1994, Giray et al. 2000).

Worker polymorphism is another form of division of labor that has been postulated to explain social insect success (Hölldobler and Wilson 1990, Steiner et al. 2010). It involves workers of different sizes performing different tasks for the colony (Oster and Wilson 1978). The body size of polymorphic workers can be influenced by patriline (Hughes et al. 2003, Rheindt et al. 2005, Smith et al. 2008) and nutrition (Smith et al. 2008). Nutrition influences the levels of juvenile hormone, which has been known to determine workers' body size (Nijhout and Wheeler 1982, ONo 1982). Polymorphic workers occur in a few species of bees (Spaethe and Weidenmüller 2002, Grüter et al. 2012, Segers et al. 2016) and wasps (Archer 1972, Spradbery 1972, O'Donnell and Jeanne 1995), but they are more prevalent in termites (Miura and Matsumoto 1995, Campora and Grace 2004, Haifig et al. 2011) and ants (Hölldobler and Wilson 1990, 2009). Among the 296 living genera of ants, 46 of them have polymorphic workers (Hölldobler and Wilson 2009).

One of the most elaborate polymorphic worker systems involving division of labor is the one exemplified by leaf-cutter ants, *Atta sexdens* L. (Wilson 1980b, Hölldobler and Wilson 2009). In general, larger workers cut and transport leaves back to

the nest, while smaller workers tend to the fungus in the nest. Extreme differences in worker sizes exist in this ant species, where the smallest workers ride on the leaves as they are carried back to the nest to remove fungal contaminants and to defend the larger workers from parasitic phorid flies (Vieira-Neto et al. 2006) while the largest workers defend the nest (Wilson 1980b). Polymorphic workers are also found in *Cataglyphis cursor* Fonscolombe (Fournier et al. 2008), in the weaver ant *Oecophylla smaragdina* F. (Crozier et al. 2010, Kamhi et al. 2015), in the *Formica rufa* L. group (Higashi 1974, Herbers 1979) and in the invasive red imported fire ant, *S. invicta* (Tschinkel 2006).

Solenopsis invicta is originally from the grasslands of South America and was introduced in the United States during the 1930s (Hölldobler and Wilson 1990, Tschinkel 2006). Since it was introduced, it has spread throughout the southern United States and can now be found in Mexico, some Caribbean Islands, Australia, New Zealand, and eastern Asia. It is not found in Africa or Europe yet (Ascunce et al. 2011, Wetterer 2013). However, climate models indicate that *S. invicta* may invade those continents (Morrison et al. 2004, Sutherst and Maywald 2005). *S. invicta* has become an invasive species likely due to lack of predators, high reproductive rate, and aggressive foraging behavior (Allen et al. 2004). This ant species causes serious impacts on agriculture, wildlife, and households (Adams 1986, Lofgren 1986, Vinson 2013).

Solenopsis invicta workers are divided into three physical castes based on body size: minors, medias, and majors (Wilson 1978). Polymorphic workers perform all colony tasks (Cassill and Tschinkel 1999), but minor and media workers are more likely to tend to the brood (Mirenda and Vinson 1981), while media and major workers defend

and forage for insect prey (Wilson 1978, Mirenda and Vinson 1981). It is suggested that larger workers are more effective at subduing and retrieving large prey. Smaller workers are also known to forage, but they specialize on small insect prey (Wilson 1978) or in the liquid portion of the prey (Mirenda and Vinson 1981). Major and media workers tend to move large objects, such as twigs, more so than minor workers and work on nest excavation (Wilson 1978). Previous studies are limited regarding how well each physical caste group of *S. invicta* performs tasks, such as digging and foraging different particle sizes.

Media workers in *S. invicta* appear to be the most versatile workers, able to tend to the brood and forage (Cassill and Tschinkel 1999). In addition, media workers move food among colony members by constantly receiving and donating it (i.e., trophallaxis), and they tend to groom other workers more so than minor and major workers (Mirenda and Vinson 1981). Overall, media workers are classified as generalists, because it is thought they can complete almost all colony tasks (Cassill and Tschinkel 1999). Such is the case for media workers of *Eciton rapax* Smith (Burton and Franks 1985), *Formica perpilosa* Wheeler (Brandão 1978), *Camponotus compressus* Fabricius (Sharma et al. 2004), and *A. sexdens* (Wilson 1980b, Wilson 1980a). It is hypothesized that media workers are vestiges of an ancestral, monomorphic condition (Wilson 1980b). In *S. invicta* they perform various tasks (Mirenda and Vinson 1981, Cassill and Tschinkel 1999), however, it is unknown whether particle size plays a role in their performing of different tasks.

Understanding particle size preference in *S. invicta* polymorphic workers can provide information aimed to improve bait efficiency (Jordan et al. 2013). A few studies have indicated that *S. invicta* workers prefer bait sizes of 1.0 mm or larger (Hooper-Bùi et al. 2002, Neff et al. 2011, Reed et al. 2015). This study combines behavioral and morphometric data to answer: (1) whether particle sizes moved by *S. invicta* workers correlate with body size when performing specific tasks, and (2) whether media workers are the most versatile workers among the three physical caste groups of *S. invicta* when handling different particle sizes.

Methods

Collection and Rearing

Colonies of *S. invicta* were collected around Bryan and College Station, Texas, along roads in farmland areas. Colonies were at least 1.2 km apart from each other. In the lab, colonies were maintained at 23°C under a 12 light: 12 dark regime. Each colony was placed in a plastic container (27 x 39.5 x 9.5 cm) with Fluon[®] (BioQuip Products, Inc., Rancho Dominguez, California) on the inner edges to prevent queens and workers from escaping. The bottom of the container was layered with paper towels. A nesting area for the queens, brood, and some workers was placed on one side of the container. The nesting area consisted of a Petri dish (14 cm in diameter) with two smaller Petri dishes within it (9 and 5.5 cm in diameter) to form three chambers for the colony (Figure 8). Queens and brood were usually found in the two inner chambers. Each chamber had one access opening. All three chambers were filled with approximately 0.5 cm of cream-

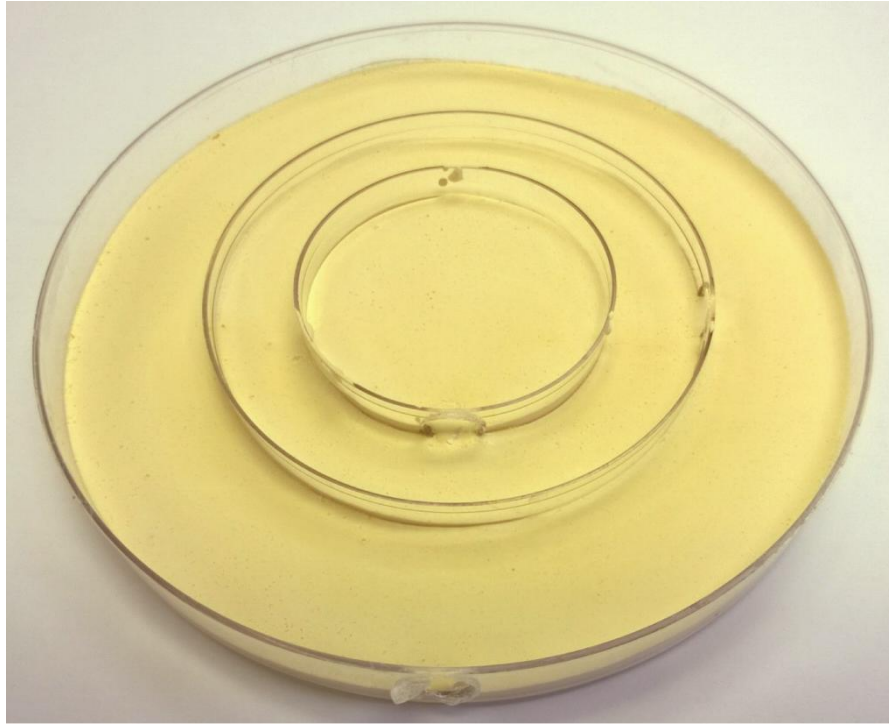


Figure 8. Structure of the nest for the queens, brood, and workers in the digging and foraging experiments. Queens and brood are normally located in the two inner chambers.

colored plaster (Castone[®] Dental Stone, Patterson Logistics, Fort Worth, Texas) that provided humidity for the brood when moistened periodically with de-ionized water. A water source was placed near the nesting area, which consisted of de-ionized water in a test tube (15 x 2 cm) stuffed with a cotton ball. Two Petri dishes (9 cm diameter) for food sources were placed opposite to the nesting area and to the water source. Frozen crickets, *Acheta domesticus*, were added to one of the small Petri dishes, while the other one contained a plastic weighing boat (5 x 1 cm) with a small amount of cotton that was used to absorb honey water (25% honey in de-ionized water).

Preparing Glass Beads

Different sizes of clear glass beads (BioSpec Products, Inc., Bartlesville, Oklahoma) were used for our experiments: 0.25, 0.50, 1.0, and 2.0 mm in diameter. Glass beads were cleaned before each trial by placing them in a sonicator (Cole-Parmer, Chicago, Illinois) with de-ionized water and Alconox[®] detergent powder for about 10 minutes. Then still within the sonicator, glass beads were rinsed six times with de-ionized water for about ten minutes each time to remove any soap residue.

Digging Behavior Experiment

A plastic cylinder container (2.5 x 2.0 cm) with an attached snap-on lid was glued to the bottom of one side of a Petri dish (9 cm diameter) (Figure 9). The cylinder container was filled with glass beads. A hole was drilled into both the bottom of the Petri dish and the lid of the cylinder container for workers to have access to the glass beads. In

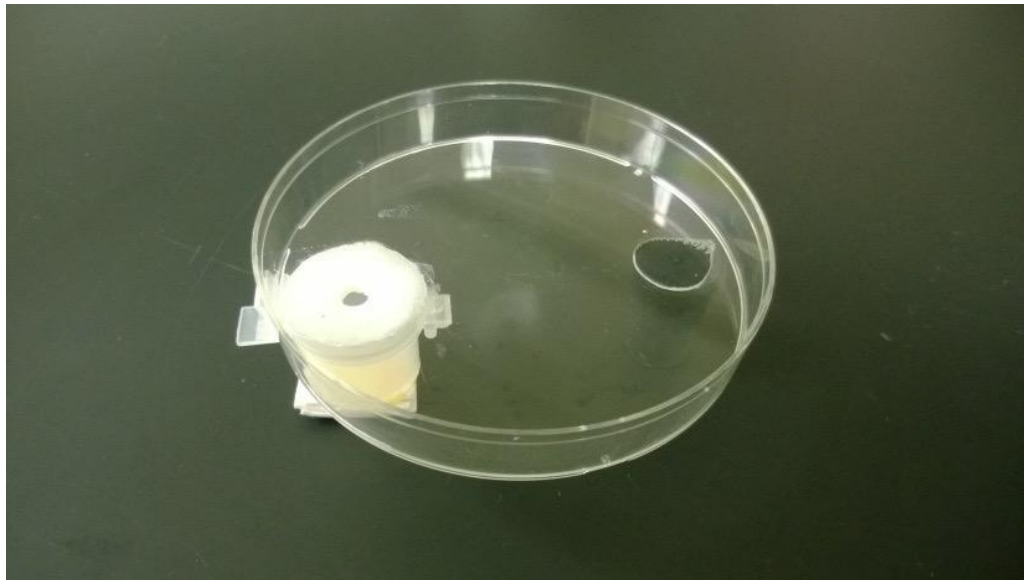


Figure 9. Set-up for the digging behavior experiment. The plastic cylinder container was filled with clear glass beads of the same size.

addition to the cylinder container, a black rubber stopper was glued to the bottom of the Petri dish opposite of the cylinder container for balance.

Workers from ten monogyne colonies (i.e., a colony having a single queen) were used for this experiment. One trial for each of the four bead sizes was performed for each colony, thus, there were a total of ten trials per bead size. For each trial, approximately 100 workers were randomly selected by exploiting their aggressive behavior using a wooden applicator stick to which they climbed or they were obtained by soft forceps. Each trial had roughly equal proportions of the physical castes. Talcum powder (Johnson & Johnson Consumer Companies, Inc., Skillman, New Jersey) was applied to the inner edges of the Petri dish to prevent workers from escaping. For each trial, the cylinder container was filled with glass beads of the same size. Workers were given the opportunity to dig into the glass beads for 1 hour. While viewing under a microscope (Olympus, SZX12, Japan), any workers that brought a glass bead up to the surface of the Petri dish were removed and placed in a freezer (-17 °C). Trials were conducted between 12:00 to 18:00. Any left over workers from each trial went back to the original colony. The heads of frozen workers were removed from the body and their head width between the eyes was measured under a microscope. Head width is a common measurement to determine ant body size (Tschinkel et al. 2003). The physical caste was determined by the head width (Table 1) (Wilson 1978).

A regression analysis was used to determine if there was a linear relationship between worker body size and glass bead size. A chi-square test of independence was used to determine if there was an association between the proportion of workers in each

Table 1. Head width measurements of the three physical caste groups of *S. invicta* for digging behavior experiment (Wilson 1978).

Minors	Media	Majors
Head width < 0.73 mm	Head width 0.73 to 0.92 mm	Head width > 0.92 mm

of the physical castes and the workers' preference of glass bead size. A chi-square test of homogeneity was used to determine if media workers were the most versatile workers among the three physical castes when dealing with the different glass bead sizes. A randomized block design and Tukey's test were used to determine if there was a difference in the average number of workers handling the different glass bead sizes. For all statistical tests, the level of significance was set at an alpha value of 0.05.

Foraging Behavior Experiment

Workers from six monogyne and four polygyne colonies (i.e., a colony having more than one queen) were used for this experiment. Original colonies were reduced to approximately 200 workers, along with one queen and some brood. This allowed all experimental colonies to be approximately equal in colony size and also allowed the ten colonies to have roughly an equal proportionality in the three physical castes. Experimental colonies had a similar environment compared to the original colonies to allow workers to forage (Figure 10). However, instead of a layer of paper towels, there was 1 cm of white sand. The two Petri dishes for food sources were also placed upside down with the edges buried in the sand. This provided a leveled environment for workers to move the glass beads laterally if they chose to.

The glass bead sizes used for this experiment consisted of 0.5, 1.0, and 2.0 mm. Glass bead size of 0.25 mm was not used due to being similar size to the white sand particles. The inside contents of the cricket *A. domesticus* were applied to the glass beads to attract foragers. Before the trials, crickets were removed from the food source 2 days

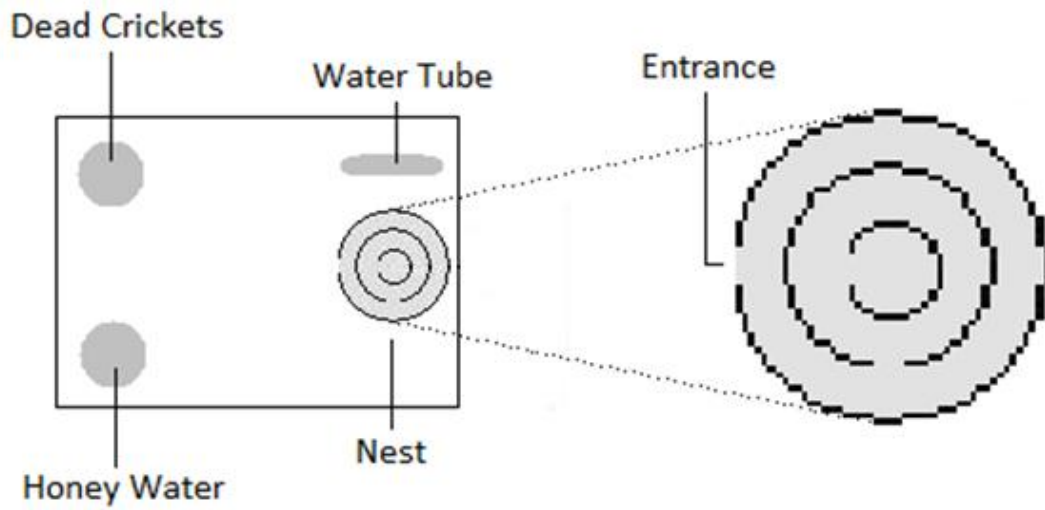


Figure 10. Experimental set-up for the foraging behavior experiment. Glass beads were introduced in the area where crickets would normally be located.

beforehand. Trials were conducted between 12:00 to 18:00. At the beginning of each trial for each colony, a cricket was introduced in the food source area where crickets were normally located and was removed once workers of different body sizes were recruited to the area.

Two components were performed for this experiment. The first component determined how long it took workers to move a glass bead to the halfway point (8.5 cm) between the center of the area, where the cricket was initially, and the entrance of the nest (Figure 10). The halfway point was designated by a piece of white string attached overhead to the edges of the plastic container containing the environment for each colony. A set of glass beads of three different sizes were introduced separately in the area where the cricket was initially. Once a worker started interacting with the glass bead, a stopwatch (StopWatch Application for Windows Phone) was started. The stopwatch was stopped when the glass bead was moved to the halfway point. However, because workers could take a while to move the glass bead to the halfway point, after 15 minutes, the time was recorded and the bead was removed. Once an entire set of beads was completed, then the trial was started on another colony. Each colony experienced five sets of beads, in which five of each bead size was introduced to each colony.

The second component of this experiment was similar to the first component, except when a worker was able to move the glass bead 1 cm by either carrying or dragging it after it was introduced, then the worker was removed and placed in a freezer. The heads of frozen workers were removed from the body and their head width between the eyes was measured under a microscope. Head width is a common measurement to

determine ant body size (Tschinkel et al. 2003). The physical caste was determined by the head width (Table 2) (Wilson 1978).

For the first component of this experiment, a randomized block design was used to determine if the type of colony (i.e., monogyne and polygyne) had an effect on foraging behavior. In addition, the Kaplan-Meier method (Rich et al. 2010) was used to determine if there was a difference between the distribution of the time that workers used to move glass beads for the three sizes of glass beads in the foraging experiment.

For the second component of this experiment, a regression analysis was used to determine if there was a linear relationship between worker body size and the size of glass bead selected by the worker. A chi-square test of independence was used to determine if there was an association between the proportion of workers in each of the physical castes and the workers' preference of glass bead size. A chi-square test of homogeneity was used to determine if media workers were the most versatile workers among the three physical castes when dealing with the different glass bead sizes. For all statistical tests, the level of significance was set at an alpha value of 0.05.

Results

Digging Behavior

The average body size of *S. invicta* workers digging in experimental arenas was significantly different among the four types of arenas as defined by their glass bead size (One-way ANOVA: $F = 117.9, p < 0.0001$; Figure 11). When *S. invicta* workers dug into four different sizes of glass beads, the average body size of the workers correlated to the

Table 2. Head width measurements of the three physical caste groups of *S. invicta* for foraging behavior experiment (Wilson 1978).

Minors	Media	Majors
Head width < 0.73 mm	Head width 0.73 to 0.92 mm	Head width > 0.92 mm

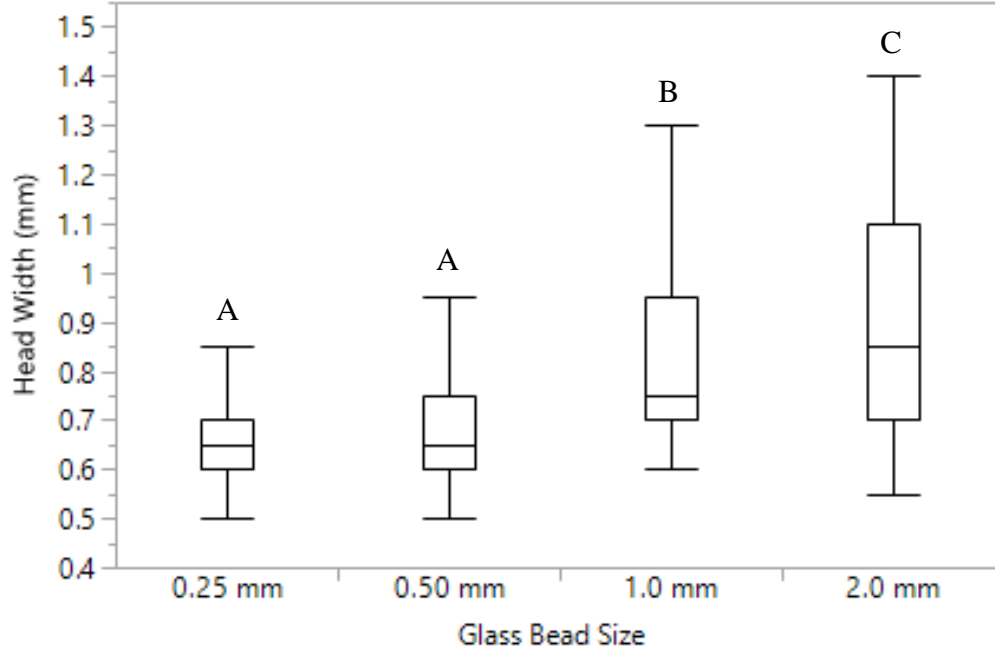


Figure 11. Head width of all the workers digging into four glass bead sizes. Similar letters indicate no difference in the head width of the workers, while different letters indicate a difference in the head width of the workers ($p = 0.05$).

glass bead sizes as expressed by a cubic polynomial regression model relating average body size of the worker to the size of the selected glass bead ($R^2 = 0.19$, $F = 117.9$, $p = 0.0005$). Different physical castes of *S. invicta* varied significantly in their preference for glass bead sizes when digging. This difference was expressed by a chi-square test of independence (chi-square = 275.1, $p < 0.0001$; Figure 12).

Media workers show a preference amongst the four glass bead sizes. The proportion of media workers selecting a 0.50 mm glass bead was significantly larger than the proportion of workers selecting a glass bead of one of the other three glass bead sizes (Chi-square test of homogeneity: chi-square = 38.3, $p < 0.0001$; Figure 13). Pairwise comparisons of the four proportions indicate that the proportion of media are not significantly different for 0.25 and 1.0 mm glass beads (Chi-square test of homogeneity: chi-square = 1.9, $p = 0.17$).

The average number of workers digging among the four glass bead sizes was significantly different (Randomized block design: $F = 16.1$, $p < 0.0001$; Figure 14). The average number of workers selecting 0.50 mm glass beads was larger than the other three bead sizes, while 2.0 mm glass beads had the smallest average number of workers.

Foraging Behavior

There was no difference in foraging behavior and body size of *S. invicta* workers between monogyne and polygyne colonies in this experiment (Two-factor ANOVA: $F = 1.4$, $p = 0.24$). There were differences in worker average body sizes among the three

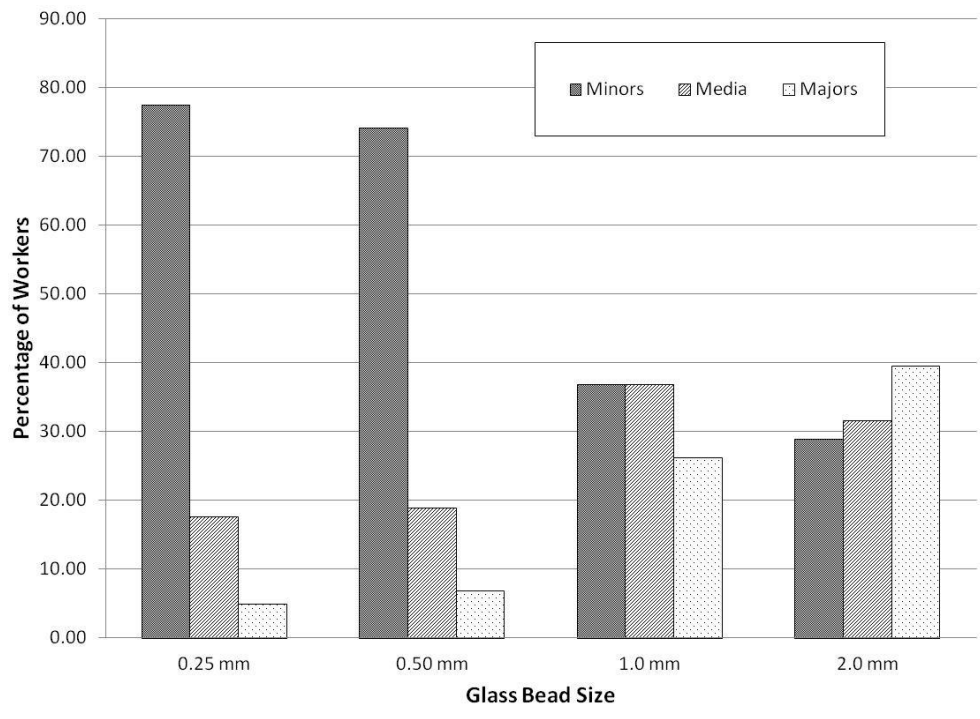


Figure 12. The percentage of workers for each physical caste group digging into four glass bead sizes. Different letters indicate a difference among the four glass bead sizes within the physical caste. ($p < 0.001$)

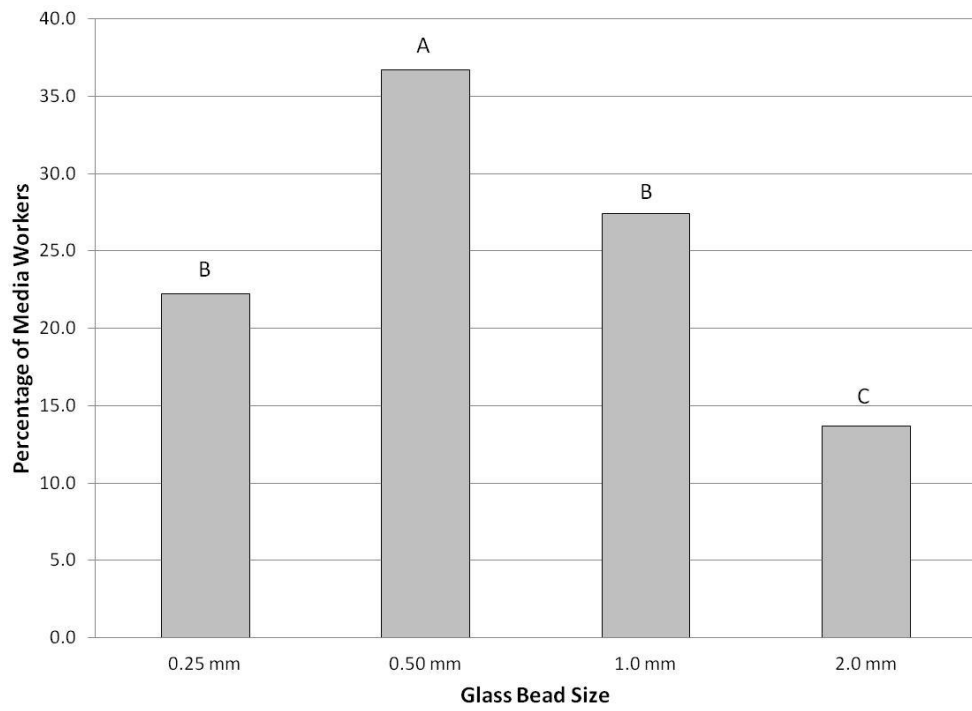


Figure 13. Percentage of the total number of media workers digging into four glass bead sizes. Different letters indicate a difference in the proportion of media workers ($p < 0.0001$).

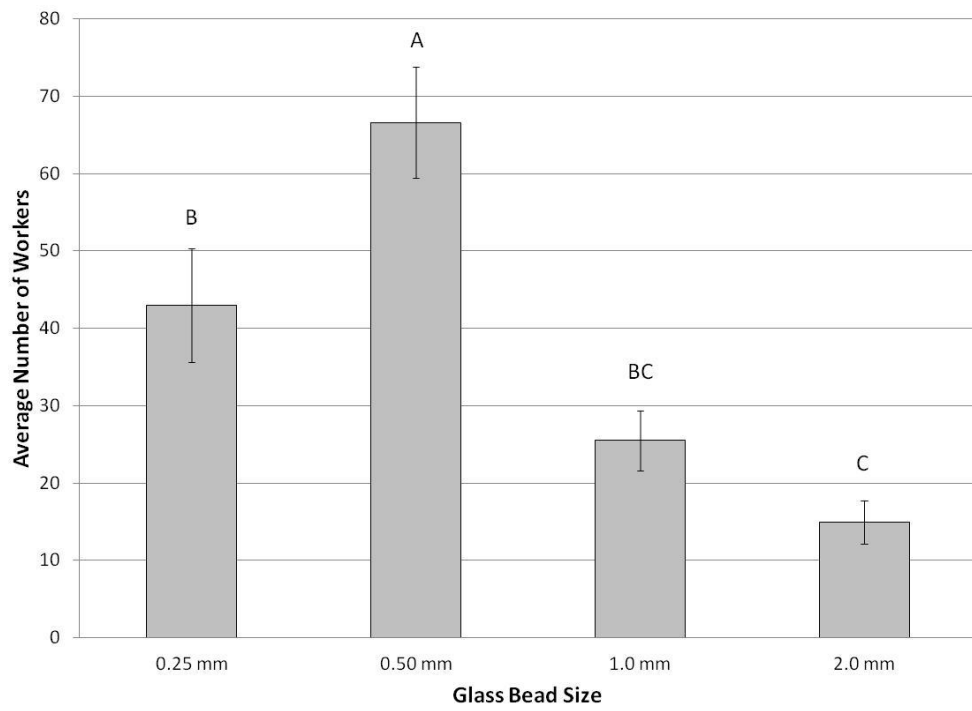


Figure 14. Average number of workers digging into four glass bead sizes. Different letters indicate a difference in the average number of workers ($p < 0.0001$).

glass bead size (One-way ANOVA: $F = 12.5$, $p < 0.0001$; Figure 15). When workers foraged for three different sizes of glass beads, the average body size of workers correlated to the glass bead sizes (Simple linear regression: $R^2 = 0.18$, $F = 320.2$, $p < 0.0001$). Different physical castes vary in their preferences for different bead sizes (Chi-square test of independence: $\text{chi-square} = 23.5$, $p = 0.0001$; Figure 16). The proportion of media workers that foraged was not significantly different for the three glass bead sizes (Chi-square test of homogeneity: $\text{chi-square} = 55.2$, $p = 0.58$; Figure 17).

There was not a significant difference in the amount of time it took the workers to move the three glass bead sizes to the halfway point in the container between the monogyne and polygyne colonies in this experiment (Kaplan-Meier: $\text{chi-square} = 0.058$, $p = 0.81$; Figure 18). However, there was a significant difference in the average amount of time it took the workers to move the three glass bead sizes to the halfway point in the container (Kaplan-Meier: $\text{chi-square} = 123.6$, $p < 0.0001$; Figure 19).

Discussion

The body size of workers in *S. invicta* correlates to the sizes of objects when digging and foraging with the exception when workers were digging in 0.25 and 0.50 mm glass beads. This result aligns with the results of previous studies. It has been observed that media and majors of *S. invicta* carry large insect prey back to the colony, while minors carry small insect prey (Wilson 1978) or take in the liquid portion of the prey (Mirenda and Vinson 1981). This study went beyond previous studies by observing how well the different body sizes of workers handle different sizes of objects. Overall,

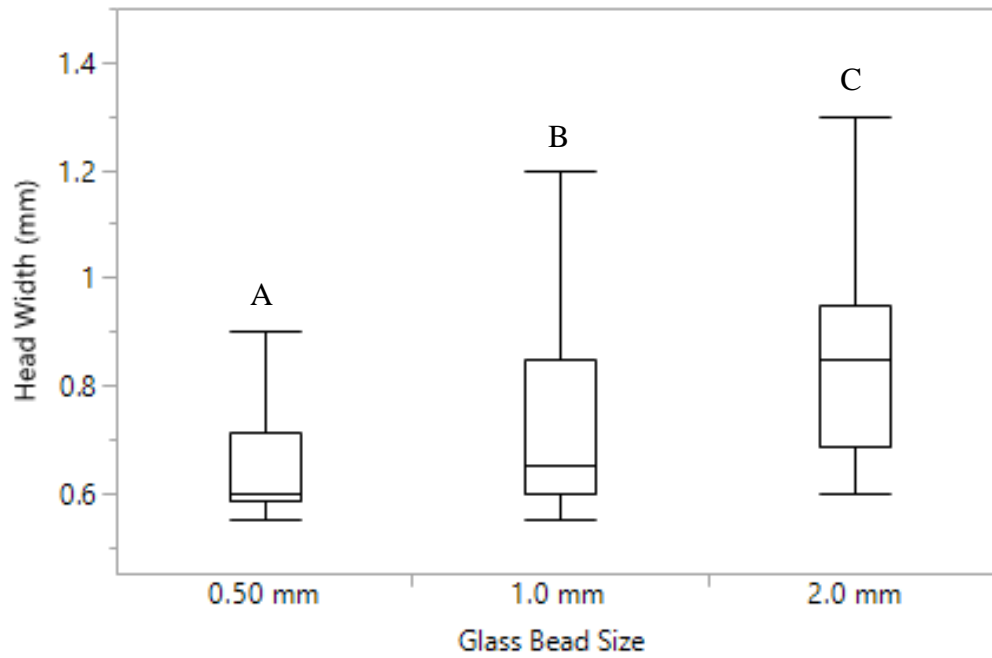


Figure 15. The head width of the all workers foraging for three glass bead sizes. Different letters indicate a difference in the head width of the workers ($p < 0.0001$).

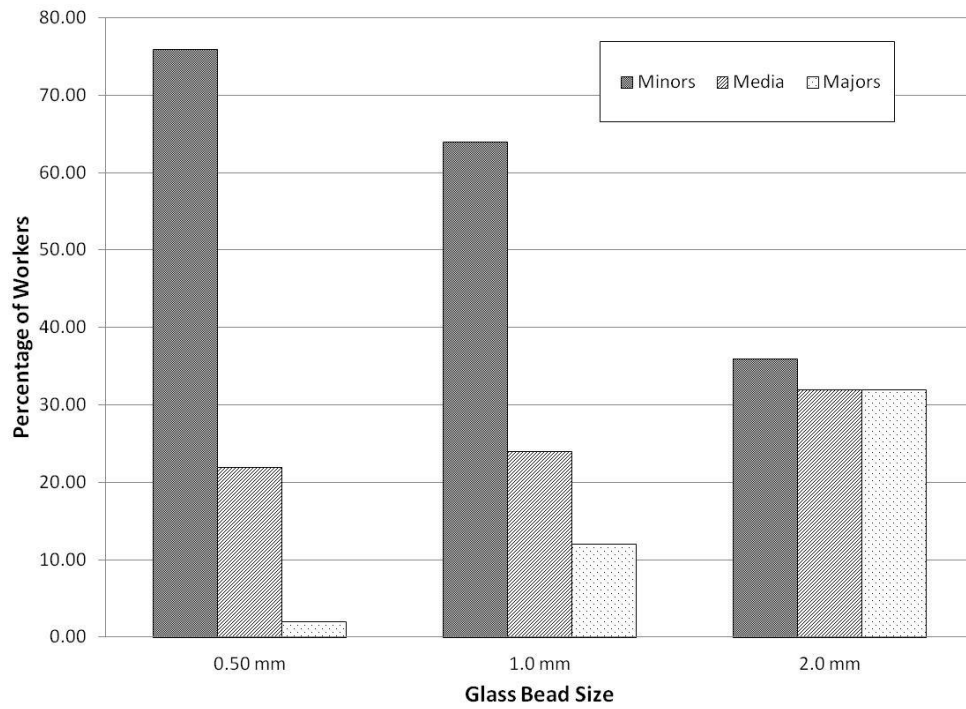


Figure 16. The percentage of workers for each physical caste group foraging for three glass bead sizes. Different letters indicate a difference among the three glass bead sizes within the physical caste ($p = 0.0001$).

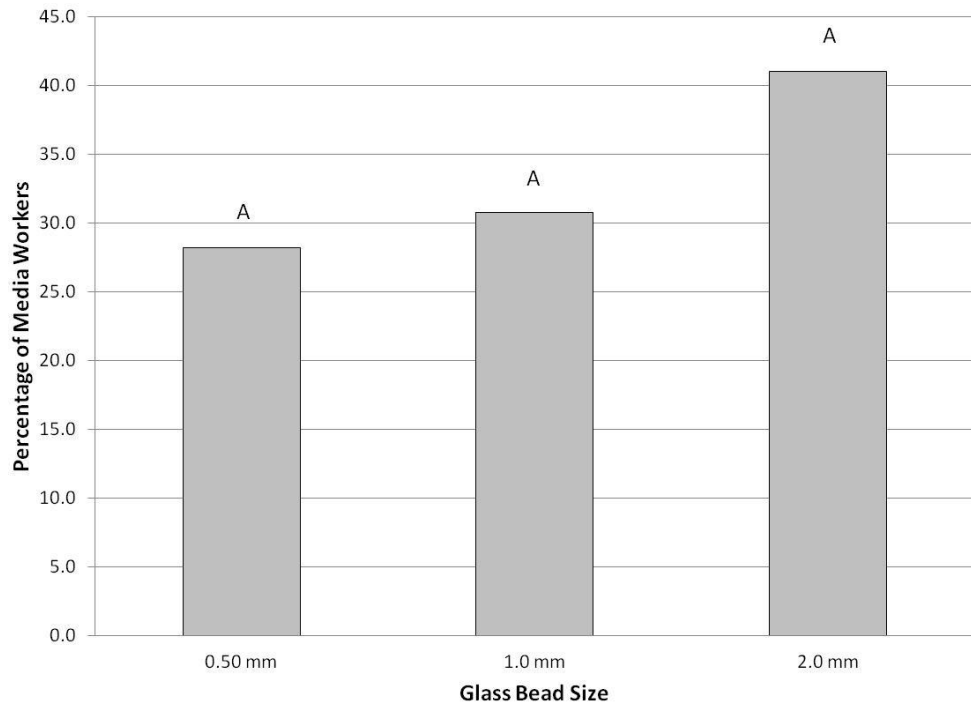


Figure 17. Percentage of the total number of media workers foraging for three glass bead sizes. Similar letters indicate no difference in the proportion of media workers ($p = 0.58$).

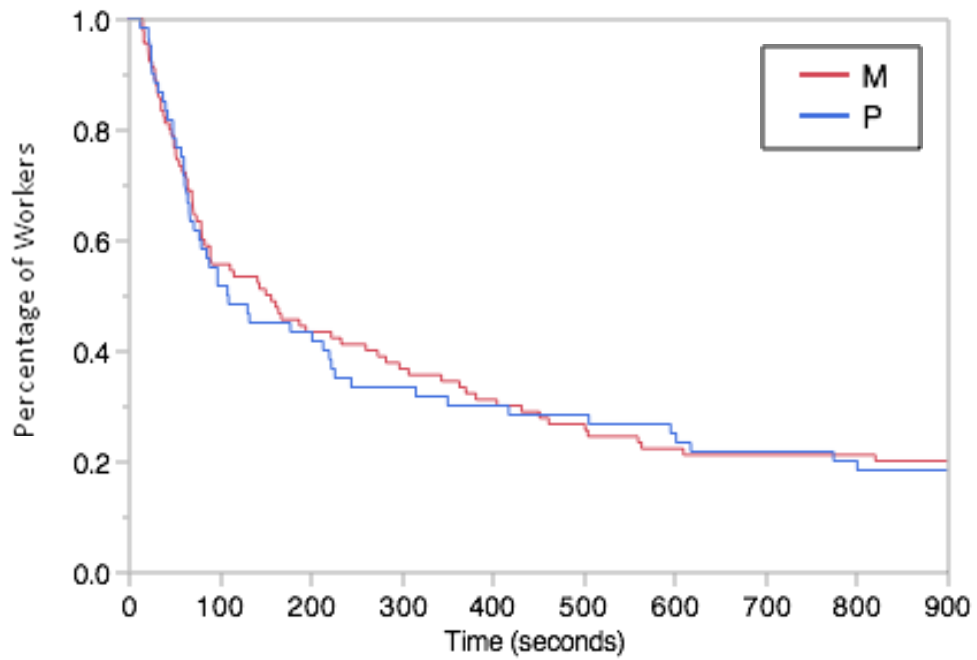


Figure 18. The time it took a percentage of workers to move the three glass bead sizes to the halfway point between the food and the entrance of the nest between monogyne and polygyne colonies. M = monogyne and P = polygyne ($p = 0.81$).

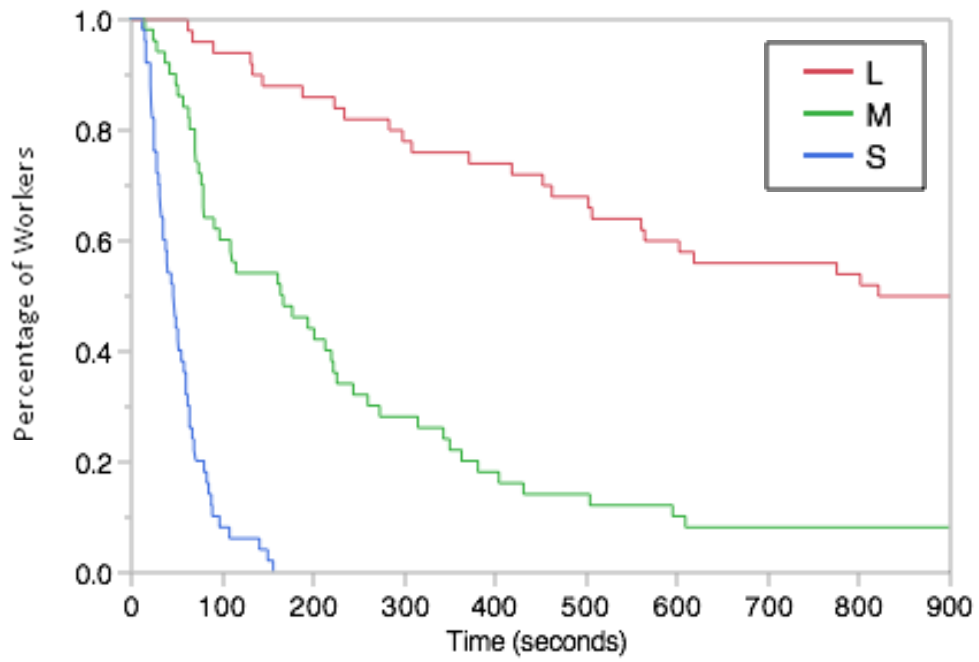


Figure 19. The time it took a percentage of workers to move the three glass beads to the halfway point between the food and entrance of the nest. S = 0.50 mm, M = 1.0 mm, and L = 2.0 mm ($p < 0.0001$).

larger workers of *S. invicta* have the ability and are more effective in moving large objects (Wilson 1978, Wood and Tschinkel 1981).

When *S. invicta* workers dig or forage, the percentage of minors involved in these behaviors decreases as the size of objects increases. On the other hand, the percentage of media and majors increases as the size of objects increases. However, the percentages of majors with the larger glass bead sizes are not as high as the percentages of minors with the smaller glass bead sizes. It appears as a whole that majors do not dig and forage as much as minors. In another study, it was observed that major workers have poor sanitary habits by rarely helping with removing waste from the colony and they are less attentive towards brood than smaller workers (Porter and Tschinkel 1985b). Overall, the role of majors in *S. invicta* is not well understood. It could be that majors have evolved to be important for performing tasks that are relevant in their native area of South America, but have become less important in non-native areas where *S. invicta* has invaded. It is also important to consider the possible behavioral differences in majors when studying field and lab populations. Some studies have suggested that majors are important for foraging insect prey, storing food, handling and transporting sexual brood, defending the colony, and building the nest (reviewed in Porter and Tschinkel 1985). It is also suggested that larger workers are important for making rafts during floods (Cassill et al. 2015).

Media workers in *S. invicta* are versatile workers when foraging for spherical objects ranging from 0.50 to 2.0 mm in diameter. However, they are not versatile workers when digging in spherical objects ranging from 0.25 to 2.0 mm in diameter.

Media workers clearly preferred 0.50 mm beads. If the experiment was more controlled, in which a set maximum number of workers were allowed to dig for each glass bead size, then the results could potentially have changed. However, by only letting a certain number of workers dig, then a size preference would not have been observed.

When digging, the lowest percentage of media workers was observed handling the 2.0 mm beads, which could be due to the fact they have problems picking up that size when removing the glass bead from the hole while digging. In contrast, when foraging, media workers preferred 2.0 mm beads. This could be because media have problems going against gravity when picking up glass beads, but less likely have problems going against friction when dragging glass beads while foraging.

A large amount of the literature generally focuses only on minors and majors in ant species that have polymorphic workers, such as *Atta sexdens rubropilosa* Forel and *Solenopsis interrupta* Santschi (Depickere et al. 2008), and *Oecophylla smaragdina* Fabricius (Chapuisat and Keller 2002). However, studies should also explore media worker behaviors, which are classified as generalists due to the fact they are thought to complete all the tasks of a colony compared to minors and majors (Mirenda and Vinson 1981, Cassill and Tschinkel 1999). The digging and foraging behavioral experiments conducted in this study can be used to determine how well media workers can handle different sizes of objects when performing different tasks in other ant species that have polymorphic workers (Brandão 1978, Burton and Franks 1985, Sharma et al. 2004).

Previous studies have indicated that *S. invicta* prefer bait sizes of 1.0 mm or larger in diameter (Hooper-Bùi et al. 2002, Neff et al. 2011, Reed et al. 2015). However,

it was indicated that *S. invicta* workers visited baits ranging from 0.59 to 0.84 mm more often compared to smaller and larger bait sizes (Hooper-Bùi et al. 2002). It was also observed that smaller workers prefer smaller bait sizes, while larger workers prefer larger bait sizes (Neff et al. 2011). Overall, my study shows that baits used for pest control would be more efficient if they were around 0.50 mm in diameter. Not only did workers prefer 0.50 mm glass beads, but they also handled and transported them faster when foraging compared to the other glass bead sizes tested.

Interestingly, as a whole in the digging behavior experiment, the larger glass bead size of 0.50 mm was more preferred than the smaller glass bead size of 0.25 mm. It is assumed that if given the choice, the workers would prefer the smallest size. It is possible that the 0.25 mm glass bead size was too small for the workers to grasp with their mandibles. During several observations when workers were digging in 0.25 mm glass bead sizes, it was observed that some workers were removing clusters of glass beads that consisted of at least two glass beads stuck together, which was also observed in previous unpublished digging behavior experiments (Asha Rao, personal communication). It appears that the workers added a substance to allow the glass beads to stick together, which could potentially make it easier to handle compared to one glass bead. Little is known about this phenomenon in ants, but it is potentially similar to the cement material used by termites when building their nests (Brossard et al. 2007, Korb 2010). This will need to be explored further with *S. invicta*, including the origin and composition of the chemical used for sticking the glass beads together.

It is unlikely that workers would handle and transport smooth, spherical objects in nature as presented in this study. Therefore, future studies should involve the use of glass beads that have a rough texture. It is then possible that the workers could grasp the glass beads with the rough textured surface more easily compared to the smooth surfaced glass beads in this study. In addition, minors may potentially be able to handle and transport more of the larger glass bead sizes with the rough textured surface when digging and foraging compared to this study.

Future studies involving minor workers in *S. invicta* should also be conducted. Minims are the smallest workers among the physical caste groups of *S. invicta* (Hölldobler and Wilson 1990, 2009). The average head width of the minims is 0.51 mm (Tschinkel 1988) with the lowest observed head width around 0.45 mm (Wood and Tschinkel 1981). Minims are the first workers to appear in an incipient colony and therefore, must perform all the tasks for the survival of the colony without any help from minors, media, and majors. Studies on this physical caste are limited. Little is known about the role of minims in tending to brood, nest maintenance, and foraging (Porter and Tschinkel 1986). This study could be repeated testing minims and observing how well they handle and move different sizes of glass beads when digging and foraging. Based on their head width measurements (Wood and Tschinkel 1981, Tschinkel 1988), it is likely that minims can handle the smaller glass beads (i.e. 0.25 and 0.50 mm) compared to the larger glass beads (i.e. 1.0 and 2.0 mm). Unfortunately, minims were not tested in this study because the study used mature colonies and to obtain minims, mated queens need to be found before they start making a nest.

Results from the digging and foraging behavioral studies reinforce the general idea that larger body sizes of workers can handle large objects, while smaller body sizes of workers can handle smaller objects. These studies went further by looking at how well each physical caste group of *S. invicta* performs tasks involving different sizes of particles, including observing that media workers are versatile workers when foraging for glass bead sizes ranging from 0.50 to 2.0 mm. However, the same conclusion cannot be stated for media workers when digging due to the possibility that they have problems picking up larger glass beads due to the mass of the glass bead and the gravitational pull being exerted on the glass bead. Overall, these results can be used to improve the efficiency of baits to control populations of *S. invicta* in non-native areas.

CHAPTER IV

BEHAVIORAL RESPONSE BY THE RED IMPORTED FIRE ANT *Solenopsis invicta* BUREN TO RECOGNITION CHEMICALS ON INANIMATE OBJECTS

Introduction

Social insects rely on chemicals for the survival of the colony. Some social insects produce alarm pheromones, such as wasps (Fortunato et al. 2004, Bruschini et al. 2006), bees (Breed et al. 2004b, Slessor et al. 2005), and termites (Šobotník et al. 2008, Costa-Leonardo et al. 2009). Chemicals are also used for making trails in wasps (Jeanne 1981, Smith et al. 2002, Jandt et al. 2005), bees (Jarau 2009), and termites (Costa-Leonardo et al. 2009, Bordereau and Pasteels 2010). Ants also rely heavily on chemicals for alarming other workers (Jackson and Morgan 1993, Hölldobler 1999) and to recruit workers to a food source (Hölldobler and Wilson 1990, 2009).

Recognition chemicals are a group of pheromones used by ants to recognize other nestmates in the colony (Hölldobler and Wilson 1990, Smith and Breed 1995, d’Ettorre and Lenoir 2010). Recognition chemicals are also used by wasps (Lorenzi et al. 1997, Ruther et al. 2002), bees (Breed 1998, Breed et al. 2004a), and termites (Haverty et al. 1999, Aguilera-Olivares et al. 2016). This recognition system helps protect the colony’s resources from competitors and parasites (d’Ettorre and Lenoir 2010, Bos and d’Ettorre 2012).

Recognition chemicals in social insects can come from multiple sources, such as from queens and workers producing the chemicals and passing them to others within a

colony, nest construction materials, floral oils, and food (Smith and Breed 1995, van Zweden and d'Ettorre 2010). The source of recognition chemicals in ants either comes from the postpharyngeal gland, which is shared by trophallaxis, or from the cuticle, which is shared by grooming behaviors (vander Meer and Morel 1998). Recognition chemicals on other ants are detected by the antennae. It is thought that workers use their antennae to identify recognition chemicals by comparing any recognizable chemical with a database of chemicals found in their colony. This database is likely stored in the workers' nervous system (Lenoir et al. 1999, Hölldobler and Wilson 2009, Bos and d'Ettorre 2012). An encountered individual will be accepted if the perceived differences in recognized chemicals do not reach a specific threshold (van Zweden and d'Ettorre 2010, Bos and d'Ettorre 2012).

Cuticular hydrocarbons are important recognition chemicals in ants (Lenoir et al. 1999, Howard and Blomquist 2005). Hydrocarbons can provide a large amount of information about an ant, such as the species and the colony that the ant belongs to, along with the ant's task group and reproductive status (Howard and Blomquist 2005, d'Ettorre and Lenoir 2010). Hydrocarbons are generally nonvolatile, and therefore, they are only efficient as a recognition cue when individuals are within 1 cm of each other (Brandstaetter et al. 2008, d'Ettorre and Lenoir 2010). Other than hydrocarbons, polar lipids could potentially be used as recognition cues by ants, such as fatty acids (Franks et al. 1990).

Solenopsis invicta uses recognition chemicals to identify nestmates (Obin 1986, Obin and Vander Meer 1988, Vander Meer et al. 2008). This ant species is originally

from the grasslands of South America and was introduced in the United States during the 1930s (Hölldobler and Wilson 1990, Tschinkel 2006). Since it was introduced, it has spread throughout the southern United States and can now be found in Mexico, some Caribbean Islands, Australia, New Zealand, and eastern Asia. It is not found in Africa or Europe yet (Ascunce et al. 2011, Wetterer 2013). However, climate models indicate that *S. invicta* may invade those continents (Morrison et al. 2004, Sutherst and Maywald 2005). *Solenopsis invicta* has become an invasive species likely due to lack of predators, high reproductive rate, and aggressive foraging behavior (Allen et al. 2004). This ant species causes serious impacts on agriculture, wildlife, and households (Adams 1986, Lofgren 1986, Vinson 2013).

For studying recognition chemicals, several studies with different ant species apply recognition chemicals to inanimate objects, which are then introduced to workers to observe their behavioral response (Akino et al. 2004, Ozaki et al. 2005, Greene and Gordon 2007, Martin et al. 2008, Tanner 2008, Tanner and Adler 2009). This method is useful because the behavioral response of workers can be determined only when the worker encounters a chemical extract instead of using other possible cues, such as acoustic or visual cues, when interacting with actual workers (Tanner 2008). Some studies just introduce the treated inanimate objects to workers (Akino et al. 2004, Ozaki et al. 2005), while other studies place the treated objects in specific locations of the colony's environment (Greene and Gordon 2007, Tanner and Adler 2009). Different studies have applied different pheromones on inanimate objects to determine the behavioral responses by *S. invicta* (Walsh and Tschinkel 1974, Howard and Tschinkel

1976, Lofgren et al. 1983, Lamon and Topoff 1985). However, little is known how workers respond to recognition chemicals on inanimate objects. It has been suggested that behavioral experiments with cuticular hydrocarbons need to be performed to determine if the cuticular hydrocarbons are used for nestmate recognition in *S. invicta* (Lavine et al. 2011).

Understanding more about the behavioral response of *S. invicta* workers to recognition chemicals can improve the efficiency of baits aimed to reduce their populations in non-native areas. Recognition chemicals can be applied to baits to improve the attraction that workers have to the baits. Studies have already observed *S. invicta* workers finding baits with recognition chemicals sooner than baits without recognition chemicals (Vander Meer 1996, Wiltz et al. 2010). A similar study from the invasive Argentine ant *Linepithema humile* Mayr had similar results (Welzel and Choe 2016).

This study involved a behavioral experiment performed in the laboratory to determine the reaction of workers to recognition chemicals placed on inanimate objects. More specifically, this study explored how workers responded to cuticular hydrocarbons and polar lipids applied to glass beads placed at different locations within the colony's environment.

Methods

Collection and Rearing

Colonies of *S. invicta* were collected around Bryan and College Station, Texas, along roads in farmland areas. Colonies were at least 1.2 km apart from each other. In the lab, colonies were maintained at 23°C under a 12 light: 12 dark regime. Each colony was placed in a plastic container (27 x 39.5 x 9.5 cm) with Fluon[®] (BioQuip Products, Inc., Rancho Dominguez, California) on the inner edges to prevent queens and workers from escaping. The bottom of the container was layered with paper towels. A nesting area for the queens, brood, and some workers was placed on one side of the container. The nesting area consisted of a Petri dish (14 cm in diameter) with two smaller Petri dishes within it (9 and 5.5 cm in diameter) to form three chambers for the colony. Queens and brood were usually found in the two inner chambers. Each chamber had one access opening. All three chambers were filled with approximately 0.5 cm of cream-colored plaster (Castone[®] Dental Stone, Patterson Logistics, Fort Worth, Texas) that provided humidity for the brood when moistened periodically with de-ionized water. A water source was placed near the nesting area, which consisted of de-ionized water in a test tube (15 x 2 cm) stuffed with a cotton ball. Two Petri dishes (9 cm diameter) for food sources were placed opposite to the nesting area and to the water source. Frozen crickets, *Acheta domestics* Linnaeus, were added to one of the small Petri dishes, while the other one contained a plastic weighing boat (5 x 1 cm) with a small amount of cotton that was used to absorb honey water (25% honey in de-ionized water).

Preparing Glass Beads

2.0 mm in diameter glass beads (Czech glass beads, Crafts, Etc!, Oklahoma City, Oklahoma) were used for this experiment. At that size, it was less likely for workers to move the glass beads during the study. Each glass bead consisted of a hole going through the entire glass bead. Different colors of glass beads (silver, blue, yellow, yellowish-orange, and red) were used to distinguish between control and experimental glass beads. Glass beads were cleaned before each trial by placing them in a sonicator (Cole-Parmer, Chicago, Illinois) with de-ionized water and Alconox[®] detergent powder for about 15 minutes. Then still within the sonicator, glass beads were rinsed ten times with de-ionized water for about ten minutes each time to remove any soap residue.

Chemical Extracts and Glass Beads

Purified and separated extractions of both cuticular hydrocarbons and polar cuticular lipids were obtained by liquid chromatography, which was provided by the lab of Dr. Robert Renthall from The University of Texas at San Antonio. The recognition chemicals were applied to the glass beads, which is a common method used to observe how ants respond to recognition chemicals on inanimate objects (Ozaki et al. 2005, Greene and Gordon 2007, Martin et al. 2008, Tanner 2008, Tanner and Adler 2009). This method is useful because the behavior of workers can be determined when a worker encounters only the chemical extract instead of another worker (Tanner 2008). For applying the cuticular hydrocarbons to the glass beads, 0.5 mL of pure pentane was added to a vial with the extract and the vial was carefully shook for the extract to be

evenly applied to the surface of the glass beads. Observations from preliminary work indicated that workers respond to glass beads equivalent to ten workers. When the experimental glass beads with cuticular hydrocarbons were used in the experiment, they were paired with control glass beads, which only had pure pentane applied to them. A similar method was used to apply the cuticular lipids to the glass beads, except acetonitrile was used instead of pure pentane. All glass beads were then placed in a fume hood to completely remove the solvents before being used in the experiment.

Experimental Set-Up

Five monogyne colonies (i.e., a colony having a single queen) and five polygyne colonies (i.e., a colony having more than one queen) were used for this experiment. Original colonies were reduced to approximately 200 workers, along with one queen and some brood. This allowed all experimental colonies to be approximately equal in colony size and also allowed for easier observations to see how workers interact with glass beads if they chose to. Experimental colonies had a similar environment compared to the original colonies. However, instead of a layer of paper towels, there was 1 cm of white sand. The two Petri dishes for food sources were also placed upside down with the edges buried in the sand and a hole (4 mm) was placed in the center of the Petri dish lid for the nest to place glass beads in the inner chamber of the nest (Figure 20).

In separate observations, glass beads were placed in three locations of the experimental set-up: (1) between the two Petri dishes for food sources, (2) near the entrance of the nest, and (3) in the inner chamber of the nest (Figure 21). These locations

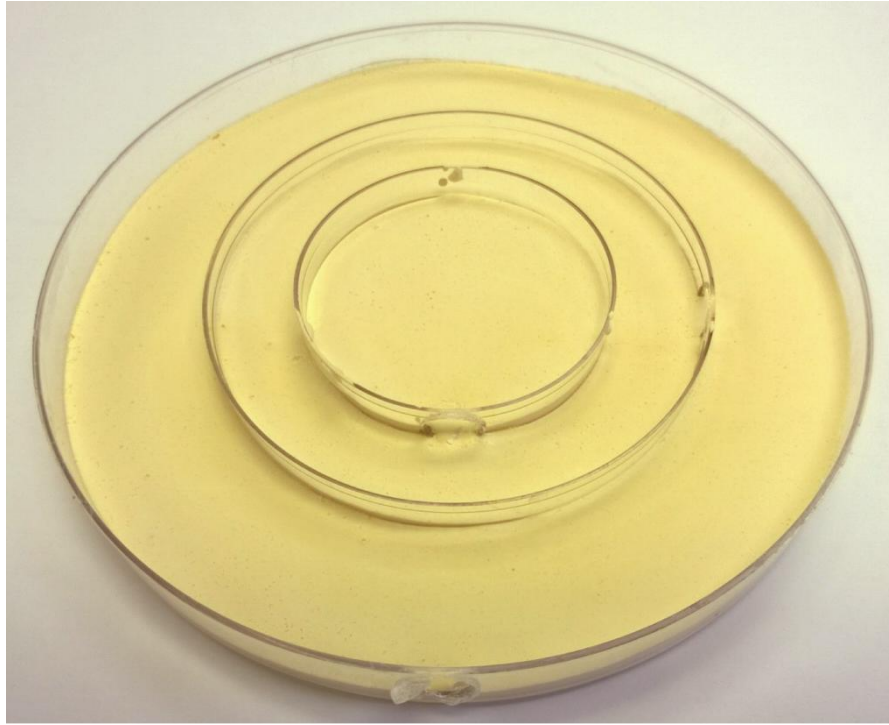


Figure 20. The structure of the nest for the queens, brood, and workers for the recognition chemical experiments. Queens and brood are normally located in the two inner chambers.

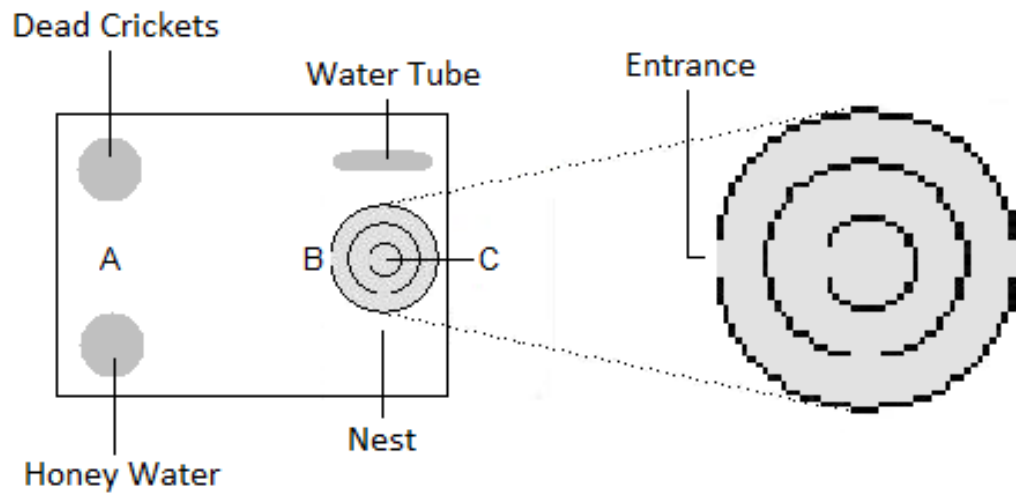


Figure 21. Experimental set-up for the recognition chemical experiments. Glass beads were placed in three locations: (A) between the food sources, (B) near the nest entrance, and (C) in the inner chamber of the nest.

can represent similar locations that workers would come across in their natural setting. Glass beads were placed on a cover slip (1.8 x 1.8 cm) if were placed between the food sources or near the entrance of the nest to prevent sand particles from touching and potentially attaching to the glass beads.

For each location, two glass beads were introduced: an experimental glass bead with recognition chemical and a control glass bead with solvent paired appropriately with the recognition chemical. Both locations and color of glass beads were randomized. Each location was video recorded for 15 minutes on a (Sony Handycam HDR-XR500V, Japan) as soon as the glass beads were introduced. The interaction time between the workers and each glass bead was determined with a stopwatch (StopWatch Application for Windows Phone), while viewing the video recordings during the 15-minute duration.

The behavioral response by the workers was also determined for each glass bead. Observing agonistic behavioral responses by workers is a common method used to determine if a chemical is useful for the purposes of nestmate recognition (Roulston et al. 2003, Howard and Blomquist 2005). A five-point behavioral scale was used to determine how the workers perceive each glass bead (Martin et al. 2008). The response behaviors for the scale consists of: 1 – ignoring the glass bead, 2 – only antennating the glass bead, 3 – mandibles open toward the glass bead, 4 – worker bite the glass bead with mandibles, and 5 – worker continuously bites the glass bead or picks up the glass bead and potentially moves it. A score of 1 or 2 was classified as a negative response, while a score of 3, 4, or 5 was classified as a positive response. While viewing the video recordings, the first 25 workers that walked within 1 cm of the glass bead in the form of

ignoring it or interacted with the glass bead were noted with a number from the scale. For each worker, the displayed behavior with the highest number was noted. The responses of the first 25 workers were used because as the workers interact with the glass beads, they could potentially be leaving their own recognition chemical on it.

Data Analysis

The excess time with the experimental bead for each pair was determined by taking the interaction time of the experimental bead minus the control bead. The excess time was then analyzed by a one-way ANOVA to determine if there was a significant difference between the average excess time across the levels of the recognition chemicals, locations, and colony type. The chi-square test of homogeneity of proportions was used to evaluate the behavioral responses of workers to the glass beads to determine if there was a significant differences between the preferences for the recognition chemicals and locations. For all statistical tests, the level of significance was set at an alpha value of 0.05.

Results

An examination of the average excess time, that is, the difference between the interaction time with experimental glass bead minus the interaction time with the control glass bead, reveals a difference among the three locations with the treated glass beads (One-way ANOVA: $F = 12.3510$, $p < 0.0001$; Figure 22). However, the three locations did not interact significantly with recognition chemicals (One-way ANOVA: $F = 0.5115$, $p = 0.6029$) or colony type (One-way ANOVA: $F = 1.0564$, $p = 0.3556$). There was a

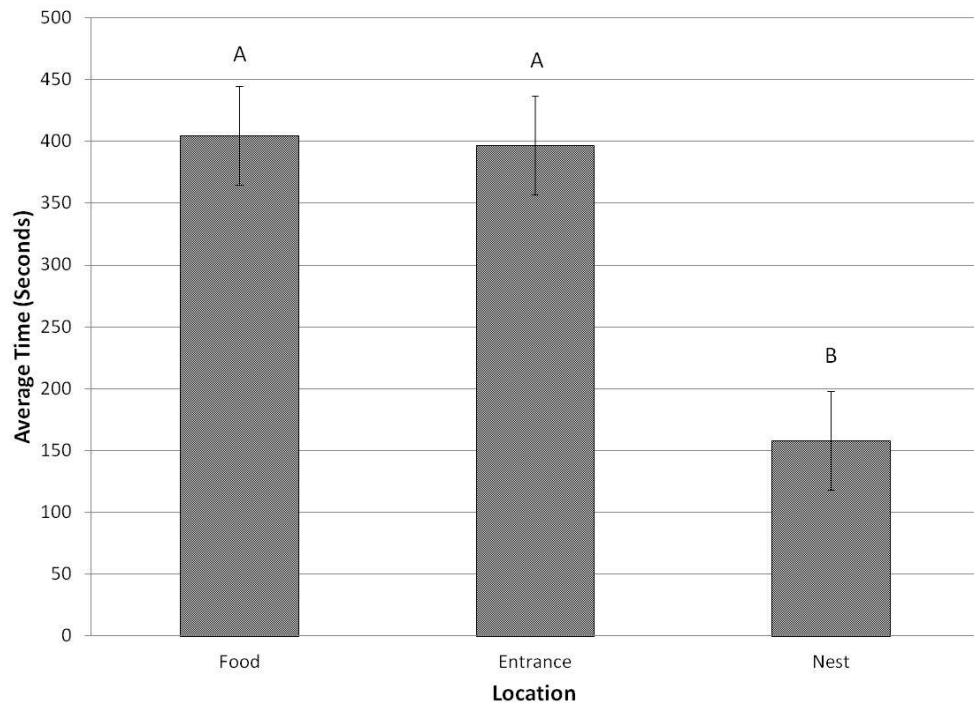


Figure 22. The average excess time of the experimental glass beads at each location. Different letters indicate a difference in the average time ($p < 0.0001$).

significant interaction between colony type and the recognition chemicals for the average excess times (One-way ANOVA: $F = 10.0406$, $p = 0.0027$). Therefore, the recognition chemicals were compared separately for each colony type (Figure 23).

There was a significant difference among the five behavioral responses from the workers between the experimental and control glass beads for all three locations (Chi-square test of homogeneity of proportions: $\chi^2 = 503.439$, $p < 0.0001$). A significant difference occurs between the behavioral responses of the workers and the recognition chemicals near the food sources (Chi-square test of homogeneity of proportions: $\chi^2 = 20.976$, $p = 0.0003$; Figure 24) and near the entrance of the nest (Chi-square test of homogeneity of proportions: $\chi^2 = 50.135$, $p < 0.0001$; Figure 25). However, there is not a significant difference between the behavioral responses of the workers and the recognition chemicals inside the nest (Chi-square test of homogeneity of proportions: $\chi^2 = 4.451$, $p = 0.3484$; Figure 26).

Discussion

Based on the calculated excess time spent with the experimental glass beads, *S. invicta* spent more time with experimental glass beads treated with cuticular hydrocarbons or polar cuticular lipids when near the food sources and near the entrance of the nest compared to control glass beads. On the other hand, the interaction time for both the experimental and control glass beads were similar inside the nest. It is likely that workers did not like any foreign objects inside their nest. This may also explain why there was no difference in behavioral responses by the workers towards the experimental

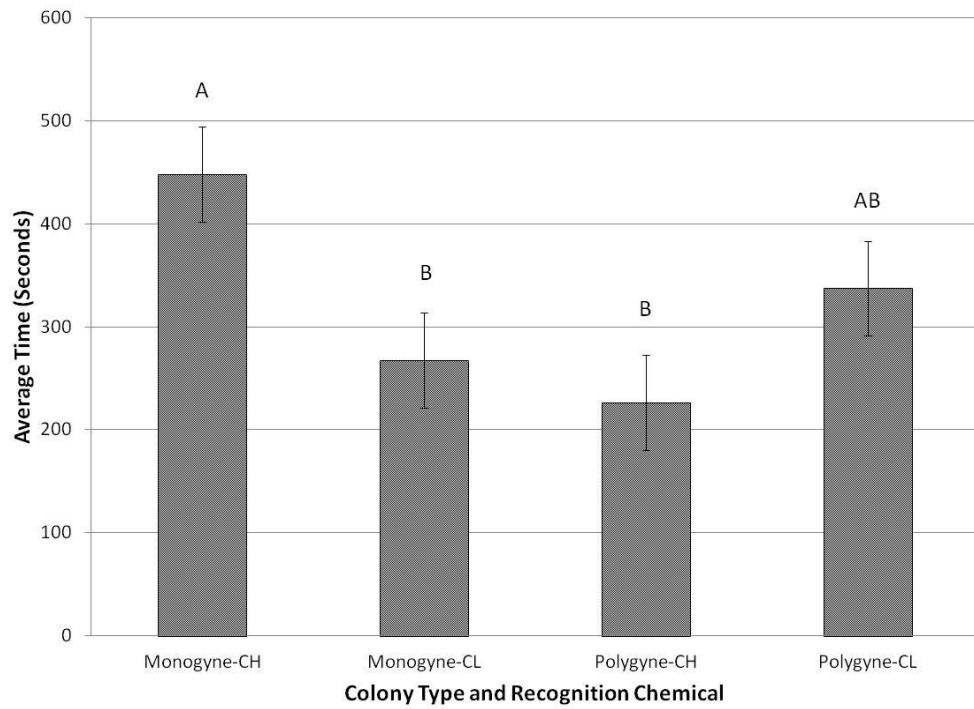


Figure 23. Average excess time of the experimental glass beads for each recognition chemical for each colony type. Different letters indicate a difference in the average time. CH = cuticular hydrocarbons and CL = polar cuticular lipids ($p < 0.05$).

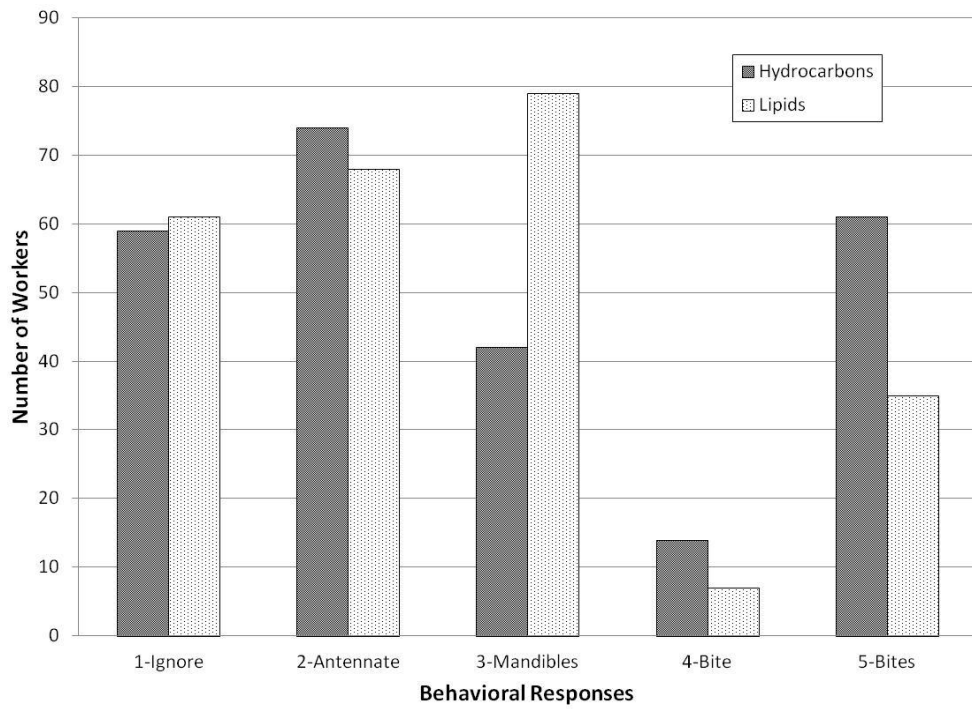


Figure 24. Number of workers observed performing different behavioral responses when exposed to cuticular hydrocarbons or polar cuticular lipids near food sources. There is a difference between the two recognition chemicals ($p = 0.0003$)

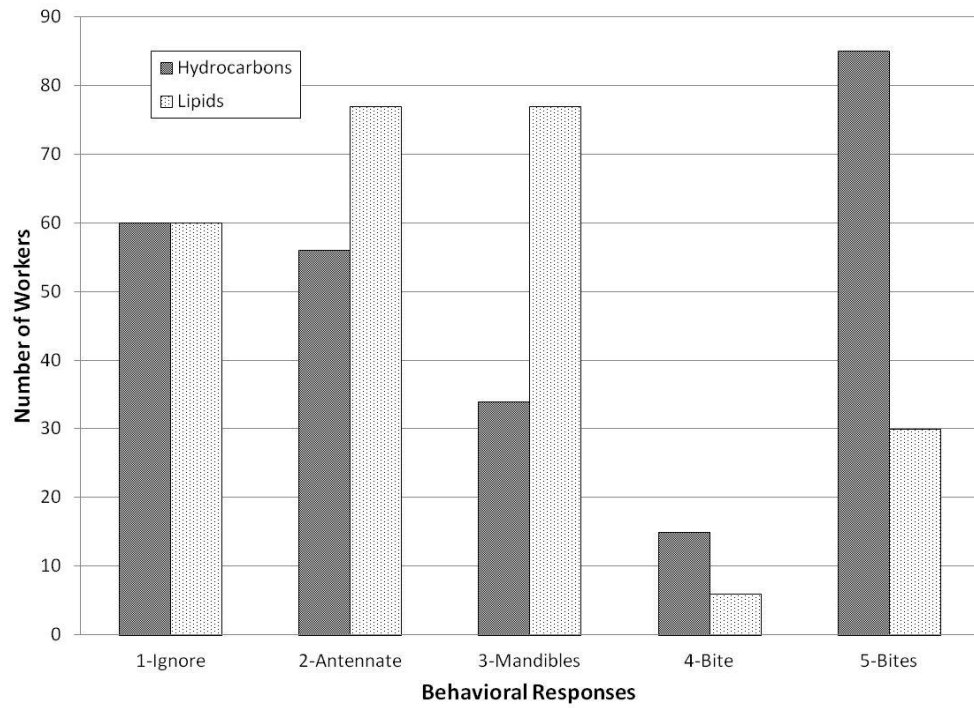


Figure 25. Number of workers observed performing different behavioral responses when exposed to cuticular hydrocarbons or polar cuticular lipids near the entrance of the nest. There is a difference between the two recognition chemicals ($p < 0.0001$)

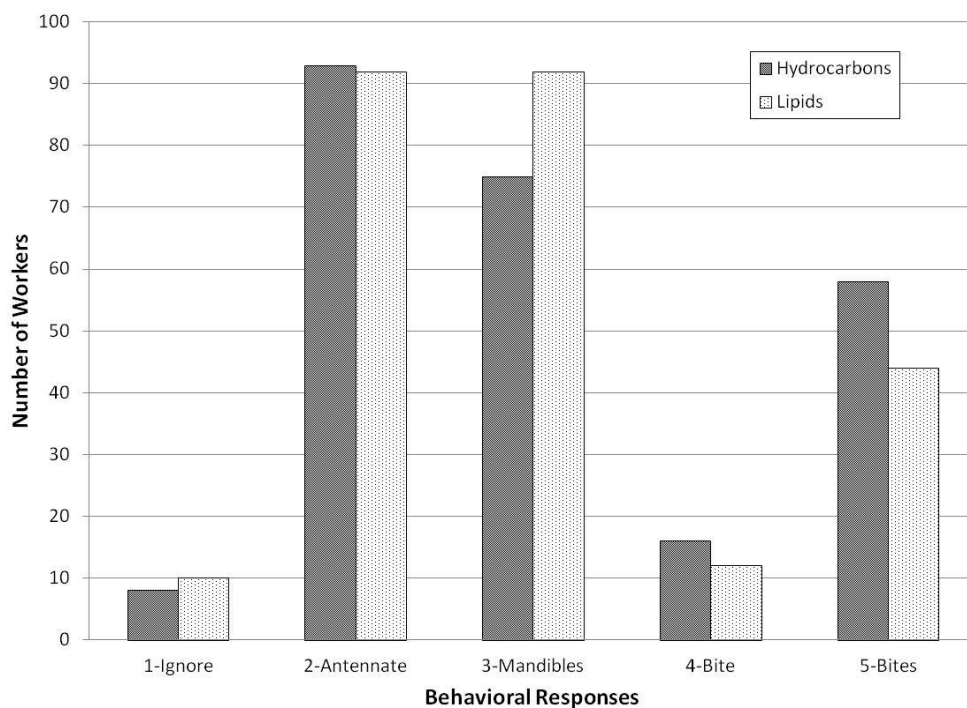


Figure 26. Number of workers observed performing different behavioral responses when exposed to cuticular hydrocarbons or polar cuticular lipids inside the nest. There is no difference between the two recognition chemicals ($p = 0.3484$)

and control glass beads inside the nest because they possibly viewed the set of glass beads as foreign objects that do not belong there.

Workers of monogyne colonies spent more time with cuticular hydrocarbons compared to polar cuticular lipids, which can indicate the cuticular hydrocarbons are used more for recognizing nestmates. Some studies indicate that cuticular hydrocarbons are important for recognizing nestmates in *S. invicta* (Obin 1986, Vander Meer et al. 1989) and other ants (Lahav et al. 1999, Liang and Silverman 2000, Wagner et al. 2000). For the polygyne colonies, there was no difference in the interaction time between cuticular hydrocarbons and polar cuticular lipids, but workers appear to interact more with polar cuticular lipids than cuticular hydrocarbons. In addition, there was no difference in interaction time between polygyne colonies with polar cuticular lipids and monogyne colonies with cuticular hydrocarbons. This may suggest that polygyne colonies of *S. invicta* rely more on polar cuticular lipids for nestmate recognition compared to cuticular hydrocarbons, but further testing needs to be performed to determine if this is actually true. It has been suggested that *Leptothorax acervorum* Fabricius uses cuticular fatty acids as recognition cues. The workers of this species usually live in polygyne colonies (Franks et al. 1990). Even though, it is suggested that hydrocarbons are used more for recognizing nestmates compared to oxygenated lipids (Dani et al. 2001), chemical compounds within polar cuticular lipids that could potentially be involved in recognizing nestmates should be explored further.

Solenopsis invicta workers displayed more aggressive behaviors towards experimental glass beads compared to control glass beads when located near the food

source and entrance of the nest. In terms of displaying aggressive behavior towards the experimental glass beads near both the food source and the entrance of the nest, more workers open their mandibles towards the glass beads with polar cuticular lipids compared to glass beads with cuticular hydrocarbons. However, for the same locations, more workers bit the glass beads with cuticular hydrocarbons once or multiple times than the glass beads with polar cuticular lipids. This provides more evidence that cuticular hydrocarbons are used for recognizing nestmates due to the higher aggressive behavior displayed by workers. In this study, only five monogyne and five polygyne colonies were observed, thus, more data would need to be collected to confirm the behavioral responses to cuticular hydrocarbons and polar cuticular lipids between monogyne and polygyne colonies. This will need to be looked at further to determine if polar cuticular lipids are important for recognizing nestmates in polygyne colonies.

A few studies already indicate that *S. invicta* workers can find baits with recognition chemicals applied to them faster than baits without recognition chemicals applied to them (Vander Meer 1996, Wiltz et al. 2010). The results of this study provide further evidence that it is likely that baits with recognition chemicals will be more attractive to workers and that it is likely that there will be no difference in the attractiveness towards baits at different locations. However, this will actually need to be further tested by applying recognition chemicals on baits instead of glass beads. In addition, if *S. invicta* workers can find the baits with recognition chemicals faster, then it is less likely for native ants to find the baits, which could potentially help the populations of the native ants compete with *S. invicta* (Vander Meer 1996).

Cuticular hydrocarbons and polar cuticular lipids should be separated into chemical compounds and tested in a similar method as performed in this study. Known chemical compounds within cuticular hydrocarbons have already been tested in other ant species (Wagner et al. 2000, Lucas et al. 2005, Martin and Drijfhout 2009) and known chemical groups within the polar cuticular lipids of *S. invicta* are already known (R. Renthall, S. Katti, and X. Gao, unpublished). As for cuticular hydrocarbons, they are important for preventing desiccation and acting as a barrier to microorganisms (Lenoir et al. 1999, Howard and Blomquist 2005), and therefore, it is suggested that not all the chemical compounds within the cuticular hydrocarbons would be used as recognition cues (Dani et al. 2001). The chemical compounds could be tested in a similar manner as in this study and eventually be tested on baits if workers are attracted to the chemical compound. However, it is possible that a single chemical compound is not used as a recognition cue, as seen with *L. humile* and *Novomessor cockerelli* André, in which two cuticular hydrocarbon chemical compounds were needed to obtain an aggressive response by workers (Greene and Gordon 2007). This will need to be taken into consideration when exploring the different chemical compounds within the cuticular hydrocarbons and polar cuticular lipids.

Follow up studies should go further by observing where workers move experimental and control glass beads. In this study, the glass beads were generally too large for most workers to handle, but smaller glass beads can be used for workers to easily handle and move if desired. This could determine if glass beads treated with cuticular hydrocarbons or lipids would go into the nest or elsewhere in the environment

of the colony. This method could also determine if the attraction to polar cuticular lipids is based on their classification as a food source for the ants instead of treated as a nestmate recognition cue. When testing, a glass bead with the inside contents of a cricket could be applied to the surface and observe if that glass bead is moved to the same destination as the glass bead treated with polar cuticular lipids. Another study could determine the interaction time and behavioral response of workers when introduced to two experimental beads, in which each will be treated with either cuticular hydrocarbons or polar cuticular lipids. This will determine if the cuticular hydrocarbons would influence how the workers respond behaviorally to the polar cuticular lipids and vice versa when both are present.

Results of the interaction time from this study agree with other studies that cuticular hydrocarbons are important for recognizing nestmates (Obin 1986, Vander Meer et al. 1989), but more so with monogyne compared to polygyne colonies. Polar cuticular lipids could potentially be used for recognizing nestmates in polygyne colonies, but further testing will need to be performed by looking at specific chemical compounds within the polar cuticular lipids, along with determining if workers are sensing the polar cuticular lipids as a food source instead of as recognition cues. Recognition chemicals can be important when making baits to reduce the populations of *S. invicta*, which also needs to be further investigated.

CHAPTER V

CONCLUSIONS

It is suggested that studying the behavior of an invasive species, such as *S. invicta*, is essential to gain more understanding of how a species establishes in non-native areas (Holway and Suarez 1999). By studying ant behavior, new methods can potentially be discovered to control this invasive species and novel methods could be used to prevent other invasive species of similar characteristics from establishing new populations outside their native area (Holway et al. 2002).

More research needs to be performed on the biology of *S. invicta*. Results from these studies can potentially contribute to developing new strategies for controlling their populations. It is nearly impossible now to eradicate *S. invicta* from the United States, but it can still be attempted to keep their populations at low levels (Holway et al. 2002, Tschinkel 2006). The studies in this dissertation explored color vision of *S. invicta*; how well polymorphic workers can handle different sizes of objects when digging and foraging; and explored the behavioral response from workers towards objects treated with cuticular hydrocarbons and polar cuticular lipids. The methods used in these studies can be performed with other ant species to learn more about their biology.

The color discrimination study has indicated that workers of *S. invicta* can discriminate colors, while based on color pairs, they generally show preference for colors with long wavelengths (i.e. orange and red). On the other hand, colors with short wavelengths were less preferred (i.e. blue and yellow) with the exception of green. It

was also observed that workers dig more in light conditions compared to dark conditions. Future studies should look at sensitivity to different wavelengths of monochromatic light, along with determining if there are any differences in color discrimination between task groups and physical caste groups.

The study looking at polymorphic workers of *S. invicta* indicated that when digging and foraging, the body size of the workers correlated to the glass bead sizes and that a relationship existed between the physical caste groups and the size of the glass beads. In addition, 0.50 mm glass beads are the most preferred by workers. Media workers are not versatile workers when digging in the different sizes of glass beads, but they were when foraging different sizes of glass beads. Future studies should look more at how workers handle 0.25 mm glass beads and how minors would handle the different sizes of glass beads when digging and foraging. In addition, future studies should look at how workers handle and carry objects that have rough, textured surfaces.

The study involving cuticular hydrocarbons and polar cuticular lipids being applied to glass beads has indicated that the interaction time with the experimental glass beads near the food sources and near the entrance of the nest is similar, but the interaction time of both locations is different compared to inside the nest. Workers from monogyne colonies spent more time with glass beads treated with cuticular hydrocarbons, while there was no difference in the interaction time between the cuticular hydrocarbons and polar cuticular lipids from workers in polygyne colonies. Behavioral responses towards glass beads were different when the glass beads were located near the food sources and near the entrance of the nest. However, the behavioral responses were

similar when the glass beads were inside the nest. In addition, workers were more aggressive with the experimental glass bead treated with cuticular hydrocarbons compared to the experimental glass bead treated with polar cuticular lipids. Future studies should observe where workers potentially move the glass beads treated with either cuticular hydrocarbons or polar cuticular lipids in their environment and observe if there is a difference in interaction time and behavioral responses when both recognition chemicals are introduced at the same time. In addition, future studies should separate out the chemical groups among the cuticular hydrocarbons and polar cuticular lipids and apply those specific chemicals to glass beads to determine the interaction time and behavioral responses by workers to the treated glass beads.

Results from these studies can be used to make more efficient baits to reduce *S. invicta* populations. Odorless dyes of the colors used in the color discrimination study can be applied to baits to determine if workers show a similar color preference as they did in the color discrimination study. In general, baits consist of yellowish corn grit particles (Reed et al. 2015). More studies would need to be conducted to determine more about the size preferences of baits by workers. Previous studies have indicated that *S. invicta* workers prefer bait sizes larger than 1.0 mm in diameter (Hooper-Bùi et al. 2002, Neff et al. 2011, Reed et al. 2015). However, it was seen that workers prefer glass beads of 0.50 mm in diameter. Bait sizes can range from small dust particles to over 2.0 mm in diameter (Hooper-Bùi et al. 2002). Finally, results from the cuticular hydrocarbons and polar cuticular lipids study provide more evidence that these recognition chemicals can

be useful in attracting workers when applied to baits, which other studies have already begun doing this (Vander Meer 1996, Wiltz et al. 2010).

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