BIOLOGY, DIET PREFERENCES, AND CONTROL OF THE DARK ROVER ANT *BRACHYMYRMEX PATAGONICUS* (HYMENOPTERA: FORMICIDAE) IN TEXAS

A Dissertation

by

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ABSTRACT

The dark rover ant *Brachymyrmex patagonicus* has within recent years expanded its range in the United States and has become more prevalent in urban environments. This ant is an invasive species that is native to South America, and very little is known about it. Therefore, the research addressed the reproduction, foraging behavior, mechanical vector potential, food lure preference, and control of *B. patagonicus*.

Field collected colonoids of *B. patagonicus* were utilized in this laboratory research and were placed in 15 cm petri dishes. The ants were held under constant environmental conditions for 4 months and number of eggs, larvae, pupae, and adults were documented. Results indicated that the optimal temperature for *B. patagonicus* is 30˚C and that development time is 33 d.

Results of the laboratory foraging bioassays indicated that *B. patagonicus* will readily move the colony to be in close proximity of food and water. Data from these trials also solidified that foragers must be in contact with queen and brood in order to forage.

Data from laboratory trials showed that *B. patagonicus* can vector *E. coli* to at least 2.0 m. Albeit, as distance increased, the number of *E. coli* colonies decreased. Based on these data, *B. patagonicus* should be considered a pest of medical concern.

Results of the laboratory trials indicated that *B. patagonicus* foragers preferred carbohydrates in the laboratory, but in field trials the foragers preferred carbohydrates in

the winter and spring and protein in the fall and summer. The data suggested that this species will switch food lure preference based on time of year.

Two field trials and one laboratory trial, were conducted as a part of this research. In field trial #1, 30 structures with exterior infestations of *B. patagonicus* were located and treated with a liquid sprayable application. At 30 d post-treatment Demand CS showed the greatest reduction in the ants. In the laboratory trial, both granular and gel baits were utilized. The gel baits caused more mortality than the granular baits at 11 d post-treatment. In field trial #2, 32 structures with exterior infestations of *B. patagonicus* were utilized. Multiple treatment strategies were used and results showed that the gel bait only application had the highest percent control at 90 d post-treatment.

DEDICATION

I would like to dedicate all of this work to my family, my beautiful wife Kay and our daughters Becca and Molly. You have been 100% supportive of my education. You are always my source of reason, love, peace, and comfort in my times of need during this process. You helped me to stay focused on the things that are most important starting with our faith and family. I love you with all of my heart.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Invasive Ants

Ants (Order: Hymenoptera: Family: Formicidae) are one of the most abundant animals found on earth and are reported on all continents except Antarctica (Suarez et al. 2001). There have been over 14,000 species of ants identified (Ebeling 1978), and scientists believe that there are another 3,000 to 6,000 yet to be discovered (Gusmao et al. 2011). Of the 14,000 ant species described, approximately 45% are considered urban pests (Klotz et al. 2008). Due to the large number and diversity of ants found all over the globe, many are invasive. The term invasive is used to describe ants that are non-native to a region that spread widely in a habitat or environment that tend to have negative effects on native flora and fauna. There are two species of urban invasive tramp ants that are found worldwide: *Linepithema humile* Mayr (Argentine ant) and *Monomorium pharaonis* Linnaeus (Pharaoh ant) (Hedges 2010). There are five species of ant that are found on the top 100 worst animal species, all of which are found in North America, including *Solenopsis invicta* Buren (red imported fire ant), *L. humile* (Argentine ant), *Anoplolepsis gracilipes* Smith (yellow crazy ant), *Wasmannia auropunctata* Roger (little fire ant) and *Pheidole megacephala* Fabricus (big headed ant) (Lowe et al. 2000).

Invasive species introduction in the United States is the second leading cause of losses in biodiversity, second only to habitat and landscape fragmentation (Walker and Steffen 1997). In fact, there are over 50,000 documented invasive species in the United

States that cause environmental damages and economic losses, which are estimated at $125 billion annually (Pimental et al. 2000, Allendorf and Lundquist 2003). The major concern of invasive species is the difficulty in controlling them once they are widely established over a region (Tsutsui and Suarez 2003). Thus, the control of invasive species is a controversial and very complex problem (Allendorf and Lundquist 2003). Control of invasive species is often dealt with a "shoot first and ask questions later" policy to hopefully reduce the expansion of the invasive species (Ruesink et al. 1995, Allendorf and Lundquist 2003).

Invasive ants have had a detrimental effect on native species in the United States, through exploitative and interference competition (Human et al. 1998, Holoway et al. 2002, Allendorf and Lundquist 2003, Tsutsui and Suarez 2003). Ant species are one of the most damaging invasive worldwide (Moller 1996), and the effects that they have on the ecology and environment are well documented (Tsutsui and Suarez 2003, Ness and Bronstein 2004). In the case of "tramp" ants (commonly transported with human goods) they are successful invaders because of a suite of characteristics they possess which can include, general nesting and dietary requirements, polygyny, colony reproduction by budding, long lived, high reproductive rates, few natural enemies (predators, competitors, parasites, and disease), genetic plasticity, and reduced intraspecific aggression (Holoway et al. 2002, Lee 2002). Tramp ants are also highly successful because humans alter environments, which allow these species to live in climates in which they would not survive under normal conditions (Suarez et al. 2001). Most invasive species have come from South America (Suarez 2010). The report of invasive

species found in urban areas has increased significantly in the past decade including reports of *Brachymyrmex patagonicus* Mayr (MacGowan et al. 2007), which is from South America (Quiran et al. 2004).

Ants and other invasive species are spread in many different ways, which is a very complex web that includes land use, weather, and transportation networks (Dukes and Mooney 1999, Crowl et al. 2008). Much of this is due to increased global travel by individuals and trade among nations, which has been brought about by transportation technology (Aide and Grau 2004). Documentation of invasive species range expansion is critical no matter what the perceived effect on native flora and fauna may be in any region. This is where people can play a role in the reporting of new invasions to the proper authorities, so that basic steps can be taken to monitor and limit negative effects (Crowl et al. 2008). Human expansion is the leading cause of extinction of plants and animals, second is the introduction of invasive species, both of which are major ecological and societal challenge (Lodge et al. 2006).

Ants play an assortment of roles (Holoway et al. 2002) as prominent members of a terrestrial ecosystem (Holldobler and Wilson 1990). Ants can affect many different aspects of the environment because they can be pollinators, predators, scavengers, herbivores, and detritivores (Holldobler and Wilson 1990, Way and Khoo 1992). Ants also prey on many animals (Holoway et al. 2002) and can act as a host to many fly (Diptera) and hymenopteran (Hymenoptera) parasitoids (Feener and Brown 1997). Ants are also important because they excavate soil, which leads to soil turnover and nutrient redistribution (MacMahon et al. 2000). It is because of those reasons that ants are an

important part of the ecosystem, and can affect many different areas of the environment in both positive and negative ways.

Ants are commonly found in close association with humans due to the urbanization of habitats, which has made it simpler for tramp ants to find resources and establish colonies (Lessard and Buddle 2005). Ants have been successful in urban areas in large part to the encroachment of humans on previously undisturbed land and then the preservation of urban forests (Thompson 2007). Although increased urbanization leads to a decline in ant diversity, tramp ant species have been able to thrive in human environments. Thus, the argument has been put forth that human activity and not invasive species may be the primary factor that negatively affects native ants (Sanford et al. 2008).

***Brachymyrmex* spp**.

In 1862, Gustav L. Mayr proposed the genus *Brachymyrmex* (Mayr 1868), which is commonly referred to as rover ants. Presently, there are 38 described species of *Brachymyrmex* in the world (LaPolla and Longino 2006), including 26 species found in Argentina (Quiran et al. 2004). There are ten species of *Brachymyrmex* in North America, of which four are undescribed (Deyrup et al. 1988, MacGowan et al. 2007), and this genus is currently under revision (Fisher and Cover 2007). In North America, *B. patagonicus* (Mayr) is found primarily in the Gulf Coast region of the United States from Florida to Texas, and also in New Mexico, Arizona, Nevada and California (MacGowan 2007 and B. Drees pers. com.). The last complete revision of the genus *Brachymyrmex* was done by Santschi (1923), while a partial revisions was more recently

completed by Quiran et al. (2004 and 2005) which led to the re-description of six species including *B. patagonicus* (MacGowan et al. 2007). Due to the lack of published literature on *B. patagonicus* very little is known about this species.

There are at least three species of *Brachymyrmex* found in Texas including *B. depilis* Emery, *B. obscurior* Forel, and *B. patagonicus* (Vinson et al. 2003). *B. patagonicus* is native to South America (Quiran et al. 2004) and was first reported in the United States in 1978 in Louisiana (Wheeler and Wheeler 1978) as *B. musculus* Forel (MacGowan et al. 2007). *Brachymyrmex patagonicus*, commonly referred to as dark rover ants (Mac Gowan et al. 2007), are small in size ranging from 1.5 to 2.5 mm in length, and the workers are monomorphic (Snelling and George 1979). They are unique as compared to most other ant species in that they have a nine- segmented antenna without a club (Creighton 1950, Klotz et al. 2008) and they have a one- segmented petiole, which is hidden under the gaster. They have a small circle of hairs at the tip of the abdomen, the thorax is uneven in shape, and the front portion of the abdomen is humped (Hedges 2010).

Brachymyrmex colonies are monogyne (Klotz et al. 2008, Hedges 2010), and colonies are small ranging from several hundred to a few thousand workers (Klotz et al. 2008). Colonies are usually located in soil or rotting wood (Vail et al. 1994), but *B. patagonicus* has been known to nest in potted plants, and wall voids (Dash et al. 2005). Mating usually occurs in the evening hours in the summer, and the alates are attracted to lights (Vail et al. 1994). *B. patagonicus* can be found virtually anywhere there are available nesting and food resources, and it is common to find *B. patagonicus* in close

proximity to dominant ant species such as *L. humile*, *Nylanderia fulva* Mayr, and *S. invicta*. This is evident due to the high density of *B. patagonicus* found in residential areas where those species are also found.

Brachymyrmex ants will invade structures, and have proven to be difficult to control in urban environments (R. Davis, J. Meyers, J. Johnson, T. Rasberry, B. Springer pers. com.). Non-chemical or cultural control should be considered when controlling populations of these ants (Keefer 2010). Infestations are usually associated with moisture and/or fungal decay, and correction of these conducive conditions will help to control these ants. It is reported that some *Brachymyrmex* tend soil dwelling Coccoidea (Hemiptera) in order to harvest their honeydew (Wheeler 1910, Santschi 1923, Dennis 1938, Smith, 1947, Warren and Rouse 1969, LaPolla and Longino 2006, Klotz et al. 2008, M. Eubanks pers. com.). Proper upkeep, rotation of plants, and control of aphid populations will reduce the abundance of *Brachymyrmex* populations around and/or in structures (Hedges 2010). *Brachymyrmex* are not known to be of medical importance; however, they are known to inhabit hospitals and other medical facilities. Therefore, the vector potential of this should be investigated. *Brachymymex patagonicus* are considered "tramp" ants and, as such, do not have a well-defined nest area and are easily transported by human commerce (LaPolla and Longino 2006). These ants are often referred to as a "nuisance" pest because they do not sting or bite (Vinson et al. 2003). Because so little is known about *Brachymyrmex patagonicus*, my research focused on the following areas:, reproduction potential, development time, foraging behavior, food lure preference, vector potential and control. In these trials *B. patagonicus* was identified

using keys from Santschi 1923, Wheeler and Wheeler 1978, Quiran 2004, and Quiran 2005.

***Objectives**:*

1) To determine the reproduction potential of *B. patagonicus* colonies held at specific temperatures in a laboratory;
2) To measure the foraging behavior of *B. patagonicus* in laboratory setting;
3) To examine the potential of *B. patagonicus* as a potential vector of a human pathogen;
4) To determine food lure preferences of *B. patagonicus* foragers in the laboratory and field; and
5) To determine effectiveness of commercial products to control *B. patagonicus* populations in the laboratory, and associated with structures in urban environments.

***Null Hypotheses**:*

1) Reproductive potential of *B. patagonicus* populations will not vary significantly under three different temperatures under laboratory conditions;
2) Foraging behavior of *B. patagonicus* workers will not differ significantly based on length of experimental units;
3) The number of pathogen colonies transferred by *B. patagonicus* to sterile agar plates will not be significantly different as compared to number of pathogen colonies on untreated controls;

4) Foragers of *B. patagonicus* will not prefer any food lure over others tested; nor from untreated controls; and
5) Treatments using commercial products will have no negative effects on *B. patagonicus* populations when compared to untreated controls.

CHAPTER II

INVESTIGATION OF REPRODUCTION POTENTIAL AND DEVELOPMENT TIME OF *BRACHYMYRMEX PATAGONICUS* (HYMENOPTERA: FORMICIDAE)

Introduction

The reproductive potential of *B. patagonicus* has not been determined and very little has been published for this species regarding this topic. *B. patagonicus* go through complete metamorphosis which entails an egg, larva, pupa and adult stages. There are several factors that can influence reproductive potential of queens and development time of immature stages, including her weight, diet, ratio of workers to brood, temperature, relative humidity, photoperiod, number of queens per colony, and age of queen (Tschinkel 1988, Arcilia et al. 2002). All of those factors are influenced by the health of the colony (Holldobler and Wilson 1990). With all these factors that can affect reproduction in Formicidae it is understandable that egg production rate is species specific (Holldobler and Wilson 1990).

Although *B. patagonicus* are monogyne it has been reported that in laboratory colonies, when multiple queens are present, egg production is higher than when a single queen is present. In laboratory studies a solitary queens produced approximately 15 eggs, but when multiple queens were present approximately 20 eggs were produced (Miguelena and Baker 2010). There are no reports of *B. patagonicus* as being polygyne in the wild (Miguelena and Baker 2010). As in other species of ants, one of the roles of workers of *B. patagonicus* is the feeding of queens; this is why the ratio of workers to

brood is critical. There must be enough workers to care for the queen as well as the brood at all times. It is also reported that in some species of ants, the queen gets nutrition from intaking larval secretions and haemolymph (Borgesen and Jensen 1995). In fact, Tschinkel 1988 and 1995 reported that in other monogyne species of ants the presence of late instars contributed to increased egg production by queens. Holldobler and Wilson (1990) reported that the queen is also fed more when late instar larvae are present in monogyne colonies. It has also been reported that the number of pupae and the number of larvae regardless of instar stimulate queen oviposition (Gibson and Scott 1990, Borgesen and Jensen 1995, Borgensen 1989). Based on that information it can be said that reproduction is stimulated by the development of larvae to pupae (Tschinkel 1995). In contrast, it has also been reported that the presence of late instar larvae decrease queen egg laying capability because resources are being allocated to the larvae instead of the queen (Tschinkel 1988 and 1995).

Temperature greatly affects reproduction in ant colonies (Wheeler 1910, Porter 1988, Williams 1990, and Abril et al. 2008). Development time for many species of ants from egg to adult has been studied and is well documented under laboratory and field conditions (Mintzer 1979 and MacKay 1981). Information regarding estimated developmental times for each stage of development is also available for many species of ants (Peacock and Baxter 1950, O'Neal and Markin 1975); however there is no published data on how climatic factors influence *B. patagonicus*. The relationship between temperature and oviposition rate is a useful component in understanding a species physiological needs and possible maximum distribution (Abril et al. 2008). The

knowledge gained in such experiments would help to determine the upper and lower temperature thresholds for *B. patagonicus*, as well as the optimal temperature for oviposition.

It has been observed that the reproduction period for *B. Patagonicus* in Texas starts in May with a spike in June/July and slows in September. Since at the time of the start of this work there was very little known about the reproductive potential of *B. patagonicus* and *Brachymyrmex* spp., and it was not know which factors would influence reproduction and any data gained would be new information. In this research, by investigating the general biology of this ant and the reproductive potential, integrated pest management (IPM) practices can be developed and implemented for seasonal control of this species.

Materials and Methods

Field colonies of *B. patagonicus* including queens, workers and brood for all objectives in this research were collected from multiple distinct sites in Bryan/College Station, TX including urban forest areas (city parks), and from around the exterior of structures (irrigated flower beds). Using a shovel, the researcher dug out a colonoid and placed it and the soil into a 19 L bucket with talc powder spread on the upper rim to prevent escape of the ants. These collected field colonies were brought back to the laboratory, separated from their substrate, by placing the soil with the colonoid into a 35 x 30 cm plastic box with Insect-a-Slip (BioQuip Products Inc.) on the sidewalls to prevent ant escape. Inside the plastic box on the opposite side of where the soil with ants was placed a 150 x 15 mm test tube was placed which was filled with water and pluged

with a cotton ball and a 2.5 cm hexagonal weighboat with a food source. This set-up allowed the ants to move from the soil and into the test tube with water, Once the ants were in the test tube they were placed in 150 x 15 cm petri dishes (KORD-Valmark, Bristol, PA) coated with Insect-a-Slip to prevent escape of ants. These arenas were provisioned with water and harborage in the form of a 16 x 150 mm test tube (Fisher Scientific, West Chester, PA) filled with water, and a cotton plug was inserted as described by (Porter 1988). In addition, three Fuji apple chunks (1.0 x 1.0 x 1.0 cm) as food (J. Cook pers. com.) were added to the arena. These ants were kept in a constant environment at 27±2°C, 80±2% R. H., 8 h of light and 16 h of dark (8:16, L/D) and allowed to adjust to laboratory conditions for a minimum of 48 hrs prior to testing. Fifteen queens were isolated, and each queen was placed in a 150 x 10 mm petri dish with 30 worker ants, apple chunks, water, and harborage (as described above). Five each of the colonoids were kept at 25±2°C, 80±2% R. H., 30±2°C, 80±2% R. H., and 35±2°C, 80±2% R. H. These colonoids were allowed to function under constant laboratory conditions for three months. Data were collected twice weekly and included the numbers of eggs, 3rd instar larvae, pupae, and adults. Photoperiod was held constant with 8 h of light and 16 h of dark (8:16, L/D). Eggs and larvae were counted using a Nikon SMZ-2T microscope fitted with a Leeds LR92240 light source.

Results

At 25°C there were 5 eggs laid in replication #4 at 100 d post-initiation of the study (Table 1). By 114 d all 5 eggs had hatched and 5 larvae were present. None of the five larvae made it to the pupal stage. The queens in replications #s 1, 2, 3, and 5 did not

produce eggs during this study (Table 1). There were no adults produced at this temperature. Three of the queens died during this study. The queen in replication #3 died at 29 d, in replication 1 at 78 d, and in replication #5 at 83 d post-initiation of the study. All five replications had worker mortality by the end of the study. There was 100% mortality of the workers in replication #1 at 78 d, replication #5 at 83 d, and replication 3 at 86 d. In replication #2 there was 20% mortality of the workers at 135 d (Table 1). The 5 eggs laid at 100 d took 14 d to develop into larvae.

Table 1. Results of investigation of reproduction of *Brachymyrmex patagonicus* at 25ºC

Rep #	# of days to queen mortality from initiation of study	% worker mortality, days from initiation of study	# of eggs, days from initiation of study	# of larva, days from initiation of study	# of pupae, days from initiation of study	# of emerged adults
1	78 d	100%, 78 d	0	0	0	0
2	0	20%, 135 d	0	0	0	0
3	29 d	100%, 86 d	0	0	0	0
4	0	80%, 135 d	5, 100 d	5, 114 d	0	0
5	83 d	100%, 83 d	0	0	0	0

At 30ºC at 71 d post-initiation the queen in replication #3 had laid 10 eggs, at 75 d the queen in replication #1 had laid 10 eggs, and at 94 d the queen in replication #5 laid 10 eggs (Table 2). The queens in replication #s 2 and 3 did not produce eggs during this study. At 89 d, 6 of the eggs in replication #3 had hatched and were larvae, at 94 d 5 of the eggs in replication #1 had become larvae, and in replication #5 at 114 d 5 of the

eggs had become larvae. At 94 d, in replication #3, three larvae had become pupae, at 107 d there were 3 pupae in replication #1 (Table 2). At 118 d there were a total of 5 pupae in replication 1 and 6 pupae in replication #3. There were no pupae discovered in replications #s 2, 4, and 5 throughout the study. In replication #3 at 100 d post-initiation 3 adults had emerged from pupa. Two of the queens died in this study. The queen in replication #2 died at 32 d and the queen in replication #1 died at 118 d. There was 100% mortality of the workers in replications #s 1 and 5 at 135 d post-initiation and 20% mortality of workers in replications s# 2 and 3, and10% mortality of the workers in replication #5 at 135 d (Table 2).

Table 2. Results of investigation of reproduction of *Brachymyrmex patagonicus* at 30ºC.

Rep #	# of days to queen mortality from initiation of study	% worker mortality, days from initiation of study	# of eggs, days from initiation of study	# of larva, days from initiation of study	# of pupae, days from initiation of study	# of emerged adults
1	118 d	100%, 118 d	10, 75 d	5, 94 d	3, 107 d 5, 118 d	0
2	32 d	20%, 135 d	0	0	0	0
3	0	20%, 135 d	10, 71 d	6, 89 d	3, 94 d 6, 118 d	3, 100 d
4	0	0, 135 d	0	0	0	0
5	0	10%, 135 d	10, 94 d	5, 114 d	0	0

At 35ºC at 8 d post-initiation of the study, the queens in replications #s 1 and 2 had both laid 10 eggs each (Table 3). The queens in replications #s 3-5 did not lay any

eggs in this study. At 11 d there was 1 larva in replication #1 and at 22 d there was 1 larva in replication #2. At 38 d there were 3 larvae in both replications #s 1 and 2, and at 44 d there were 4 larvae in replication 1 and there were 5 larvae in replication 2. At 50 d there were 8 larvae in replication #1 and 10 larvae in replication #2. In replication #2 there was 1 pupa at 15 d and in replication #1 there was 1 pupa at 29 d (Table 3). No adults emerged from the pupae. Three queens died during this study, the queen in replications #s 3 and 5 died at 50 d and the queen in replication #4 died at 61 d post-initiation of the study. All 5 replications had mortality of workers during this study. At 50 d there was 100% mortality of the workers in replication #5 and 33% mortality of the workers in replication #3. In replication #4 there was 30% mortality of the workers at 58 d and 50% mortality at 61 d. At 68 d there was 7% mortality of the workers in replication #1 and 33% mortality of the workers in replication #2 (Table 3).

Table 3. Results of investigation of reproduction of *Brachymyrmex patagonicus* at 35ºC.

Rep #	# of days to queen Mortality from initiation of study	% worker mortality, days from initiation of study	# of eggs, days from initiation of study	# of larva, days from initiation of study	# of pupae, days from initiation of study	# of emerged adults
1	a	7%, 68 d	10, 8 d	1, 22 d 3, 38 d 4, 44d 8, 50 d	1, 29 d	0
2	a	33%, 68 d	10, 8 d	1, 11 d 3, 38 d 5, 44 d 10, 50 d	1, 15 d	0
3	50 d	33%, 50 d	0	0	0	0
4	61 d	30%, 58 d 50%, 61 d	0	0	0	0
5	50 d	100%, 50 d	0	0	0	0

a=queen did not die during study

There were eggs laid by *B. patagonicus* queens at all three temperatures (Table 4). The mean number of days from the initiation of the study to the first clutch of eggs laid by queens at each temperature ranged from 8 to 100 d. Larvae developed at 30°C 18 d after the appearance of the first eggs and at 35°C the first larvae appeared 7 d after the appearance of the first eggs (Table 4). Pupae developed at 30°C 9 d after the first appearance of larvae and adults appeared 6 d after the first appearance of pupae (Table 4). The total development time from egg to larvae at 30°C was 33 d.

Table 4. Mean development time of each life stage of *Brachymyrmex patagonicus* in days at multiple temperatures

Temp.	Mean time to 1st eggs from initiation of study	Mean time to 1st larvae from appearance of 1st eggs	Mean time to 1st pupae from appearance of 1st larvae	Mean time to 1st adults from appearance of 1st pupae	Total development time from egg to adult
25°C	100	14	a	a	b
30°C	80	18	9	6	33
35°C	8	7	5.5	a	b

a=no development of that life stage at that temperature
b=could not be calculated

Discussion

In the laboratory studies, there were eggs laid by the queens and larval development at 25°, 30°, and 35°C. There was development from egg to larva at all temperatures. There was pupa development at 30º and 35ºC; however, the only temperature where there was complete development from egg to adult was 30ºC.

Egg production was lower in this study than expected and as reported by Migulena and Baker (2010) who documented that a single *B. patagonicus* queen laid 17 eggs in her first clutch. In the current study, regardless of temperature the mean number of eggs laid by a queen was 9. Egg production in the current study could have been negatively affected by the time of year when the study was conducted (August 2013 and 2015) in the laboratory. Other factors that could have negatively influenced the size of first clutch of eggs in the current study were temperature, relative humidity, photoperiod, and ratio of workers to queen. Temperatures were held constant ±2°C, relative humidity was 80±2%, and photoperiod was (8 h light, 16 h dark). In the current study the optimal

temperature was 30°C in which eggs were laid and fully developed to adults. This is consistent with Migulena and Baker (2010) who found the optimal temperature to be 29°C for *B. patagonicus* reproduction in the laboratory. In their study they had solitary queens produce up to 18 eggs in the first clutch, which was more than in the current study, where the largest first clutch of eggs was 10.

Based on the results of the current study, temperature played a substantial role in the development of the *B. patagoncius* brood, and this was consistent with Porter (1988) who reported that temperature greatly affected the development of *S. invicta* in a laboratory study. This is supported by data which are that in the current study at 35°C the time from initiation of the study to the first batch of eggs was 8 d, which was much sooner than at 25°C (100 d) and 30°C (80 d), respectively. It was also evident because the duration of the egg development to larvae and larvae to pupa was sooner at 35°C than at 25°C or 30°C. At 30°C time from egg to larvae (9 d) was almost twice as long as it was at 35°C (5.5 d). Time required to develop from egg to larvae varied at all three temperatures and the development time required to reach pupae from larvae varied from 30°C (9 d) and 35°C (5.5 d).

Although development time of life stages varies greatly by species of ant, without any baseline knowledge of *B. patagonicus*, the only comparisons that can be made are with other ants. At 27°C it is reported that *M. pharaonis* complete development from egg to pupae is 41 d (Peacock and Baxter 1950), this is much slower than the results in the current study in which *B. patagonicus* developed from egg to adult in 33 d at 30°C. Porter (1988) reported that *Pheidole bicarinata* development time was 35-41 d

at 27°C, which is also very similar to *B. patagonicus* development time of 33 d at 30°C. Newell (1909) reported that *Iridomyrmex humilis* had a development time of 41 d at 27°C, which is much slower than *B. patagonicus* which developed from egg to adult in 33 d at 30° C.

Development of eggs to adults is a measure of fitness and is important in understanding the reproduction of *B. patagonicus*. Survivorship of eggs to adult in this study was low, but it was undoubtedly much higher in wild populations. If the development of *B. patagonicus* is better refined through research it could lead to integrated pest management strategies for this species that would help minimize their reproduction and lead to better control in urban environments.

Understanding and documenting the reproductive cycle of *B. patagonicus* is of the utmost importance because this knowledge can help pest management professionals (PMP) to know when to be aggressive with treatments focused toward dark rover ants at accounts that have historically had issues with this ant. Based on the data of the current study the optimal temperature for sustaining *B. patagonicus* colonies in a laboratory setting is 30°C. Now that this optimal temperature has been confirmed, more extensive laboratory studies can be established to continue investigation into *B. patagonicus* development from egg to adult. I believe that *B. patagonicus* does need to be studied more extensively because it is likely that this species will continue to expand its range across the United States and become more prevalent in urban environments. Which will lead to more consumer calls to PMPs who will need to educated on the biology of this ant.

CHAPTER III

INVESTIGATION OF *BRACHYMYRMEX PATAGONICUS* FORAGING BEHAVIOR

Introduction

Since very little is known about *B. patagonicus*, it is important to describe the foraging behavior. By determining the behavior, and movement of populations, pesticide applications can be used more effectively to control this ant in urban environments. Understanding foraging behavior is key to gaining knowledge on an ant species morphology, ethology, and biochemistry (Carroll and Janzen 1973). There are several components of foraging that must be recognized, first is that a single ant is not a measure of the fitness of a colony (Wilson 1968, Carroll and Janzen 1973). Second is that ants forage for a myriad of particulate food sources and third that most ants are scavengers and omnivorous (Carroll and Janzen 1973). There are many aspects that can determine foraging by ants which include the kinds of food preferred, hunt live arthropods, gather deceased arthropods, gather particulate food sources (Janzen 1968), or tend aphids for honey dew. The characteristics of the food are of importance as well in selection by the individual workers because it can determine foraging behavior (Carroll and Janzen 1973). Factors related to the characteristic of food that can effect foraging include ability of worker to pick food up and worker load capacity of that food (Rosengren 1971), availability of labor (timing) (Steyn 1954), time required to manipulate food so that it can be passed to others (Brian 1957), storage and longevity of

that food (Tevis 1958), and preference of food to short and long term needs of the colony (De Bruyn and Mabelis 1972).

Just as import in finding resources, is bringing that resource back to the nest (Carroll and Janzen 1973). Communication is of the utmost importance within an ant colony. Most ants are capable of communicating where a food source is located to the rest of the colony, but it is unclear to what extent an individual worker within a colony relies on communication (Carroll and Janzen 1973). It is known that in some species of ants that a worker when searching for food will touch their gaster to the substrate which is a trail laying technique for recruitment to food resources (Holldobler and Wilson 1970). This trail laying behavior results in non-repetitive searching of an area for food, recruitment of ants to a food source in order to defend it while it is being collected and returned to the nest (Carroll and Janzen 1973), and it aides the worker ants in returning to the nest (Haskins and Haskins 1950). Another method of recruitment to a food resource is "tandem running" (Wilson 1959), which is reported in at least two genera of ants including *Camponotus* and *Carcdiocondyla*. This is a behavior where one worker ant follows another worker ant to a resource that is too large for one ant to manipulate (Wilson 1959). Another method of recruitment is the use of short-lived chemical trail lying. This short-lived method is widely used among ants and the pheromones only lasts for a few hours (Wilson 1971). It is hypothesized that ants that form small colonies like *B. patagonicus* may utilize the short-lived trail lying method (Carroll and Janzen 1973). In fact it is theorized that small colonies rely on the individual to learn behavior on "what it must do" and "where it must go" for the betterment of the colony (Beckers et al.

1989, Falibene et al. 2009). This is based on a simple fact that the smaller the colony the more the colony must rely on individual foragers for resources (Beckers et al. 1989).

B. patagonicus has small colonies which contain a queen, eggs, larvae, pupae and workers. The foraging trails of this species are loose, erratic and can be inconsistent. Infestations can seem large because there can be satellite colonies and multiple distinct colonies within a small area such as a residential home lot (Miguelena and Baker 2010). Another reason the foraging behavior of this ant is difficult to determine is that this species is very cryptic and subterranean, and often times nests cannot be located. The foraging behavior of urban pest ants is important to understand in order to develop management strategies that are efficacious (Kafle et al. 2008). Very little is known or understood about the foraging habits of *B. patagonicus* therefore laboratory trials were used to look at the foraging behavior of this ant.

Materials and Methods

Brachymyrmex patagonicus colonoids (1 queen, 0.10 g brood, and 100 workers) were placed in a 100 x15 mm plastic petri dish (KORD-Valmark, Bristol, PA) (nest) coated with Insect-a-Slip to prevent insect escape. Attached to this nest dish was one of the following (treatments): a 1.0 m, 5.0 m, or 10.0 m piece of Tygon® tubing (0.75 OD. cm, 0.5 cm ID, Saint-Gobain Plastics, Akron, OH) to which a food dish was connected at the end (Fig. 1). Each food dish was provisioned with a test tube 16 x 150 mm (VWR Inc.) filled with water and a cotton plug inserted (harborage), and a 2.5 cm hexagonal weigh boat (VWR Inc.) with 3.0 grams of maple pancake syrup (food). This set-up was replicated five times for each length of tubing. Data collected included presence or

absence of ants in the food dish, and whether the colony was moved from the nest dish to food dish.

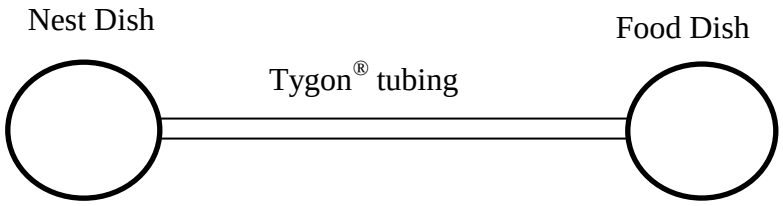

Figure 1. Nest dish with a food dish connected with a length of Tygon® tubing (either 1.0, 5.0 or 10.0 m) to determine foraging distance of *Brachymyrmex patagonicus*. Nest and food dish were 150 x 15 mm petri dishes.

***Statistics*:** Analylsis of variance (ANOVA) was used to analyze the foraging data. The software used was SPSS for Windows, Rel. 21.0.01.2008. (SPSS Inc, Chicago, IL). The design accounted for the variation which occurs in the data from having three different tube lengths. Means were separated by Tukey's honestly significant difference (HSD) test. *P*-values were considered statistically significant with $\alpha \leq 0.05$.

Results

***Foraging Colonoids*:** In the foraging arenas that had colonoids (queen, brood, and workers), the ants in the 1.0 m arenas had moved from the nest dish to the food dish at the 1 d reading (Table 5, Fig. 2). In those arenas, 95% of the worker ants were in the food dish along with the queen and brood. The ants in the 5.0 m arenas were still in the nest dish at the 1 d reading. In those arenas 65% of the workers were still in the nest dish along with the queen and brood, while 25% of the ants were in the food dish. In the 10.0

m arenas, 78% of the workers were in the nest dish along with the queen and brood at the 1 d reading (Table 5). At the 5 d reading, the colonoids in the 1.0 m arenas remained in the food dish (Fig. 3). The ants in the 5.0 m arenas at 5 d were in the nest dish with 49% of the workers and the queen and brood, while 43% of the workers were in the food dish. The ants in the 10.0 m arenas at 5 d were in the food dish with 68% of the workers and the queen and brood, while 21% of the workers were in the nest dish (Fig 4). At the 10 d reading the colonoids in the 1.0 m arenas remained in the food dish (Fig. 5). The ants in the 5.0 m arenas at 10 d were located in the food dish with 70% of the workers and the queen and brood. In the 10.0 m arenas at 10 d 79% of the workers and the queen and brood were located in the food dish (Table 5).

Table 5. Number and location of *Brachymyrmex patagonicus* colonoids with queen, brood and workers

Treatment	Day 1			Day 5			Day 10		
	# of ants in nest	# of ants in food	Location of colony	# of ants in nest	# of ants in food	Location of colony	# of ants in nest	# of ants in food	Location of colony
1.0 m	0.0±0.0b	95.0±0.0a	F	2.5±2.8a	92.5±2.8a	F	21.2±8.5a	76.2±7.5a	F
5.0 m	65.0±23.8a	25.0±23.8b	N	48.7±45.1a	42.5±43.5a	N	23.7±24.2a	71.2±24.6a	F
10.0 m	77.5±20.6a	16.2±16.0b	N	21.2±39.2a	67.5±38.4a	F	8.7±2.5a	78.7±6.2a	F

F=food dish
N=nest dish
Location of colony=location of queen and brood
Means followed by the same letter in the same column are not significantly different (p=0.05) per Tukey's HSD.

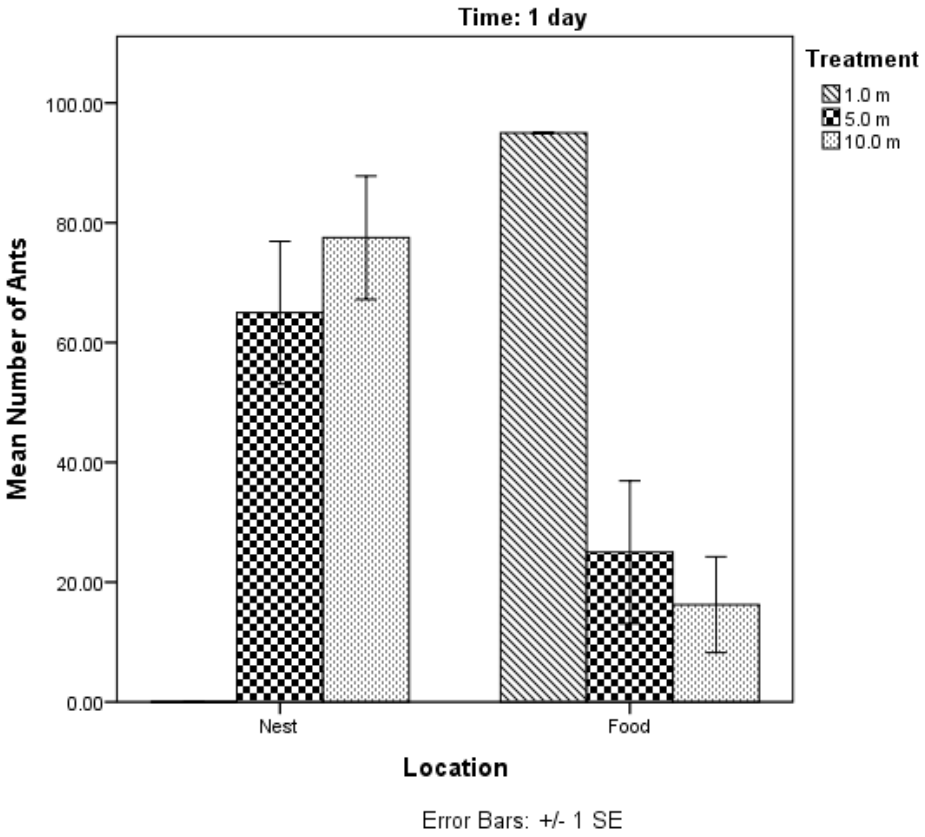

Figure 2. Number and location of *Brachymyrmex patagonicus* workers with queen and brood present at 1 day post-initiation of study.

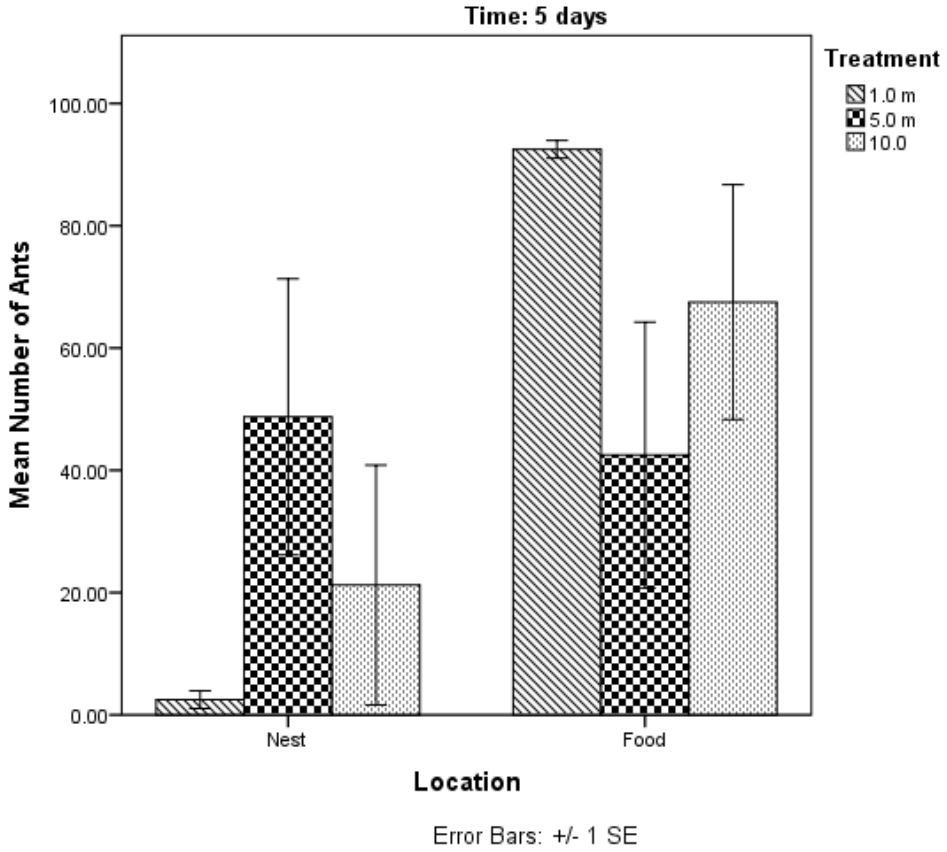

Figure 3. Number and location of *Brachymyrmex patagonicus* workers with queen and brood present at 5 days post-initiation of study.

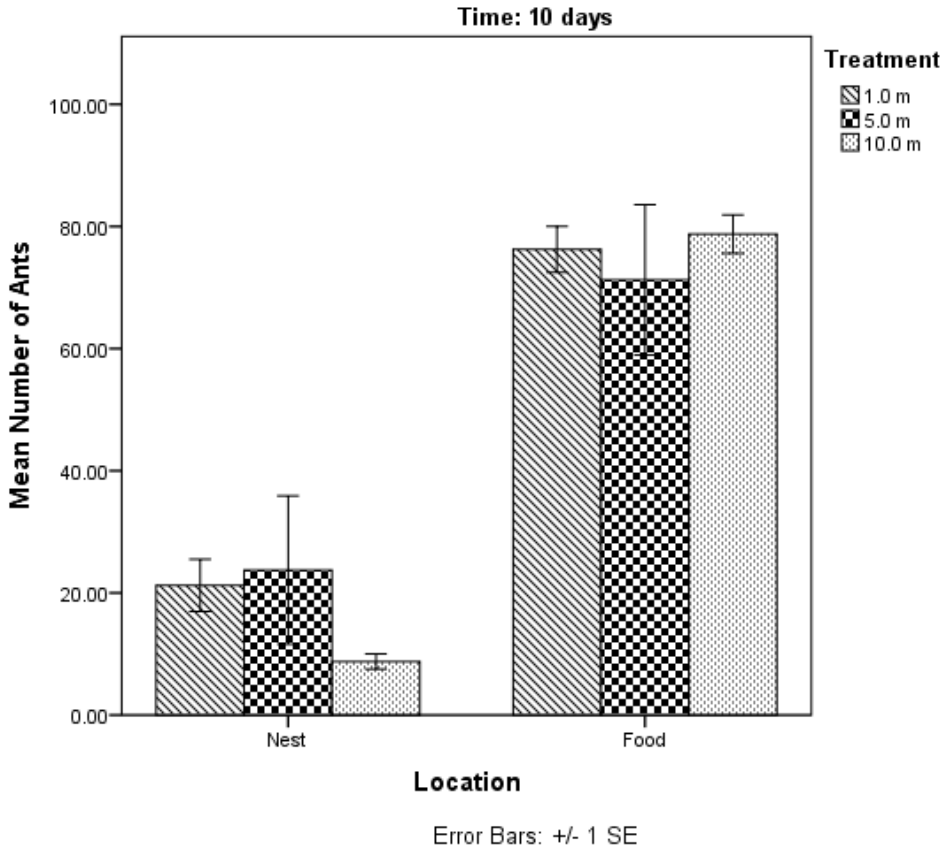

Figure 4. Number and location of *Brachymyrmex patagonicus* workers with queen and brood present at 10 days post-initiation of study.

Foraging Workers and Brood: In the foraging arenas that had workers and brood only (no queen present) the ants in the 1.0 m arenas had moved from the nest dish to the food dish at the 1 d reading (Table 6, Fig. 5). In those arenas, 79% of the worker ants were in the food dish along with the brood. The ants in the 5.0 m arenas were still in the nest dish at the 1 d reading. In those arenas, 65% of the workers were still in the nest dish along with the queen and brood, while 25% of the ants were in the food dish. In the 10.0 m arenas, 78% of the workers were in the nest dish along with the brood at the 1 d reading (Table 6). At the 5 d reading the workers and the brood in the 1.0 m arenas

remained in the food dish (Fig. 6). At 5 d 20% of the workers were in the nest dish, while 73% of the workers and 100% of the brood were in the food dish (Table 6). At 5 d 38% of the workers and 100% of the brood remained the nest dish, with 23% of the workers in the food dish (Table 6). At the 10 d reading the colonoids in the 1.0 m arenas remained in the food dish (Fig. 7). The ants in the 5.0 m arenas at 10 d were located in the food dish with 73% of the workers and 100% of the brood (Table 6). In the 10.0 m arenas, at 10 d 43% of the workers and 100% brood were located in the nest dish (Table 6).

Table 6. Number and location of *Brachymyrmex patagonicus* workers with brood present through time

	Day 1			Day 5			Day 10		
Treatment	# of ants in nest	# of ants in food	Location of colony	# of ants in nest	# of ants in food	Location of colony	# of ants in nest	# of ants in food	Location of colony
1.0 m	22.0±10.7b	79.0±7.5a	F	3.5±1.9a	87.7±2.0a	F	4.5±4.2a	92.5±2.8a	F
5.0 m	67.0±34.7a	20.0±25.8b	N	20.7±39.5a	73.2±38.9a	F	17.5±28.4a	73.7±32.5a	F
10.0 m	89.5±6.4a	0.0±0.0b	N	38.0±25.0a	23.0±19.8b	N	43.7±22.8a	25.0±15.8b	N

F=food dish
N=nest dish
Location of colony=location of workers and brood
Means followed by the same letter in the same column are not significantly different (p=0.05) per Tukey's HSD.

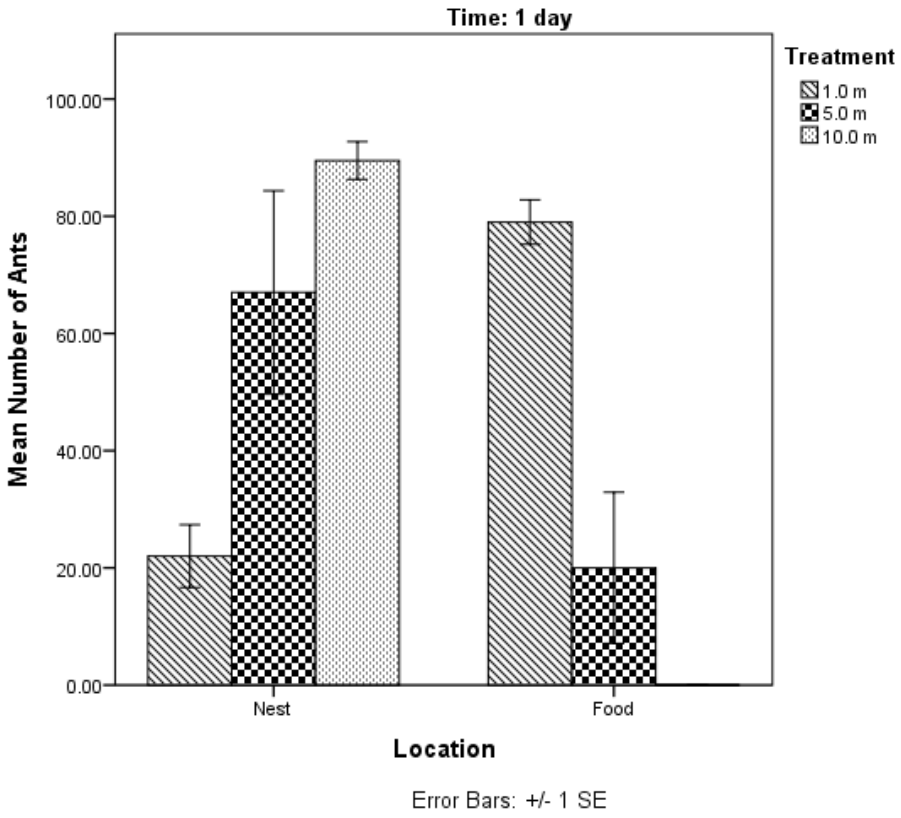

Figure 5. Number and location of *Brachymyrmex patagonicus* workers with brood present at 1 day post-initiation of study.

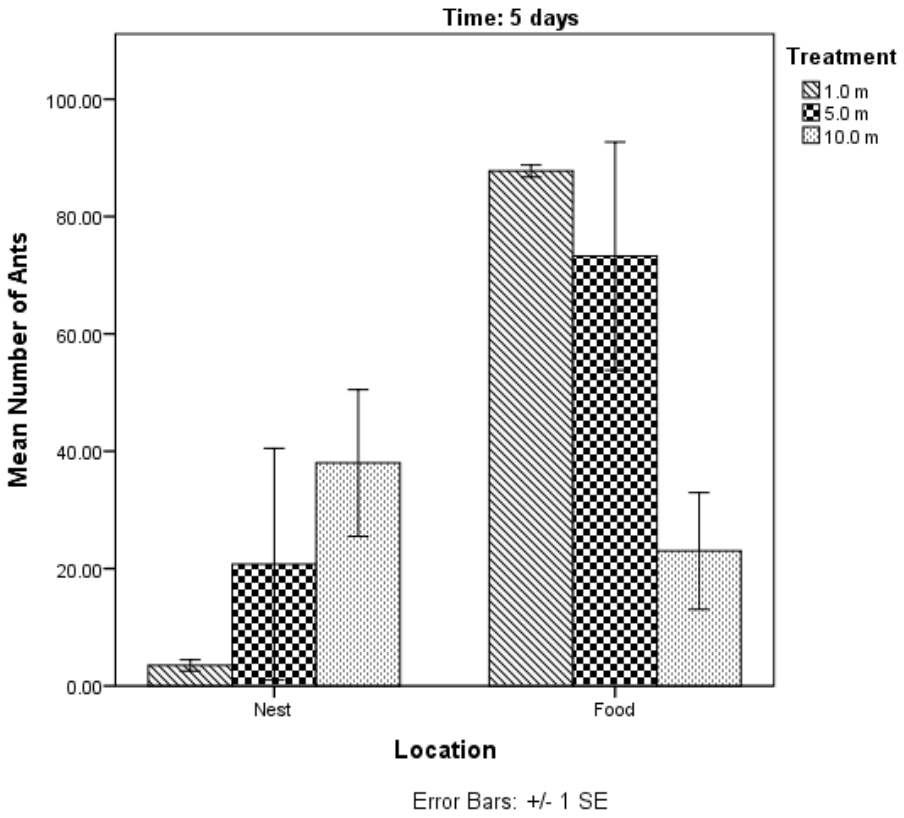

Figure 6. Number and location of *Brachymyrmex patagonicus* workers with brood present at 5 days post-initiation of study.

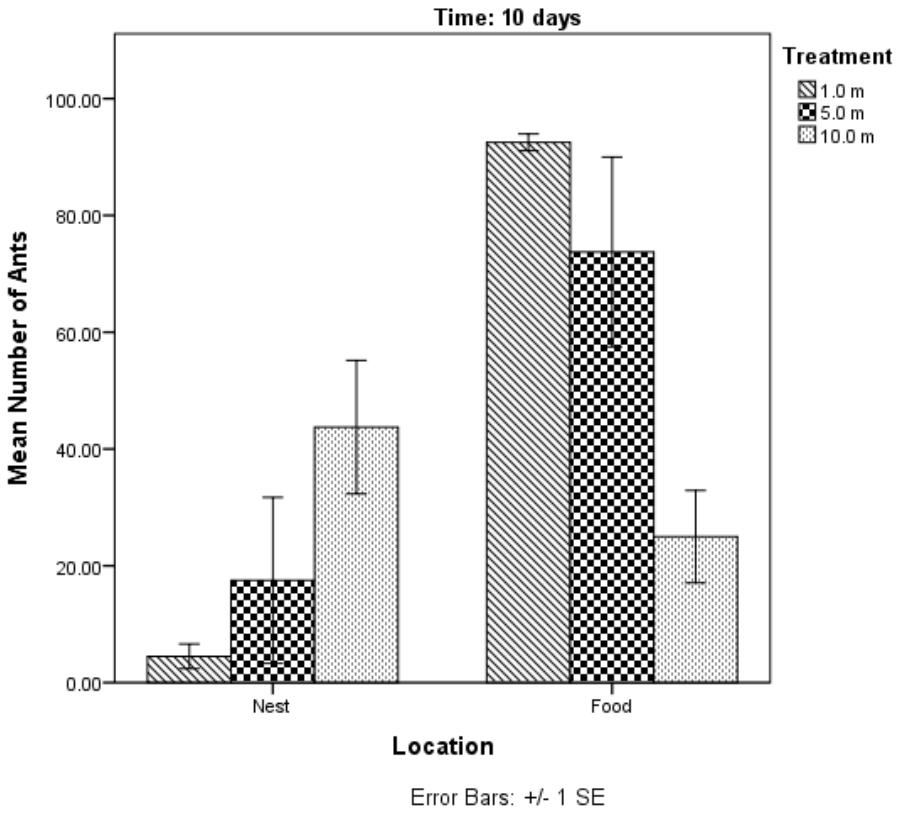

Figure 7. Number and location of *Brachymyrmex patagonicus* workers with brood present at 10 days post-initiation of study.

***Foraging Workers Only*:** In the foraging arenas that had workers only, in the 1.0 m arenas at the 1 d reading, 91% of the workers were in the nest dish (Table 7). In the 5.0 m arenas at the 1 day reading 76% of the workers were in the nest dish and 80% of the workers were in the nest dish in the 10.0 m arenas (Fig 9). At the 5 d reading in the 1.0 m arenas 78% of the workers were in the nest dish and 10% were in the food dish (Table 7). In the 5.0 m arenas at the 5 d reading 78% of the workers were in the nest and 12% were in the food dish (Fig. 10). In the 10.0 m arenas at the 5 d reading 88% of the workers were in the nest dish and 6% were in the food dish (Table 7). At the 10 d reading in the 1.0 m arenas 84% of the workers were in the nest and 6% were in the food dish (Fig. 11). In the 5.0 m arenas at 10 d 76% of the workers were located in the nest dish and 16% were in the food dish. At the 10 d reading in the 10.0 m arenas 83% of the workers were in the nest dish and 7% were in the food dish (Table 7).

Table 7. Number and location of *Brachymyrmex patagonicus* workers through time

	Day 1			Day 5			Day 10		
Treatment	# of ants in nest	# of ants in food	Location of colony	# of ants in nest	# of ants in food	Location of colony	# of ants in nest	# of ants in food	Location of colony
1.0 m	70.7±10.7a	12.0±7.7a	N	78.5±6.6a	10.0±2.9a	N	74.0±4.9a	15.5±6.1a	N
5.0 m	80.0±9.8a	9.7±4.7a	N	78.7±9.7a	12.0±8.7a	N	76.0±6.0a	16.5±7.9a	N
10.0 m	84.5±7.7a	6.0±5.2a	N	88.5±7.1a	6.5±6.3a	N	83.0±7.9a	7.5±8.5a	N

F=food dish
N=nest dish
Location of colony=location of greatest number of ants
Means followed by the same letter in the same column are not significantly different (p=0.05) per Tukey's HSD.

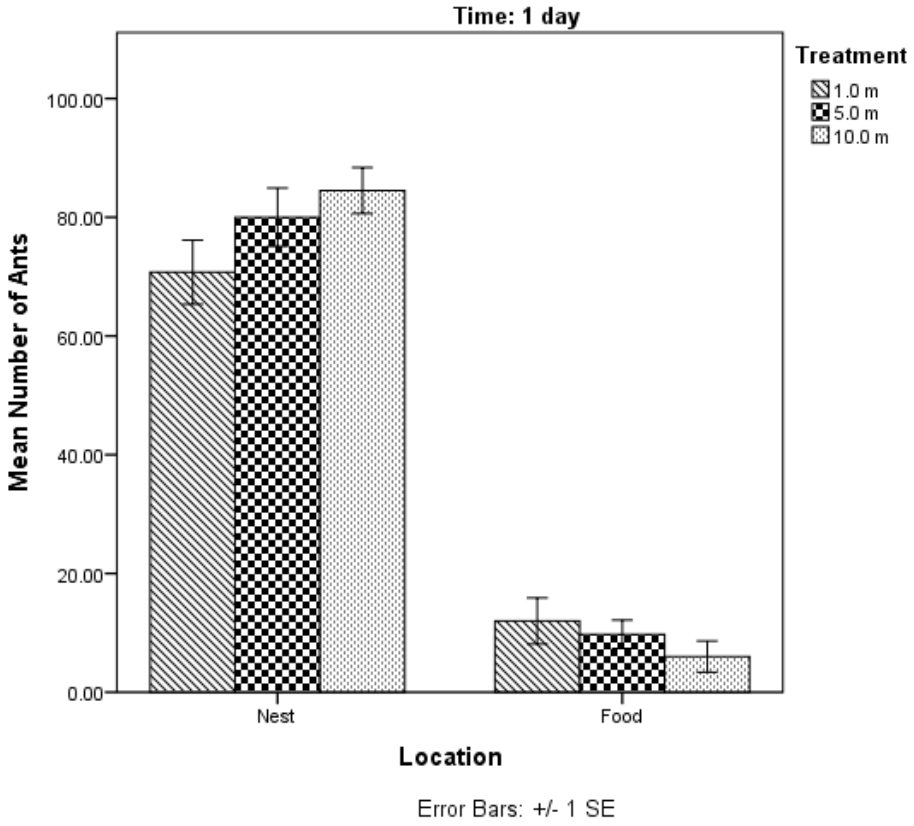

Figure 8. Number and location of *Brachymyrmex patagonicus* workers at 1 day post-initiation of study.

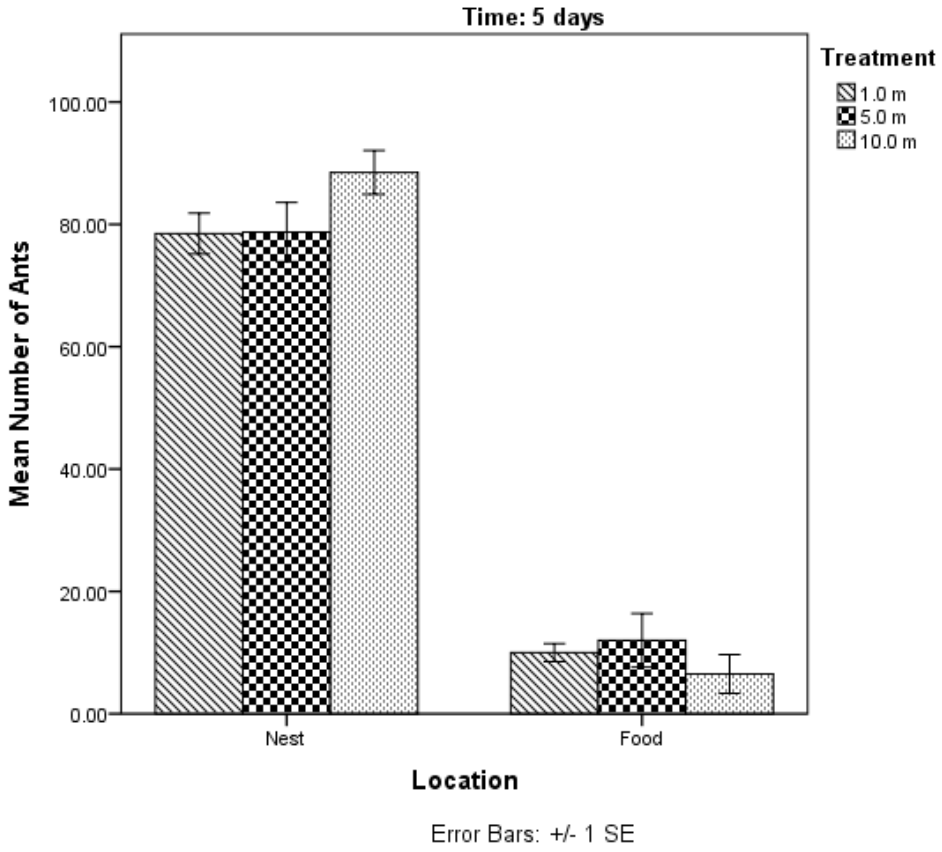

Figure 9. Number and location of *Brachymyrmex patagonicus* workers at 5 days post-initiation of study.

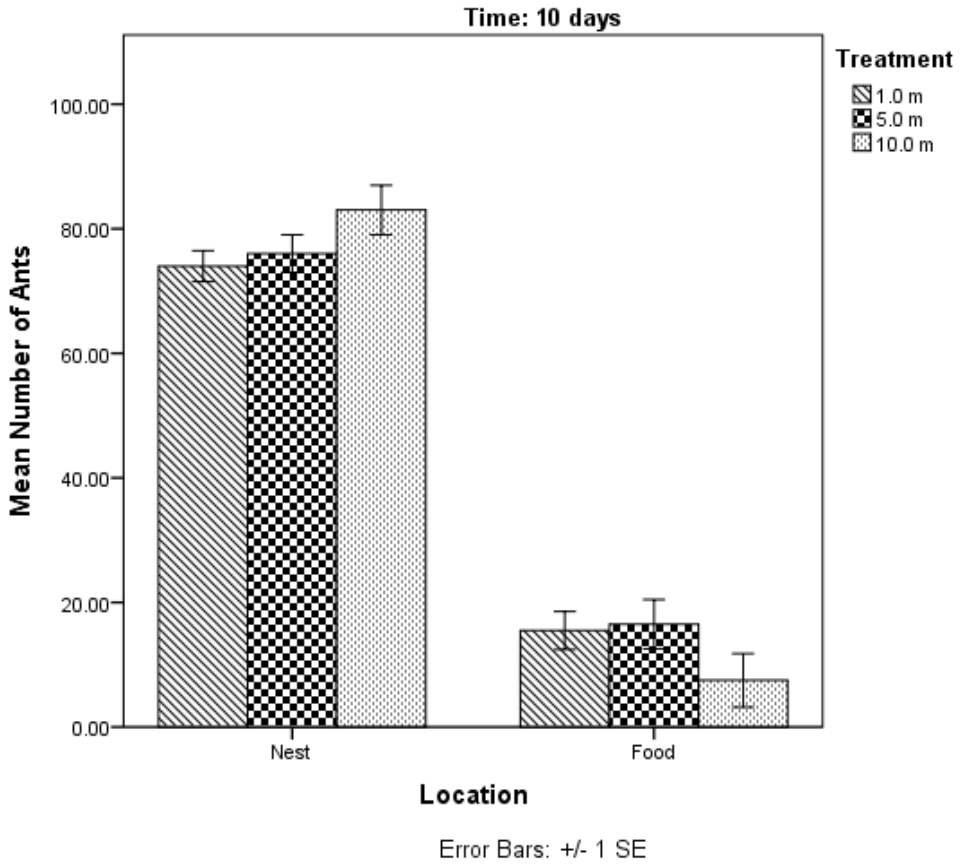

Figure 10. Number and location of *Brachymyrmex patagonicus* workers at 10 days post-initiation of study.

Discussion

As mentioned earlier there are several foraging strategies that are well documented in ants which include trail laying behavior, tandem running, and short lived chemical trails. I believe *B. patagonicus* utilizes short lived chemical trails in order to facilitate foraging. The data that support this hypothesis are that *B. patagonicus* have small colonies ranging in size from several hundred to thousands of ants (Beckers et al.1989). My personal observations of *B. patagonicus* foraging in the laboratory and in

the wild indicated that foraging is erratic and trails are loosely organized. It is also documented that ant species that have small colonies rely on individual foragers more so than ant species that have large colonies such as *S. invicta* (Carrol and Janzen 1973, Beckers et al. 1989, Falibene et al. 2009). Ant species with larger colonies tend to have more inefficient individuals and in other words the smaller the colony the more foraging is individually based and the individual forager is coordinated by chemical communication and is more efficient. Individual foraging means that there is no mass recruitment to a food source, so the individual discovers the resource without systematic communication with thousands of other individuals within the colony (Aron et al. 1989). *B. patagonicus* does this because their trails do not have a long residual and are not constantly remarked by individuals within the colony. An advantage of this technique could be that other colonies of *B. patagonicus* or other species of competing ants cannot detect the trails and therefore reduce competition for food sources.

Based on the results of the current study it is believed that *B. patagonicus* are indeed tramp ants, and as such will readily move the colony to a food source. Work completed by Migulena (2014) confirms this in which it showed that *B. patagonicus* colonoids (queen, brood, and workers) will readily move from a nest dish and inhabit and establish permanent satellite nests when resources are available that sometimes include eggs and larvae but no queen. In the current study colonoids (queen, brood, and workers) moved from the nest dish to the food dish much quicker in the 1.0 m arenas than in the 5.0 and 10.0 m arenas. At the end of the study (10 d) there were no differences in the location of colonoids based on the length of the arena, all of the

colonoids had moved to the food dish. At 10 d there was a difference in the location of colonoids of queen, brood and worker, and worker and brood, as compared to the worker only replications regardless of length of arena. The replications with workers only did not forage and move like the other colonoids did to the food dish. Instead the worker only colonoids stayed in the nest dish for the duration of the study. Therefore it is perceived that the recruitment was not substantial and trails were not heavily marked in the worker only replications as it was in the colonoid replications with queen, brood and worker and brood and workers.

It is well documented in many ant species that in order for the worker to forage there has to be communication between the queen and the workers and that the workers will also forage based on chemical ques from the larvae (Wilson 1971). This could explain why there was very little foraging in the worker only foraging assays in this study. In the assays with queen brood and workers there was much more foraging and the ants moved the colony to the food dish faster than the other two set-ups within this assay.

Future work that should be investigated with *B. patagonicus* foraging should include foraging with workers and queen only in the laboratory. This work could complement the foraging studies completed in the current study to give a full picture on the foraging strategy of *B. patagonicus*.

CHAPTER IV

POTENTIAL OF *BRACHYMYRMEX PATAGONICUS* AS A VECTOR OF A PATHOGEN

Introduction

The number of invasive ant species reported in urban environments has been on the rise in recent years (Rust and Su 2012). In fact, many ants go unnoticed for years and suddenly become a significant problem for humans such as *Myrmica rubra* (L.), *Pachycondyla chinensis* (Emery) and *B. patagonicus* (Mayr) (Groden et al. 2005, Nelder et al. 2006, MacGown et al. 2007). Ants are a major pest in urban areas and are of medical importance because some species can sting and bite humans, and others are reported as mechanical vectors of pathogens (Santos Lima et al. 2013). The relationship between ants and pathogens was initially reported in England in the early 1960's, and what was Czechoslovakia and Germany in the late 1960's and 1970's (Beatson 1972, Fonseca 2010). In more recent years in the western hemisphere, ants have been noted as vectors of pathogens in Chile and the United States (Costa et al. 2006, Fonseca 2010).

The order Hymenoptera, which include ants, is ranked third on a list of medically important groups of insects, behind only Diptera and Blattodea (Fotedar 2001, Immamura et al. 2003). Ants are social insects and some are parasitic, mutualistic and have interactions with animals and plants which give plenty of opportunity to come in contact with pathogens (Ehud and Gross 2000, Santos and Ueno 2008). Ants also carry

microorganisms on their cuticle and therefore have been long implicated as vectors of pathogens (Beastson 1972, Josens et al. 2014).

Brachymyrmex ants are major pests in urban environments including hospitals, and they have the same characteristics of other small ants that are known as vectors of human pathogens such as *M. pharaonis* (Santos and Ueno 2008, Pantoja et al. 2009). Ants, when present in sensitive areas such as hospital surgery and burn units, can create pathways of resistance by vectoring bacteria that are resistant to antibiotics (Rodovalho et al. 2007). Ants are the perfect mechanical vector of many pathogens because of their adaptability and the free contact they can have with medical waste and patients (Pantoja et al. 2009). Ants have been found in hospital for decades including in sterile areas such as in intravenous fluids and in dressings of post-operative patients (Edwards and Baker 1981).

Many ant species including *M. pharaonis*, *Tapinoma melanocephalum*, and *Paratrechina longicornis*, have been reported to carry up to 21 different pathogens/bacteria including *Staphyloccus*, *Streptoccus*, *Serratia* sp., *Acinetobacter*, *Enterococcus, Pseudomonas* and *Klebsiella.* of which many of the strains were antibiotic resistant (Ebeling 1978, Moreira 2005, Lise et al. 2006). In studies by Pantoja (2009) and Santos (2011), 75% of ants collected from hospitals and analyzed for pathogens contained either *Aspergillus* or *Candida* yeast. This is alarming to doctors, patients, and pest management professionals because ants could be directly associated with hospital infection problems (Fonseca et al. 2010). Factors that lead to the presence of ants in sensitive areas include building practices (voids), proximity of structure to residences,

human alteration of climate, human preferred temperatures, and packaging of hospital supplies that are ideal for ant nests (Beatson 1972, Zarzuela et al. 2002).

Some insects including ants possess many characteristics and behaviors that make them prime candidates to vector bacteria including minute size, as compared to larger cockroaches, flies, and mosquitoes (Josens et al. 2014). Ants also form forage trails over long distances in search of resources. This can expose hundreds or thousands of individuals within a colony to a pathogen that could allow each individual exposed to mechanically move the pathogen in sensitive areas such as hospitals, nursing homes, and commercial kitchens (Beatson 1972, Josens 2014). *B. patagonicus* has been reported in hospitals in large numbers and has been implicated as a mechanical vector of pathogens (Josens et al. 2014). In fact, Josens et al. (2014) reported that *B. patagonicus* was the most frequent species found indoors, and that they were very difficult to control. Other important information noted in Josens et al. 2014 were that *B. patagonicus* were found in kitchens, offices, living quarters, and laundry rooms (throughout the structures) and this species showed intense foraging in those areas. Lima et al. (2013) found a correlation between bacteria found on ants collected in hospitals and patients in the vicinity of where the ants were collected. This suite of characteristics along with tramp ant relationships with humans make ants a prime candidate to spread pathogens (Beatson 1972, Josens 2014). Experiments in this research were done in the laboratory to examine the vector competence of *B. patagonicus* of human pathogens.

Materials and Methods

This study was conducted in a Bio-Safety Level 1 laboratory, at the Rollins Urban and Structural Entomology Facility located in College Station, TX. Experimental arenas consisted of two 60 x 15 mm P. dish (KORD-Valmark, Bristol, PA) (Fig. 2) which were connected via Tygon® tubing (0.75 OD. cm, 0.5 cm ID, Saint-Gobain Plastics, Akron, OH). Dish #1 had LB (lysogeny broth) agar and ampicillin product number L5667 (Sigma-Aldrich St. Louis, MO) with fluorescently labeled *Escherichia coli*. Dish #2 had LB agar ampicillin and no *E. coli*. The LB agar with ampicillin was comprised of the following components; agar, tryptone, NaCl, yeast extract and ampicillin which forms into a gel that is nutrient rich microbial broth that promotes bacterial growth. The *E. coli* was a laboratory strain (Top10, Life, Technologies, Grand Island, NY) labeled with DSRed-Expression protein (Clonetech, Mountain View, CA) and an ampicillin resistant gene. Fluorescent *E. coli* was obtained from the Department of Biochemistry and Biophysics at Texas A&M University in College Station, TX. The *E. coli* was held at 37°C in constant darkness in an environmental chamber. Both dishes were lined with Insect-a-Slip (BioQuip Products Inc.) to prevent ant escape. Ants were allowed to travel through one of three lengths of Tygon® tubing (0.5 m, 1.0 m, or 3.0 m) to reach Agar Dish #2. These arenas were held in constant darkness.

Fifteen colonoids, each consisting of a queen, 0.10 g of brood and 50 worker ants were assembled. The colonoids were placed in Dish #1 and ants were then allowed to move about the arenas for 48 h after which time the arenas were disassembled, and Dish #2 was incubated for 36 h at 37°C, with lights out, and then fluorscent *E. coli* (Sarocco et

al. 2003) colonies were assessed. The three transmission distances (0.5 m, 1.0 m, or 2.0 m) were assessed and replicated five times each (Fig. 2). Simultaneously, there were ten 60 x 15 mm P. dishes with LB ampicillin agar (controls), five with 25 ants and no fluorescent *E. coli* and five with no ants and no fluorescent *E. coli*. After 48 h the ants were removed and these 10 plates were incubated for 36 h at 37°C, with lights out, and fluorescent and *E. coli* colonies were assessed with the naked eye by counting the number of colonies. An *E. coli* colony for this study was defined as a red, shiny, circular, raised, small smooth, defined margin growth on the agar.

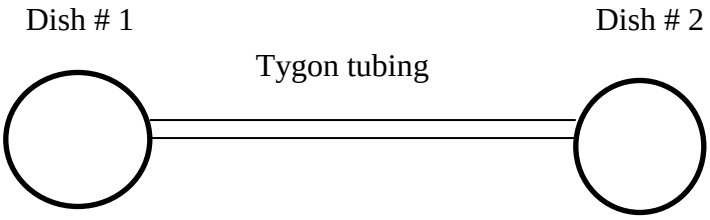

Figure 11. Arena set-up for evaluation of the potential of *Brachymnyrmex patagonicus* as a vector of a disease pathogen. Dish #1 with fluorescent *Escherichia coli*, 25 *B. patagonicus*, and LB agar with antibiotic, Tygon® tubing, and Dish #2 with LB agar and antibiotic. Dish #1 and Dish #2 are both 60 x 15 mm P dishes. Tygon® tubing lengths were one of the following 0.5, 1.0 or 2.0 m.

***Statistics*:** Analylsis of variance (ANOVA) was used to analyze the the mechanical vector data. The software used was SPSS for Windows, Rel. 21.0.01.2008. (SPSS Inc, Chicago, IL). The design accounted for the variation which occurs in the data from having three different tube lengths. Means were separated by Tukey's honestly

significant difference (HSD) test. *P*-values were considered statistically significant with $\alpha \leq 0.05$.

Results

In the 0.5 m replications the ants transferred *E. coli* to dish #2 in 100% of the arenas and the mean number of colonies transferred to dish #2 was 28.2±13.2 (Table 8). In the 1.0 m arenas the ants transferred *E. coli* from dish #1 to dish #2 in 60% of the replications. The mean number of *E.coli* colonies transferred to dish #2 was 25.0±26.4 (Fig. 13). In the 2.0 m replications the ants transferred *E. coli* to dish #2 in 60% of the arenas (Table 8) and the mean number of colonies transferred to dish #2 was 4.0±4.6 (Fig. 13). The mean number of *E. coli* colonies transferred from dish #1 to dish #2 in the 0.5 m and 1.0 m replications was significantly different (p=0.05) from the number of *E. coli* colonies transferred in the 2.0 m replications (Table 8). In the control replications that contained ants and no red *E.coli* in dish #1, there was no detection of red *E. coli* in dish #1 or #2 throughout the study, and there was no detection of red *E. coli* colonies in dish #2 in the control replications that contained no ants and no red *E. coli* in dish #1 throughout this study (Table 8).

Table 8. Number of times and mean number of fluorescent red *E. coli* colonies transferred from Dish #1 to Dish #2 through Tygon tubing by *Brachymyrmex patagonicus*

Treatment (length of tubing)	*E. coli* Transferred to Dish # 2	Mean # of *E.coli* colonies in Dish # 2 (SD)	Mean # *of E. coli* colonies in controls (ants w/ no *E.coli*)	Mean # of *E. coli* colonies in controls (no ants, no *E. coli*)
0.5 m	5 out of 5 (100%)	28.2±13.2a	0.0±0.0a	0.0±0.0a
1.0 m	3 out of 5 (60%)	25.0±26.4a	0.0±0.0a	0.0±0.0a
2.0 m	3 out of 5 (60%)	4.0±4.6b	0.0±0.0a	0.0±0.0a

Means followed by the same letter are not significantly different (p=0.05) per Fisher's LSD test.

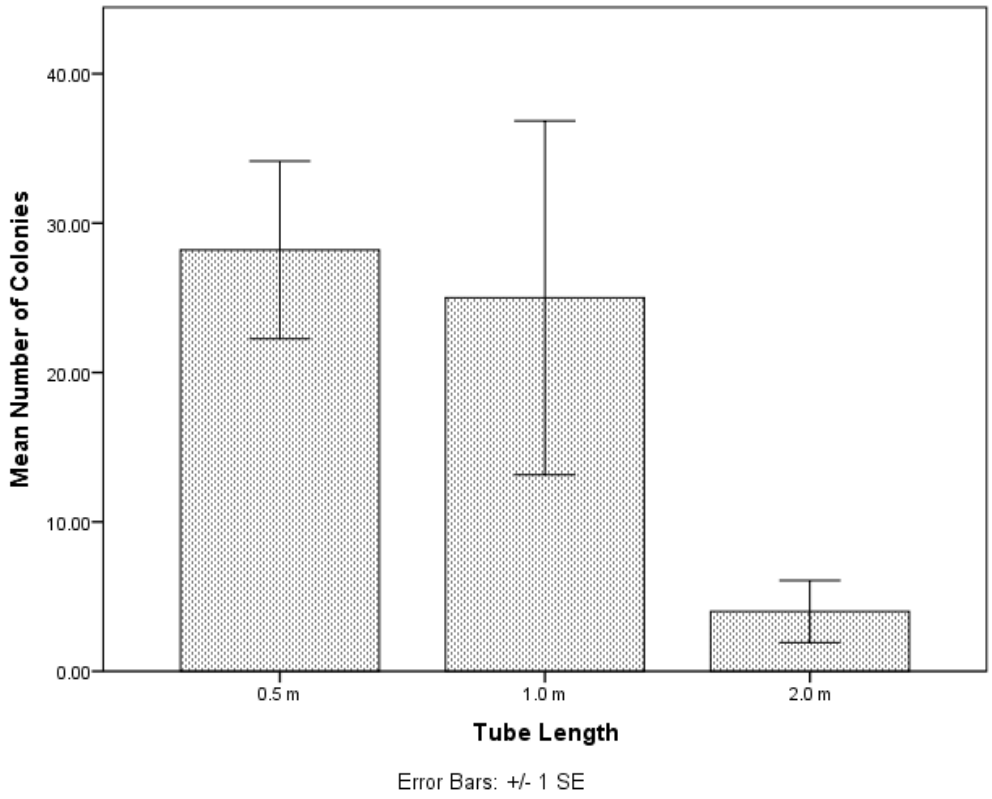

Figure 12. Mean number of *E. coli* colonies transferred from nest dish to food dish.

Discussion

There are several examples of insects that are vectors of human pathogens including urban ants such as *M. pharoanis* and (Imamura et al. 2003). One theory is that pathogens that affect humans have origins within wild and domesticated animals and crossed over to the human population due to close association (Wolfe et al. 2007). Some well-known and worldwide examples include acquired immune deficiency syndrome, malaria, plague, Chagas disease, cholera, dengue fever, and yellow fever, all were of significance for two decades or more during peak outbreaks (Wolfe et al. 2007). Today scientists and doctors conclude that humans are being bombarded by novel animal pathogens that could infect human populations (Wolfe et al. 2004). *B. patagonicus* is documented as a species that tends soil and plant dwelling aphids (Hedges 2010), and therefore *B. patagonicus* could potentially also be a mechanical vector of plant disease agents.

The results of the current study indicated that *B. patagonicus* are potential vectors of *E. coli* and are of medical concern particularly in hospitals and other sensitive accounts. In these studies *B. patagonicus* foragers transferred *E. coli* for at least 2.0 m. Albeit that as distance increased, the transmission of *E. coli* decreased. There was no red *E. coli* detected in the controls after the 48 hr incubation period, so it was concluded that *B. patagonicus* were the source of the red *E. coli* that was found in Dish # 2 in these trials.

The social aspect of *B. patagonicus* and other tramp ant species are of major concern in and around sensitive structures, including hospitals and elderly care facilities.

B. patagonicus is very similar in size and behavior to *M. pharaonis* which is a known mechanical vector of human pathogens (Santos and Ueno 2008, Pantoja et al. 2009). Both species are also considered tramp ants that move their colonies when disturbed. This information is important to the pest management industry because PMPs need this knowledge in order to know to use non-repellent pesticides to control *B. patagonicus* so that the colony will not be disturbed by the treatment and escalate the problem. It is documented that the pathogen *Serratia marcescens* was recovered from a nest of *M. pharaonis* after 21 d from the initial infection. The pathogen *B. globiglii* was detectable for 30 d (Beatson 1972) in *M. pharaonis* colonies. Ant nests can be a viable incubator for bacteria if within the nest R. H. is at or near 80% and temperatures are maintained between 27-38ºC (Beatson 1972). The ant behavior of trophallaxis is also a method of maintaining a sustainable pathogen population within a nest for weeks or months of time. Foraging ants that return to the nest often times will regurgitate food, which is then pliable for others individuals with in the colony to eat which could pass pathogens. Foragers will also regurgitate food for storage which could also be a source of pathogens within the nest.

Ants can move quickly and can travel long distances in search of resources and can come into contact with pathogens in their daily routines. Therefore ants can act as a dispersal unit for pathogens to reach humans, and in severe cases ants can act as a vector for drug resistant bacteria (Pecanha et al. 2000). This can be a highly dangerous situation, which poses many risks to humans if integrated pest management practices are

not followed such as the continued monitoring and identification of insects in and around sensitive structures (Bueno and Campos-Farina 1998).

Some of the future work that should be done to further investigate *B. patagonicus* as a mechanical vector of pathogens is to document the bacteria they naturally carry and determine if they are of medical interest. Another area to investigate should include, if a forager picks up a pathogen and returns to the nest area, how long is that pathogen viable within the nest and is it possible for the environmental conditions and the sociality of the ant to keep the pathogen viable for a long period of time and therefore, keeping the pathogen circulating among ants within the nest and the nests acts as a reservoir for the pathogen. Determine the minimum pathogen load in order to successfully vector a disease and the maximum distance possible that *B. patagonicus* can vector a pathogen. Compare bacteria found on *B. patagonicus* to those in hospitals (sensitive structures), and in natural settings; to demonstrate that if *B. patagonicus* can transfer those bacteria that are naturally found on them and the bacteria that are found on them in sensitive structures; and, finally, see if there is a correlation between bacteria found on *B. patagonicus* in and around sensitive accounts and the bacteria found on patients within those accounts.

CHAPTER V

EVALUATION OF DIFFERENT FOOD LURES TO DETERMINE DIET PREFERENCES OF *BRACHYMYRMEX PATAGONICUS* IN A LABORATORY AND FIELD SETTINGS

Introduction

B. patagonicus is found throughout much of the southeastern United States and is seemingly more difficult to control than other urban ants (R. Davis, B. Springer, J. Meyers, personal communications) due to the lack of information about the basic biology and preferences in diet. Food collection is the major duty of foragers; therefore, determining their diet preferences could help in the development of commercial bait products, which would be effective for control of this ant as it continues to spread throughout the United States. Ants require nutrients in proper ratios for reproduction and fitness (Cook et al. 2010). There are many factors that can contribute to the food lure preference by foragers including, species of ant, size of colony, season, climate, colony distance from food source, general fitness of colony, size of workers, age of worker, and presence of brood, (Holldobler 1976, Kafle et al. 2008, Human et al. 1998, Trainello 1989, Hooper and Rust 1997, Nyamukondiwa and Addison 2014). However these factors and there effects on *B. patagonicus* have not been studied. Ants of many urban species can be found feeding on a wide range of edible materials including most food consumed by humans (Edward and Abraham 1990). Many studies have been carried out

to determine food preference of ants utilizing food consumed by humans (Granovsky and Howell 1983, Edwards and Abraham 1990, Eow and Lee 2007).

Ants are social insects and live in colonies and therefore require many different nutrients in various amounts and ratios to promote proper growth and fitness of the colony (Cook et al. 2010). The two most important needs of an ant colony are carbohydrates and proteins (Cassil et al. 1998). Foragers must collect food for themselves and for other members of the colony that require different nutrients based on age and stage of growth, therefore, food collection is very complex at the colony level (Cook et al. 2010). This phenomenon is a challenge for foraging ants as they must make decisions, which are controlled by internal and external factors at the individual and colony level (Cook et al. 2010). These factors could include the fitness of the individual forager (Blanchard et al. 2000), and at the colony level, the needs of brood that are communicated through the colony to the forager (Kay 2004). In theory these ques from the colony should match up with the individual's needs and therefore result in the same acquisition of food (Judd 2006). To further complicate food finding and transport by foragers, these colony communications can vary markedly within the colony at any given time (Cook et al. 2010). How social insects maintain resources at both a colony and individual level is not completely understood (Dussutour and Simpson 2008).

When an invasive species is found in colder climates as compared to its native range, it has been reported that foragers will search simultaneously for immediate and long term nutritional needs (Judd 2006). This could be considered the case with *B. patagonicus*, which is native to warm humid regions of South America. Thus they must

collect resources that meet their immediate nutritional requirements and foods that can be stored that will meet future requirements (Judd 2006). In species that do cache food, the cues that stimulate these behaviors are different from those that stimulate the individual immediate needs (Judd 2006). There are at least two factors that help the colony regulate foraging: 1) immediately needed nutrients are collected first and 2) independent cues by foragers are detected so that cached food is of high quality (Judd 2006). These cues can come from the colony through chemical communication (Cassill and Tschinkel 1995) or the forager can respond directly to its own nutritional needs (Blanchard et al. 2000). If a species was determined to be a cache species, this behavior could be utilized to determine nutrient requirements based on time of year (Ricks and Vinson 1972).

It is important to understand the relationship between an ant and it required resources in order to better understand the biology, ecology and how to control a species. But first simple questions must be answered such as what is the preferred food lure of *B. patagonicus* foragers under laboratory and field conditions. No testing of attractiveness of *B. patagonicus* foragers to food materials has been tested and published, therefore, experiments in this research were done to examine the preferred food lure of *B. patagonicus* foragers in the laboratory and the field.

Materials and Methods

In a laboratory setting, ten different food lures were used to determine the dietary preference of *B. patagonicus*. (30°C±2 80%RH±2) Arenas for this study consisted of plastic pans (Best Plastics Englewood, NJ) that were 17 x 40 cm with the sides coated

with Insect-a-Slip (BioQuip Products Inc.) to prevent ant escape. A queen and 100 worker ants (colonoid) were placed in the plastic pan along with six 2.5 cm hexagonal weigh boats (VWR Inc.) with 0.5 g (Sartorius Scale model GD-503-NTEP) of a food lure in five and one empty (untreated control). Food lure placement in the plastic box was randomly assigned. A second set of 2.5 cm weigh boats with the same weights of each of the food lures were placed on the exterior of each arena to account for natural loss or gain of weight of the food lure. The arena was photographed six times daily using a Canon PowerShot D20 camera (three times in the AM and three times in the PM) for 5 d, and the number of foraging ants on/in the weigh boats were counted. At the end of 5 d, the remaining food lure in each weigh boat was removed and weighed, and the difference in weight was determined by subtracting the end weight from the beginning weight measured in grams. The food lures in the exterior weigh boats were also removed and weighed at the end of 5 d. The end weights of the exterior food lures were compared to their time zero (initial) weights; the differences accounted for the natural weight gain or loss, which helped quantify the actual amount of food lure removed by ants in the interior weigh boats. These data were used as another measure of acceptability for any given food lure.

In a field setting, five hexagonal weigh boats (2.5 cm) with 0.5 g of a food lure in each weigh boat were placed near a known colony of *B. patagonicus*. A second set of weigh boats with the same weights of the each of the food lures were placed to account for natural loss or gain of the food lures. There was also one empty weigh boat which served as an untreated control. Food lure placement was randomly assigned. All of the

food lures were placed out in the field at 0500 h. Photographs were taken at 0600 h am then hourly through 1000 h at which time the food lures were retrieved. The food lures were placed back in the field at 4 pm and were photographed hourly starting at 1700 h and ending at 2200 h. The number of foraging ants in the weigh boats was determined. After the 2200 h photograph, the remaining food lure in each weigh boat was weighed, and the difference in weight from the pre-treatment weight reflected the amount of food lure removed. The end weights of the food lures in the second set were compared to their time zero (initial) weights; the differences accounted for the natural weight gain or loss, helped quantify the actual amount of food lure removed by ants. This set-up was replicated five times.

***Statistics*:** Analylsis of variance (ANOVA) was used to analyze the food lure preference data. The software used was SPSS for Windows, Rel. 21.0.01.2008. (SPSS Inc, Chicago, IL). The design accounted for the variation which occurs in the data from having six different food lures. Means were separated by Fishers least significant difference (LSD) test. *P*-values were considered statistically significant with $\alpha \leq 0.10$.

Results

In the laboratory study, the *B. patagonicus* foragers preferred the honey spread and pancake syrup over all other food lures (Table 9). There were 107 foragers that were documented as visiting the honey spread and 80 foragers that were documented as visiting the pancake syrup food lure (Fig. 13) over the 5 d period. The forager activity by day is given in Figure 14, the ants visited eight of the food lures on day 1 and preferred the honey spread on days 1, 3, 4, and 5, and on day 2 the ants preferred the pancake

syrup (Fig. 14). The mean number of ants that visited the honey spread per inspection period was 4 and for pancake syrup it was 4 (Fig. 15). The mean amount of food lure removed in grams from each food lure by the ants at the end of the 5 d period was as follows; 0.05 g of tuna, 0.03 g of pineapple preserves, 0.02 g of honey spread, 0.02 g of pancake syrup, and 0.00 g sweet and sour sauce (Table 9 and Fig. 16).

Table 9. Laboratory results of *Brachymyrmex patagonicus* forager preference of different food lures.

Food Lure	Total # of Ants	Mean Wt. Removed in Grams
Sunflower oil	4	-0.01 a
Shrimp sauce	6	0.01 a
Pineapple preserves	19	0.03 a
Honey spread	107	0.02 a
Sunsweet butter and oil	8	-0.02 a
Apple butter	6	-0.06 a
Sesame seed oil	5	-0.18 b
Sweet and sour sauce	0	0.00 a
Tuna	5	0.05 a
Pancake syrup	80	0.02 a
Total	240	

Means followed by the same letter in the same column in the same season are not significantly different (p=0.10) per Tukey's Honest Significant Difference Test.
Note: a negative number indicates that the food lure gained weight.

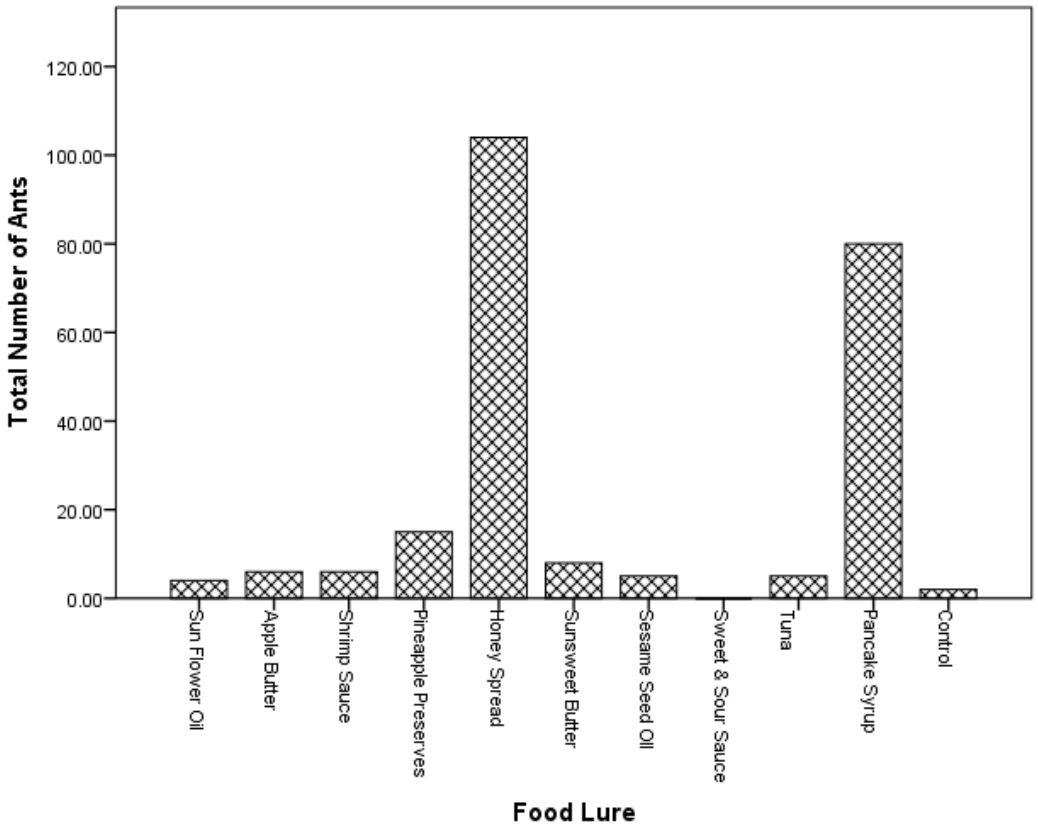

Figure 13. Total number of *Brachymyrmex patagonicus* foragers at each food lure for the 5 d period.

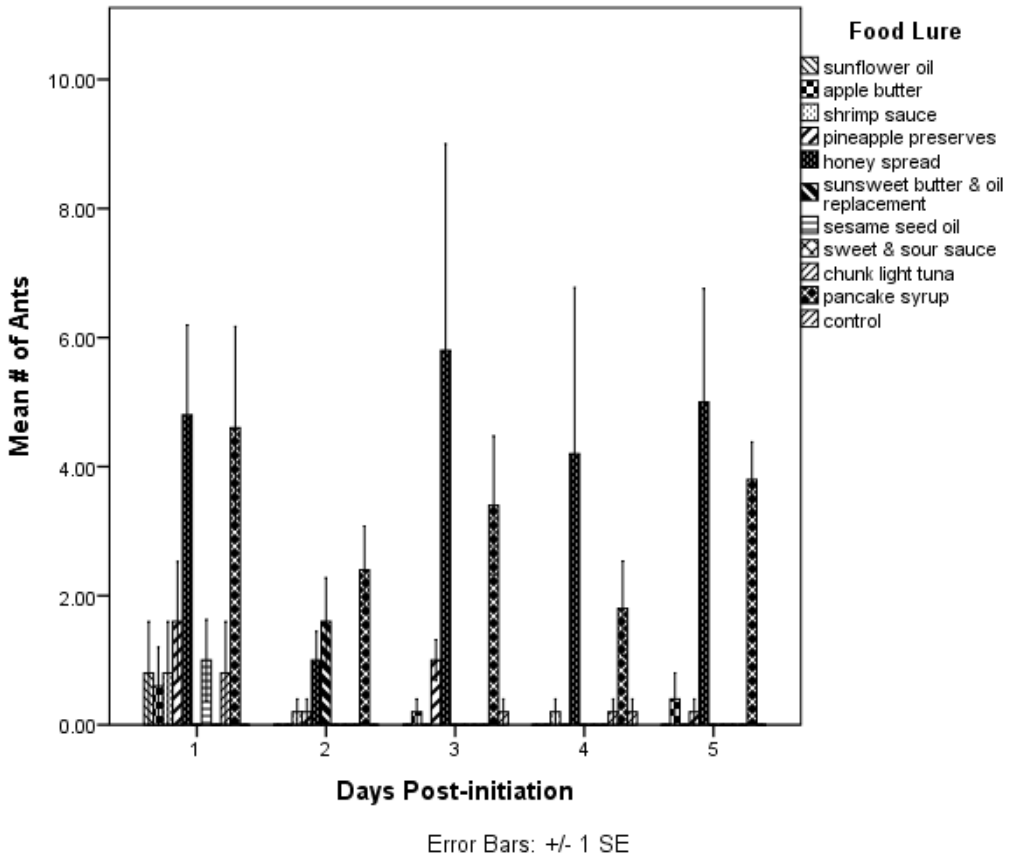

Figure 14. Mean number of *Brachymyrmex patagonicus* foragers at each food lure by day.

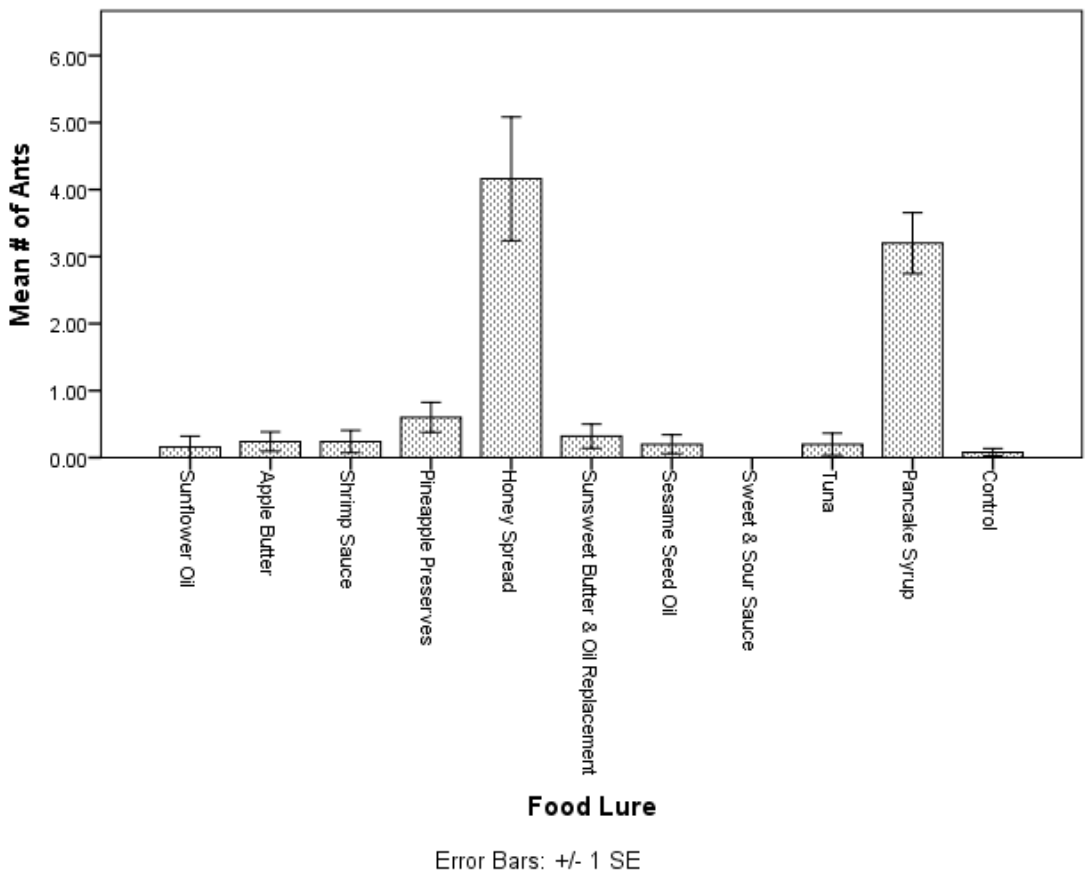

Figure 15. Mean number of *Brachymyrmex poatagonicus* at each food lure at inspection period.

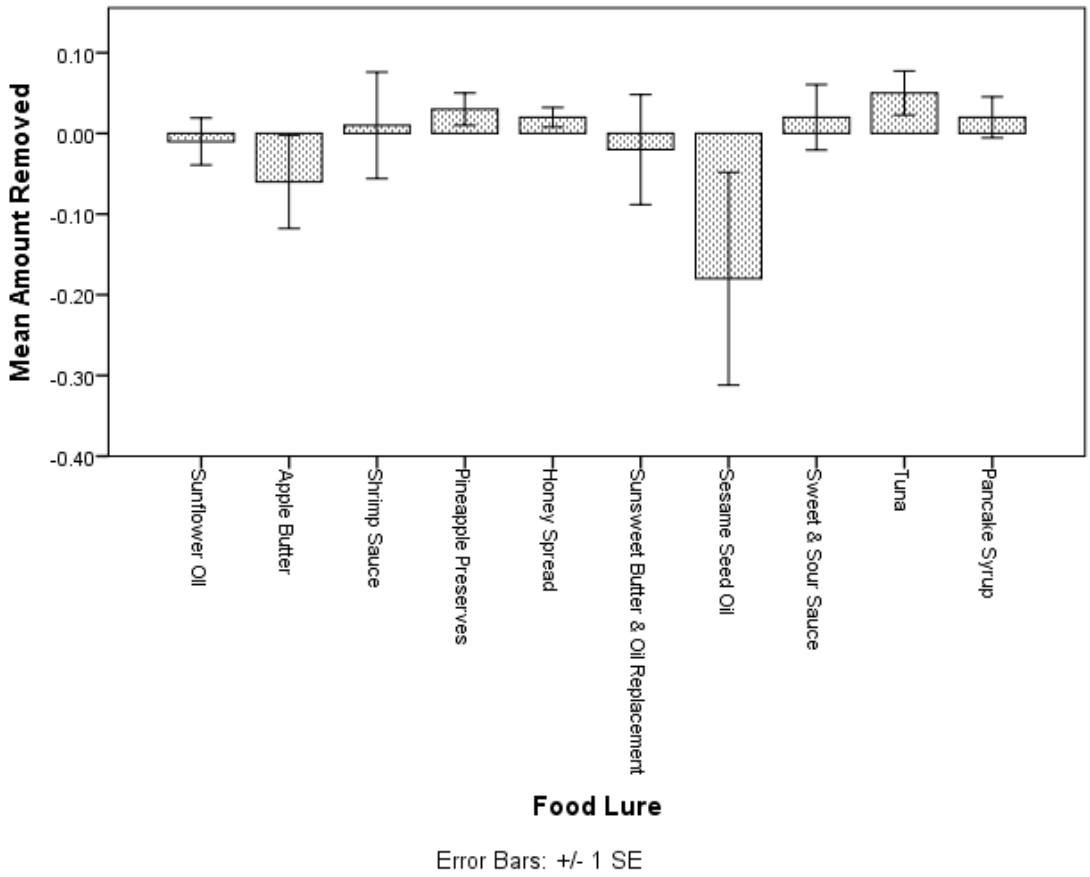

Figure 16. Mean amount of food lure removed by *Brachymyrmex patagonicus* foragers in grams at the end of the 5 d period.
Note: a negative number indicates the food lure gained weight.

Field Trials: There were a total of 7,093 *B. patagonicus* that visited the food lures during the field trials, 508 in the AM and 6585 in the PM (Table 10). There were significant differences (p=0.05) in the weight of food lures removed by the ants in the summer study (Table 10) and there were differences in the number of ants at each food lure by season per observation period (Table 10). In the winter study, over 2000 *B. patagonicus* visited the food lures followed by over 1600 in the Fall study (Fig. 17). In

the summer ants removed more tuna than any other food lure in any season followed by pancake syrup in summer (Fig. 18).

Winter: In the winter field study there were a total of 2103 *B. patagonicus* that visited the food lures (Table 10). There were 1,427 *B. patagonicus* that visited the pineapple preserves, 287 visited the honey spread, and 202 that visited the pancake syrup (Table 10). No *B. patagonicus* foragers visited the food lures in the morning hours. During the evening inspections, the ants visited all five food lures (Fig. 19). The mean amount of food lure removed in grams from each food lure by the ants at the end of the 5 d period was as follows; 0.16 g of pineapple preserves, 0.13 g of honey spread and 0.05 g of pancake syrup (Table 10).

Spring: In the spring field study there were a total of 1,354 *B. patagonicus* that visited the food lures. There were 529 *B. patagonicus* that visited the honey spread, 373 visited the pancake syrup, and 370 that visited the tuna (Table 10). No *B. patagonicus* foragers visited the food lures in the morning hours, in the evening hours the ants visited all five food lures and the untreated controls (Fig. 20). The mean amount of food lure removed in grams from each food lure by the ants at the end of the 5 d period was as follows; 0.43 g of honey spread, 0.33 g of pineapple preserves and 0.21 g of pancake syrup (Table 10).

Summer: In the spring field study there were a total of 1,559 *B. patagonicus* that visited the food lures. There were 733 *B. patagonicus* that visited the tuna, 368 visited the pancake syrup, and 284 that visited the honey spread (Table 10). *B. patagonicus* foragers visited the food lures at all of the morning inspections except at 6:00 am. In the

evening hours, the ants visited all five food lures and the untreated controls (Fig. 21). The mean amount of food lure removed in grams from each food lure by the ants at the end of the 5 d period was as follows; 0.82 g of tuna, 0.69 g of pancake syrup and 0.53 g of honey spread (Table 10).

Fall: In the fall field study there were a total of 1,632 *B. patagonicus* that visited the food lures. There were 1,093 *B. patagonicus* that visited the tuna, 266 visited the pancake syrup, and 242 that visited the honey spread (Table 10). *B. patagonicus* foragers only visited the food lures at the 9:00 am and 10:00 am inspections. In the evening hours, the ants visited all five food lures and the untreated controls (Fig. 22). The mean amount of food lure removed in grams from each food lure by the ants at the end of the 5 d period was as follows; 0.41 g of pancake syrup, 0.45 g of tuna and 0.33 g of honey spread (Table 10).

Table 10. Total number of *Brachymyrmex patagonicus* foragers at each food lure by season and time, mean amount of food lure removed and mean number of foragers at each food lure per observation period.

Food Lure	Season	Total # of ants at food lures by time of day		Mean weight of each food lure removed in grams (p=0.10)	Mean # of ants per observation period (p=0.05)
		AM	PM		
Pancake Syrup	Winter	0	202	0.05 a	6.7 b
Tuna		0	137	-0.14 a	4.6 b
Honey Spread		0	287	0.13 a	9.6 b
Pineapple Preserves		0	1427	0.16 a	47.6 a
Sweet & Sour Sauce		0	41	-0.37 a	1.4 b
Control		0	9		0.3 a
Total		0	2103		
Pancake Syrup	Spring	0	373	0.21 a	12.4 ab
Tuna		0	370	0.08 a	12.3 ab
Honey Spread		0	529	0.43 a	17.6 a
Pineapple Preserves		0	72	0.33 a	2.4 b
Sweet & Sour Sauce		0	10	0.18 a	0.3 b
Control		0	0		0.0 b
Total		0	1354		
Pancake Syrup	Summer	73	368	0.69 ab	6.7 ab
Tuna		267	733	0.82 a	13.3 a
Honey Spread		48	284	0.53 ab	5.2 b
Pineapple Preserves		94	79	0.14 b	2.6 b
Sweet & Sour Sauce		0	8	0.09 b	0.2 b
Control		14	24		0.4 b
Total		496	1496		
Pancake Syrup	Fall	12	266	0.41 a	8.5 b
Tuna		0	1093	0.45 a	36.4 a
Honey Spread		0	242	0.33 a	8.1 b
Pineapple Preserves		0	21	0.14 a	0.7 b
Sweet & Sour Sauce		0	4	0.09 a	0.1 b
Control		0	6		0.2 b
Total		12	1632		
Total # of Ants Overall		508	6585		

Means followed by the same letter in the same column in the same season are not significantly different (p=0.10) per Tukey's Honest Significant Difference Test.
Note: a negative number indicates that the food lure gained weight.

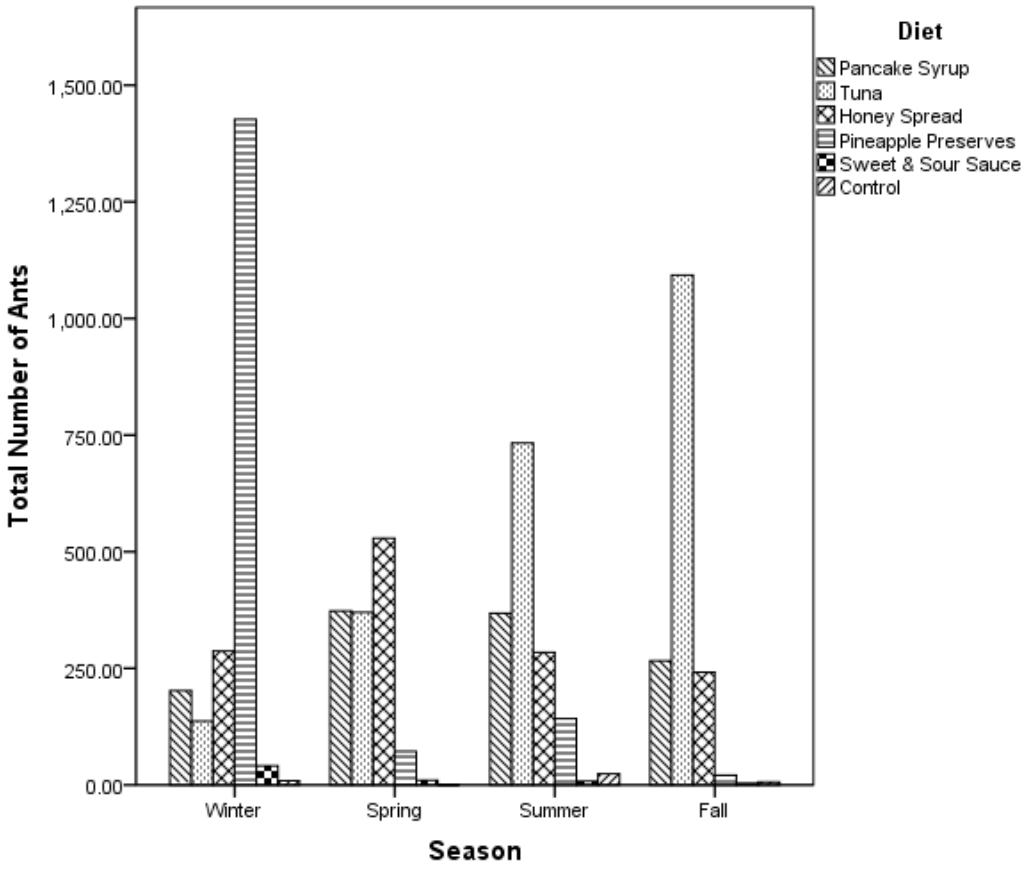

Figure 17. Total number of *Brachymyrmex patagonicus* foragers by season at each food lure.

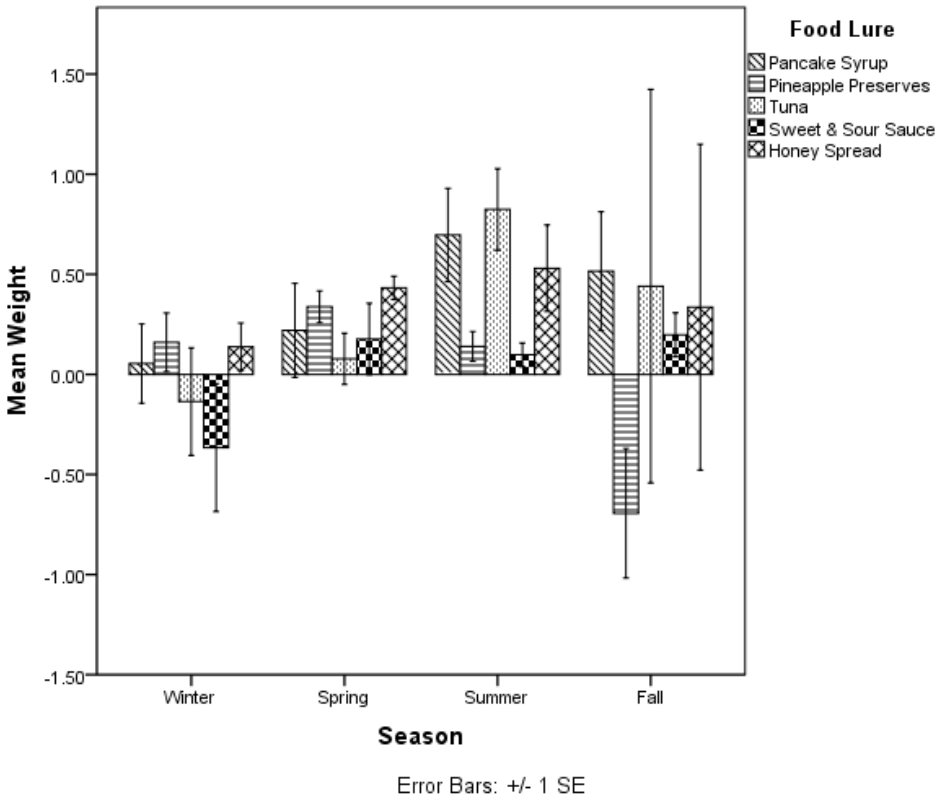

Figure 18. Mean amount in grams of each food lure removed by *Brachymyrmex patagonicus* foragers by season.

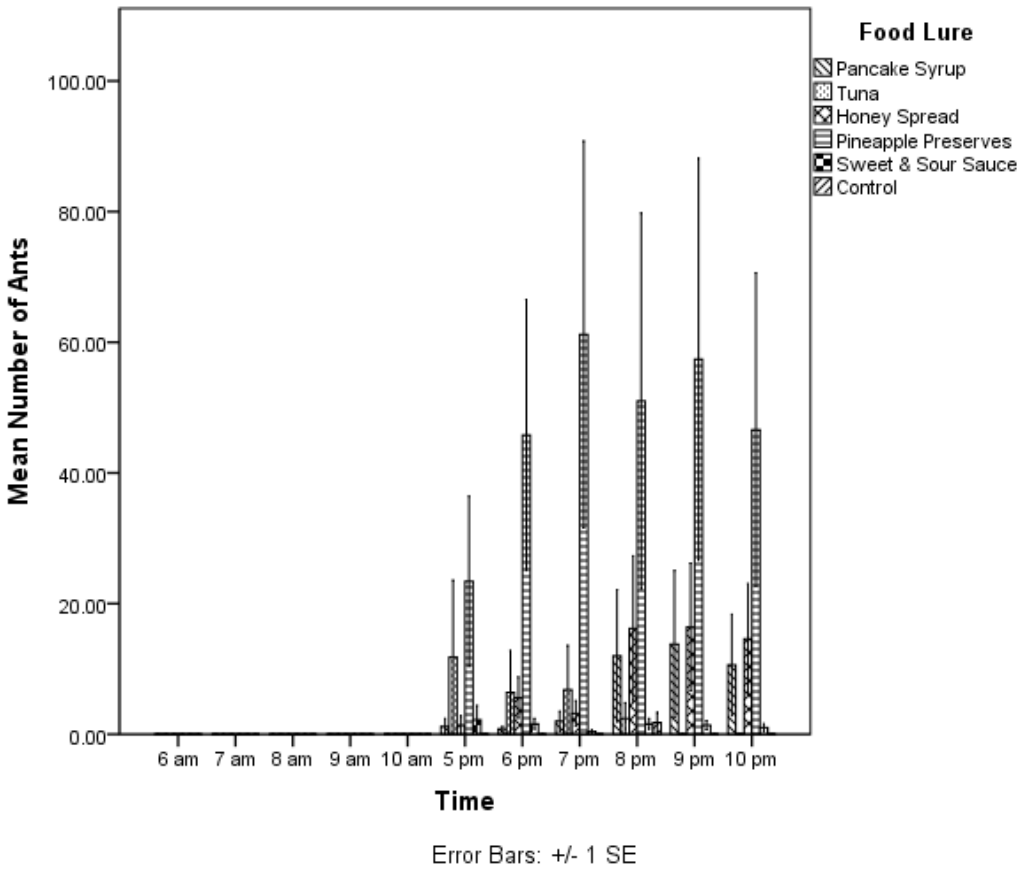

Figure 19. Mean number of *Brachymyrmex patagonicus* foragers through time by hour at each food lure in the winter.

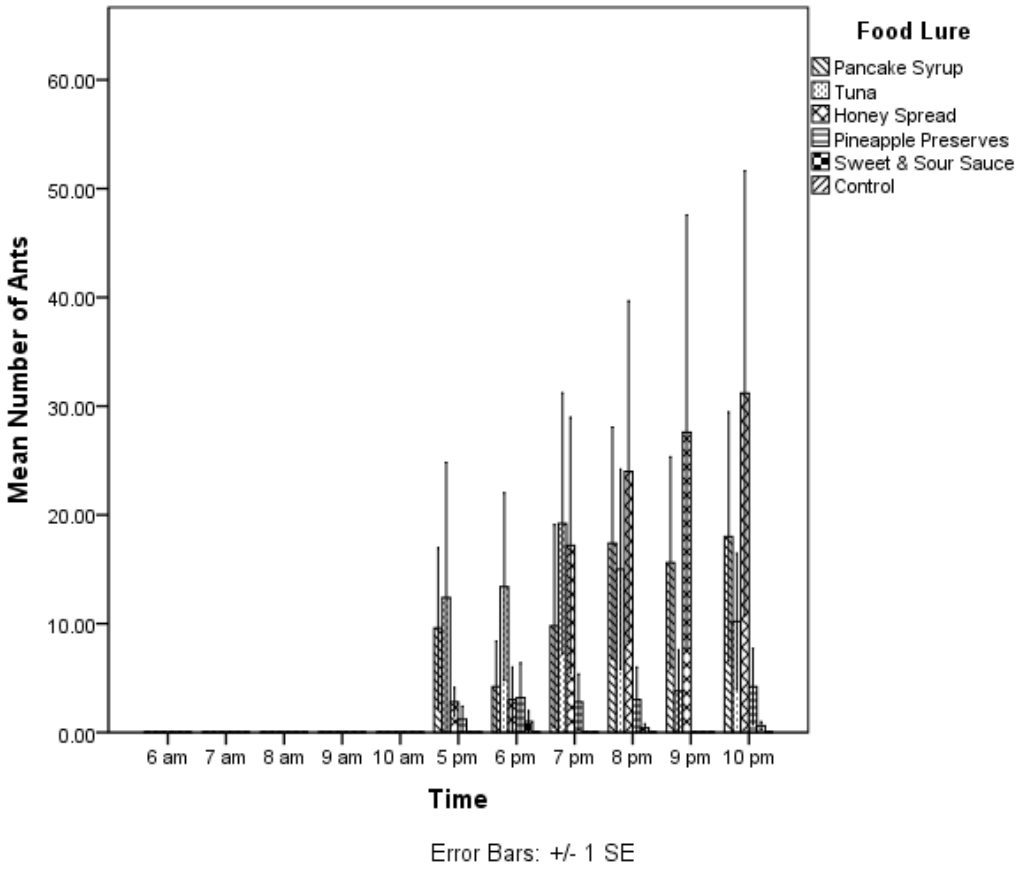

Figure 20. Mean number of *Brachymyrmex patagonicus* foragers through time by hour at each food lure in the spring.

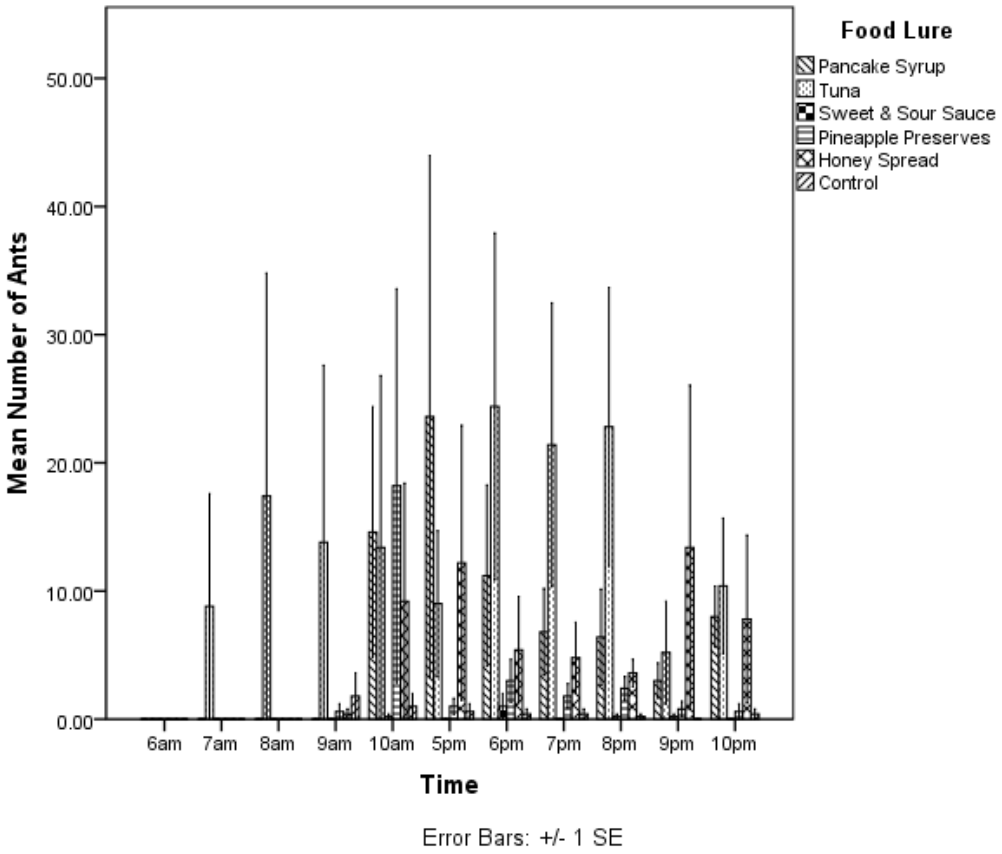

Figure 21. Mean number of *Brachymyrmex patagonicus* foragers through time by hour at each food lure in the summer.

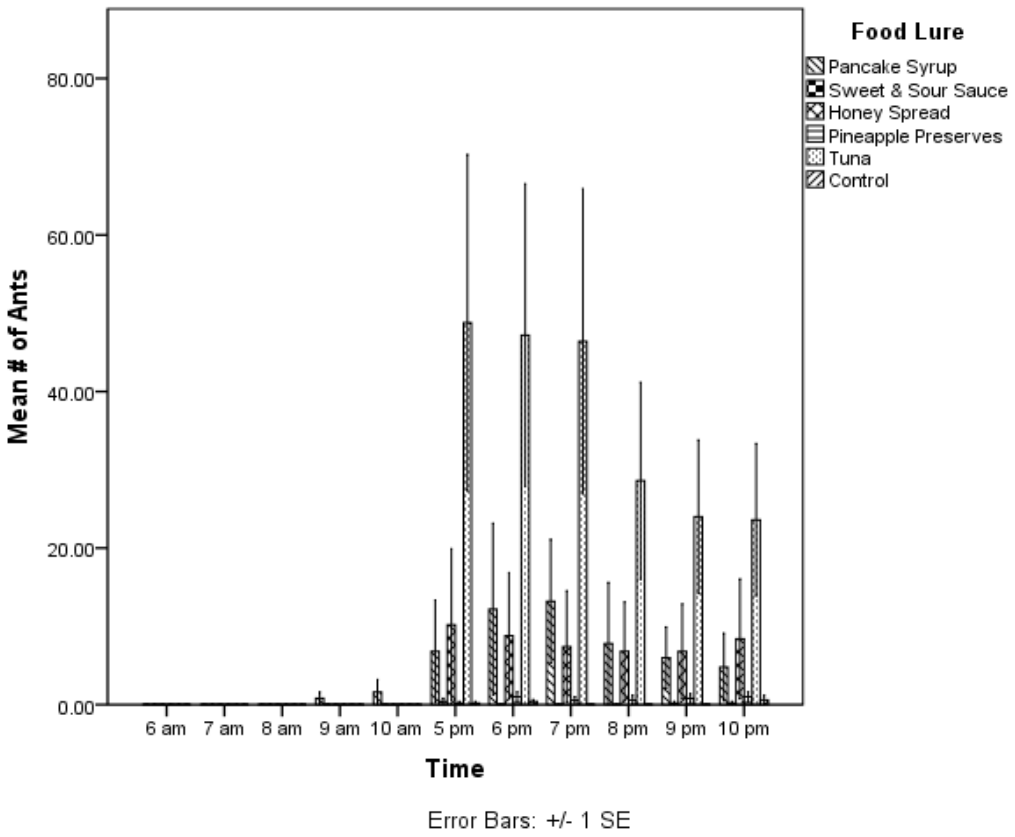

Figure 22. Mean number of *Brachymyrmex patagonicus* foragers through time by hour at each food lure in the fall.

The only species of ant documented as visiting the field study sites was *B. patagonicus*. The weather for each day of the seasonal field work can be found in Table 11.

Table 11. Weather data by season for the field trials

Season	Mean Temperature in Celsius	Mean Rainfall (cm) for the 24 h period	Mean Relative Humidity	Sunrise	Sunset
Winter	11°	0.0	64	0650	1754
Spring	17°	0.0	56	0640	2014
Summer	28°	0.0	75	0555	2052
Fall	18°	0.0	72	0710	1908

Discussion

In the laboratory trials, the data showed that the *B. patagonicus* choose the honey spread based on the total number of ants, followed by the pancake syrup, and the pineapple spread. The ants visited most of the food lures during the laboratory study. On day one of the study more ants visited the honey spread and the pancake syrup, more than the other food lures, likely because these resources are sugar based and offer quick energy. This trend continued through the end of the study. Based on the time zero data and the 5 d data as compared to the environmental controls the ants removed more of the tuna followed by the pineapple preserves, the honey spread, and the pancake syrup. The tuna is a protein source and is utilized for reproduction and growth of the colony. So overall the ants choose the honey spread based on the total number of ants documented as visiting the food lures and the tuna based on the weight of the food lures at 5 d post-initiation.

Based on the laboratory study pancake syrup, honey spread, pineapple preserves, and tuna were utilized in the field study. I also added sweet and sour sauce to round out the available food lures. In the winter more ants visited the food lures than in the spring,

summer and fall. In the winter the ants preferred the pineapple preserves over all other food lures based on the total number of ants that visited each food lure. In the spring the ants chose the honey spread, and in the summer and fall the ants chose the tuna based on the total number of ants that visited each food lure. This information is important because it can be used by PMPs to let them know when to use sugar based baits and when to use protein based baits during the year to control *B. patagonicus* infestations. Food preference based on time of year is a phenomenon that is also documented in *M. pharaonis* (Edward and Abraham 1990)

In the winter the ants removed more of the pineapple preserves which correlates with the number of ants that visited the pineapple preserves in the winter. This data is similar to findings by Chong and Lee (2006) in which *Tapinoma indicum* Forel foragers preferred pineapple jam in a field study over six other food lures. In the spring the ants chose the honey spread based on number of ants that visited the food lures and the weight removed of the honey spread. In the summer the ants removed more of the pancake syrup but more ants actually visited the tuna. In the fall the ants preferred the tuna based on number of ants and the amount of tuna removed. This seasonal preference of food lures can be caused by changes in colony development (Lee 2002) or food satiation (Edwards and Abraham 1990).Other studies have found similar results including Granovsky and Howell (1983) who found that field colonies of *M. pharoanis* readily switched between peanut butter and honey based on seasons of the year.

The only significant *B. patagonicus* activity on the food lures in the morning hours was during the summer. I believe this is so because this ant is native to areas in

South America which are tropical which typically have warmer temperatures than what we experience in the spring, fall and winter in central Texas. Therefore this species will become active in central Texas during the afternoon, evening hours once temperatures have escalated during the day to stimulate foraging. This is supported by research done by Ruano et al. (2000) in which they found that as surface temperatures increased in the environment that ant foraging increased. Similar findings were found by Jayatilaka et al. (2011) in which they found that as ambient temperatures increased, ant foraging increased. Jayatilaka et al. (2011) found critical temperature thresholds for foraging of *Myrmecia* ants that were directly linked to activity based on time of day and season. In the fall the ants visited the tuna starting at 1700 h more than any of the other food lures and this trend continued through 2200 h reading. In the winter the ants visited the pineapple preserves starting at 1700 h more than any of the other food lures and this trend continued through the 2200 h reading. In the spring the ants visited all of the food lures at the 1700 h reading. The ants chose the tuna at the 1700, 1800, and 1900 h readings and the honey spread at the 2000, 2100 and 2200 h readings. In the summer the ants visited all of the food lures during the morning and evening readings with a preference for the tuna based on to the number of ants visiting each food lure. The activity of *B. patagonicus* by season and hour is important to the pest management industry because it can be utilized by PMPs to know when peak foraging occurs and they can pass this information to their clientele to educate on when to expect activity on any bait placements in and around a structure.

In conclusion, in the laboratory study *B. patagonicus* chose the honey spread and the pancake syrup based on the number of ants at the weigh boats during the trial. Although laboratory trials are limited in variables such as population, and pheromones, and environmental conditions are held constant, this data can be used as a baseline of information for future studies. In the field study the ants chose the tuna in the fall, pineapple preserves in the winter, honey spread in the spring. In the summer the ants choose the tuna based on the total number of ants counted at the weigh boats at the inspection periods, but based on the volume removed of food lure the ants choose the pancake syrup. The foraging intensity was greatest in the afternoon inspection during all seasons and the foraging was greatest in the spring based on total number of ants counted at the weigh boats. The field study did not correlate with the laboratory study because of the limitations of a laboratory study, which include the population of ants, the pheromones emitted, and in the field the changes in environmental conditions that may occur. The switching of food preference by season ensures that colonies receive a diet that is balanced in order to fit the nutritional needs year round.

CHAPTER VI

DETERMINE EFFECTIVENESS OF COMMERCIAL PRODUCTS TO CONTROL *BRACHYMYRMEX PATAGONICUS* IN THE FIELD ASSOCIATED WITH STRUCTURES IN URBAN ENVIRONMENTS

Introduction

Brachymyrmex patagonicus populations are difficult to control, and there are few published papers on control of this species with current integrated pest management practices. The control of ants in urban environments has historically relied on baits (liquid and granular) and perimeter treatments (Mallis 1969). Baits are a logical choice of formulation to control ants based on cryptic nesting habits of many ant species and social behaviors (Williams et al. 2001). In order for baits to be successful in the control of ants it is thought that four important properties of the active ingredient must be met which are it is 1) non-repellent, 2) can be formulated with multiple food lures, 3) has a delayed toxicity, and 4) is effective at multiple concentrations (Stringer et al. 1964). To expand on the above four properties, non-repellent active ingredients are readily available on the market including fipronil, imidacloprid, indoxacarb, acetamiprid, and chlorantranilprole, as well as others. The active ingredient must be easily formulated with multiple food lures because some ant species prefer carbohydrates and proteins at different times of the year. The active ingredient must also have a delayed toxicity so that the bait can be picked up by a forager and carried back to the colony and distributed before causing mortality. Finally, the active ingredient must be efficacious at multiple

concentrations because as the forager is returning to the colony with the bait, it (active ingredient) is being diluted by secretions and physiological processes, and when the bait is shared by trophallaxis it is regurgitated so that it can be consumed by others within the colony.

There are many advantages to utilizing a bait to control ants in urban areas (Davis and Schagen 1993a, Collins and Calcott 1998). Baits are ready to use, no mixing is required as with liquid pesticides that must be added to water to make a final solution. Second, is that the soil type has little to no effect on baits, but in contrast it could have significant effects on a liquid treatment. An example being if a soil is sandy then the liquid treatment could leach down into the soil and not be available to the target pest. Third, it usually only requires one or two bait applications to gain long-term control of an ant population. Fourth the bait will spread to the rest of the colony via trophallaxis or simple grooming. Fifth, the bait can be strategically placed so that only the target species is negatively affected and sixth a bait treatment usually requires less active ingredient being placed in the environment as opposed to a liquid spray treatment (Davis and Schagen 1993a, Collins and Calcott 1998). One final advantage of a bait application is that elimination can be achieved without locating the nest (Suiter et al. 1997).

There are many components in developing efficacious bait for ant control. Ant baits have for four parts, which are the attractant, carrier, toxicant and additives (Klotz et al. 1997a). The attractant is the food or pheromone, which lures the ant to the bait. The carrier is the physical characteristics of the bait. The toxicant is the active ingredient,

which causes mortality, and the additives are the components such as dyes, emulsifiers, and preservatives (Klotz et al. 1997a).

Granular baits are often utilized to control ants on the perimeter of structures and can provide moderate control (Rust et al. 2004). Historically, mirex and sulfluramid (active ingredients) were used with great success in the control of, but both were removed from the market by the United States Environmental Protection Agency in 1978 (mirex) and 2016 (sulfluramid) (Williams 1990 and Myles 2004). Current active ingredients utilized in granular bait form to control ants include indoxacarb, hydramethlynon, abamectin, fipronil, along with many others.

Granular baits have been used to control *S. invicta*, *L. humile*, *Tetramorium caespitum*(Linnaeus), *Pheidole megacephala* (Fabricius), along with many other species. Because ants vary in size it is important to find the optimal particle size of a granular that a species will gather (Hooper-Bui et al. 2002, Furman and Gold 2006a). It is also important to know if the species you are attempting to control is monomorphic or polymorphic, the size range of the workers could determine what sizes of granular a species will readily pick up (Hooper-Bui et al. 2002). Another important aspect of any bait is that the finished solution is only attractive to the target species so that beneficial arthropods are not negatively affected by the bait application (Hooper-Bui et al. 2002).

Liquid baits have been utilized to control many species of ants including *T. melanocephalum*, *L. humile*, *M. minimum*, *Camponotus* spp. and *M. pharaonis* (Klotz et al. 1996a, Klotz et al. 1997b, Alder and Silverman 2005). *B. patagonicus* has shown an affinity to liquid baits in the field, but control is inconsistent (Robbins and Miller 2009).

Liquid baits are advantageous for control of ants because many ants including *B. patagonicus* naturally feed on honeydew produced by aphids (Klotz et al. 1997b). It has been estimated in some species of ants that over 99% of food coming into the nest is in liquid form (Markin 1970), therefore liquid baits are a logical choice for formulation.

Today, common active ingredients found in liquid ant baits include boron, imidacloprid, thiamethoxam, dinotefuran, and indoxacarb (Rust et al. 2004, Meyers and Gold 2007, Chong and Lee 2009). Boron or boric acid is a common active ingredient in liquid baits, but without multiple applications control is erratic (Klotz and Williams 1996). Disodium octaborate tetrahydrate (a derivative of boron) is another common active ingredient found in liquid ant baits and it has been found to be effective against *L. humile* and *M. pharaonis* (Klotz et al. 1996b, Klotz et al. 2007). Boron and its derivatives have been utilized in ant baits for over 100 years (Quarles 1992), and it is currently used in several commercial liquid ant baits. Boron is advantageous because it has low mammalian toxicity, has delayed effects, and is effective against many ant species at low concentrations (Klotz and Moss 1996, Klotz 1997b). Indoxacarb is highly toxic to insects (McCann et al. 2001) and, has been classified as a reduced risk active ingredient by the U.S. EPA (Furman and Gold 2006b). Boron and indoxacarb as well as imidacloprid, thiamethoxam, and dinotefuran all fit the model for a successful bait as put forth by Davis and Schagen (1993a, Collins and Calcott 1998).

Historically, liquid barrier spray treatments have been utilized to control ants in urban settings (Ebeling 1978, Rust and Su 2012). In the past, liquid treatments were applied to repel ants from gaining access to structures, but did not cause mortality

(Knight and Rust 1990) thus causing ants to become trapped or colony budding (Buczkowski et al. 2005). Active ingredients such as chlorinated hydrocarbons, and synthetic pyrethroids were common but were repellents. Chlorinated hydrocarbons were very effective for ant control but were removed from the market in the 1960s and 1970s and more recently synthetic pyrethroids have come under scrutiny by the United States Environmental Protection Agency (Davis and Schagen 1993a). The ideal liquid treatment would be a non-repellent that has delayed deleterious effects and can be transferred to naive nestmates by contact or trophallaxis such as fipronil (Vail et al. 2003, Klotz et al. 2007 and Klotz et al. 2009). One disadvantage of a liquid treatment is that only a small percentage of a colony of ants foraging will come in contact with the application and be controlled. Therefore, the colony itself may not be eliminated (Davis and Shagen 1993b). Unless properly applied, a liquid treatment can have deleterious effects on benefical organisms, therefore it is recommended that applications be strategically made to areas of known infestation as opposed to a wide spread "blanket" treatment (Phillips and Sherk 1991, Rust et al. 1996).

Barrier perimeter spray treatments can provide control of ants for up to several weeks in urban settings (Klotz et al. 2002). Barrier treatments do have environmental limitations including temperature, irrigation, and ground cover (Rust et al. 1996). Another limitation of barrier treatments is that the treatment area can soon be invaded by outlying ants that were not affected by the treatment (Silverman and Brightwell 2008). Also many ant species found in urban areas disperse from a cryptic nest location and can forage out to 60 m, so reaching a majority of individuals within a colony is very difficult

(Markin 1968, Vega and Rust 2003). There are six factors which can determine whether a treatment protocol will succeed or fail (Myers et al. 1998). First, the treatment protocol must focus on the target pests biology. Second, the target pest must be detectable at low densities to ensure efficacy of treatment as opposed to relocation of pest because of treatment (Myers et al. 1998). Third, must have access to all necessary resources to accomplish the treatment goal. Fourth, there must be cooperation among all parties as far as treatment of their property in which the pest is inhabiting (Lodge et al. 2006). Fifth, continued long term monitoring after treatment to prevent re-invasion and sixth that all native fauna should be restored to levels prior to any treatment to help prevent a re-invasion. Therefore in this research we evaluated products in multiple formulations in both laboratory and in the field against *B. patagonicus*.

Materials and Methods

Field Trial #1: Thirty structures with active exterior infestations of *B. patagonicus* were located in Bryan/College Station, TX. Pre-treatment infestation assessments were made by visually counting the number of *B. patagonicus* on the exterior perimeter of each structure at known foraging trails for 90 seconds. A structure had to have a pre-treatment count of at least 25 *B. patagonicus* in order to qualify for the study. Five replications per treatment were used for this study which included the following: Temprid® SC 0.075% (Bayer Environmental Science, Research Triangle Park, NC), Termidor® SC 0.06% (BASF Corporation, Research Triangle Park, NC), Talstar® P 0.06% (FMC Corporation Philadephia, PA), Demand® CS 0.015% (Syngenta Crop Protection Inc., Greensboro, NC), Arilon® 0.05% (Syngenta Crop

Protection Inc., Greensboro, NC), and untreated controls. All commercial products were used in a manner consistent with current label instructions. All treatments were applied using a 3.8 L B&G hand-held air compressed pump sprayer (B&G Equipment Company). Treatments were made by a pest management professional licensed through the Texas Department of Agriculture. All post-treatment assessments were made at the exact locations on each structure and in the same manner as pre-treatment assessments at 2, 14, and 30 d.

Laboratory Trial: Ants utilized in this trial were recently field collected *B. patagonicus* and allowed to adjust to laboratory conditions for a minimum of 48 h. Arenas in this study were 17 x 40 cm plastic pans (Best Plastics Englewood, NJ) with the sides lined with Insect-a-Slip (BioQuip Products Inc.) to prevent insect escape. These arenas were provisioned with food (2.0 ml of 10% honey water solution and 3 dead crickets) in a 2.5 cm weigh boat (VWR Inc.), water, and harborage in the form of a 16 x 150 mm glass tube (VWR Inc.) filled with water and plugged with cotton. Baits of a known weight (0.5 g) were provided in a 2.5 cm weigh boats on the interior of the arena and simultaneously a weigh boat with the same bait and same weight was placed in a 2.5 cm weigh boat on the exterior of the arena. Baits included in this laboratory study were; Advance® ant granular bait (abamectin 0.011%), Extinguish® Plus granular bait (hydramethlynon 0)365% and s-methoprene 0.250%), experimental granular bait (dinotefuran), Terro® PCO gel bait (sodium tetraborate decahydrate 5.40%), and an experimental gel bait (dinotefuran). The Advance® granular bait was sieved through American Society of Testing Materials nested sieves to the following sizes #25 (0.71

mm), #18 (1.0 mm) and #14 (1.4 mm), to determine if *B. patagonicus* would prefer one size granule over any other size. The weigh boat with bait on the exterior of the arena was to account for bait weight gain or loss due to environmental conditions. After a 24 h starvation period, *B. patagonicus* colonoids (1 queen, 0.10 g brood, and 100 workers) were placed in the arenas. Data regarding worker mortality was collected at 1 and 3 hrs and then at1, 3, 5, 7, 9, 11, 13, and 15 d post-treatment. There were four replications of each treatment and the untreated controls in this study.

Field Trial #2: Thirty two structures with active exterior infestations of *B. patagonicus* were located in the Bryan/College Station, TX. Pre-treatment infestation assessments were made by visually counting the number of *B. patagonicus* on the exterior perimeter at known foraging trails on the structures for 90 seconds. A structure had to have a pre-treatment count of at least 25 *B. patagonicus* in order to qualify for the study. Eight replications per treatment were used for this study which included the following: Temprid™ SC (imdiacloprid and cyfluthrin 0.075%) (liquid sprayable), Max Force Quantum Gel Bait (imidacloprid 0.03%), Temprid™ SC 0.075% and Max Force Quantum Gel Bait 0.03%, and untreated control. All commercial products were used in a manner consistent with current label instructions. All liquid treatments were applied using a hand-held air compressed pump sprayer. Temprid only treatments were made by applying finished solution on the structure 0.30 m up and 0.30 out from the foundation, in cracks and crevices, around all window frames, door frames, eaves, and overhangs. Max Force Quantum treatments were applied only as crack and crevice. Treatments that involved both Temprid and Max Force Quantum were made in the following manner;

Temprid was sprayed 0.30 m up and 0.30 m out from the foundation (not into cracks and crevices) and Max Force Quantum was applied in all cracks and crevices. Treatments were made by a pest management professional licensed through the Texas Department of Agriculture. Post-treatment assessments were made at the exact locations on each structure and in the same manner as pre-treatment assessments at 2, 14, 30, 60, and 90 d.

Statistics: Analysis of variance (ANOVA) was used to analyze the control data. The software used was SPSS for Windows, Rel. 21.0.01.2008. (SPSS Inc, Chicago, IL). The design accounted for the variation which occurs in the data from having multiple treatments in each trial. Means were separated by Tukey's honestly significant difference (HSD) test. *P*-values were considered statistically significant with $\alpha \leq 0.05$.

Results

The size of the structures utilized in this study was 156±39 m^2 with a range of 103-293. All structures in this study were treated with 3.8 L of finished solution except for one which was 293 m^2 and received 5.7 L. At the pre-treatment counts of *B. patagonicus* at each structure there were no significant differences (p=0.05) in the mean number of ants at structures associated with the different treatments (Table 12 and Fig. 23). At the two day observation period all of the treatments had caused 90% control and they were significantly different (p=0.05) from the untreated control (Table 13). At the 14 d observation period, Temprid was the only treatment with at least 90% control (Fig. 24). At the 30 d observation period, all of the treatments were significantly different (p=0.05) from the untreated control except for Altriset which had 48% control (Table 13).

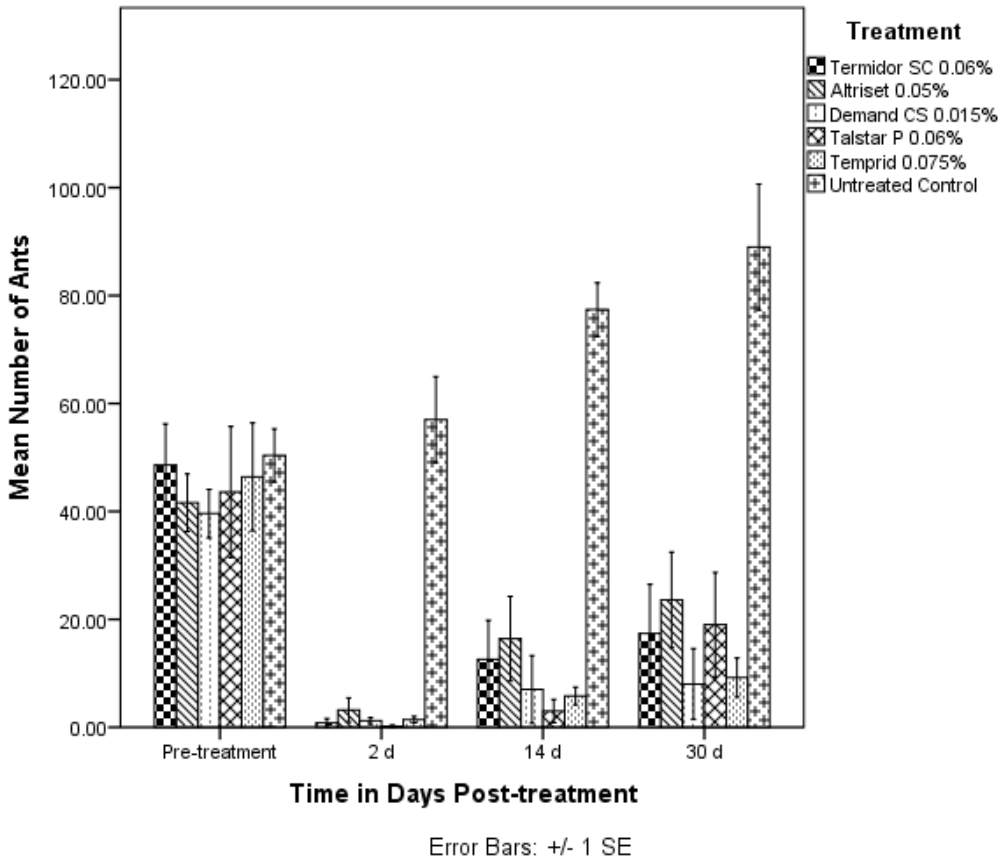

Figure 23. Mean number of *Brachymyrmex patagonicus* through time after a treatment with selected pesticide.

Table 12. Mean number of *Brachymyrmex patagonicus* through time on the exterior of structures after a treatment with a selected pesticide.

Treatment	Days Post-treatment			
	Pre-treatment	2	14	30
Termidor SC 0.06%	48.6 a	1.0 b	12.6 b	17.4 b
Altriset 0.05%	41.6 a	3.2 b	16.4 b	23.6 b
Demand CS 0.015%	39.6 a	1.2 b	7.0 b	8.0 b
Talstar P 0.06%	43.6 a	1.0 b	3.0 b	19.0 b
Temprid 0.075%	46.4 a	1.4 b	5.8 b	9.0 b
Untreated Control	50.4 a	57.0 a	77.4 a	89.0 a

Means followed by the same letter in the same column are not significantly different (p=0.05) per Tukey's Honest Significant Difference Test.

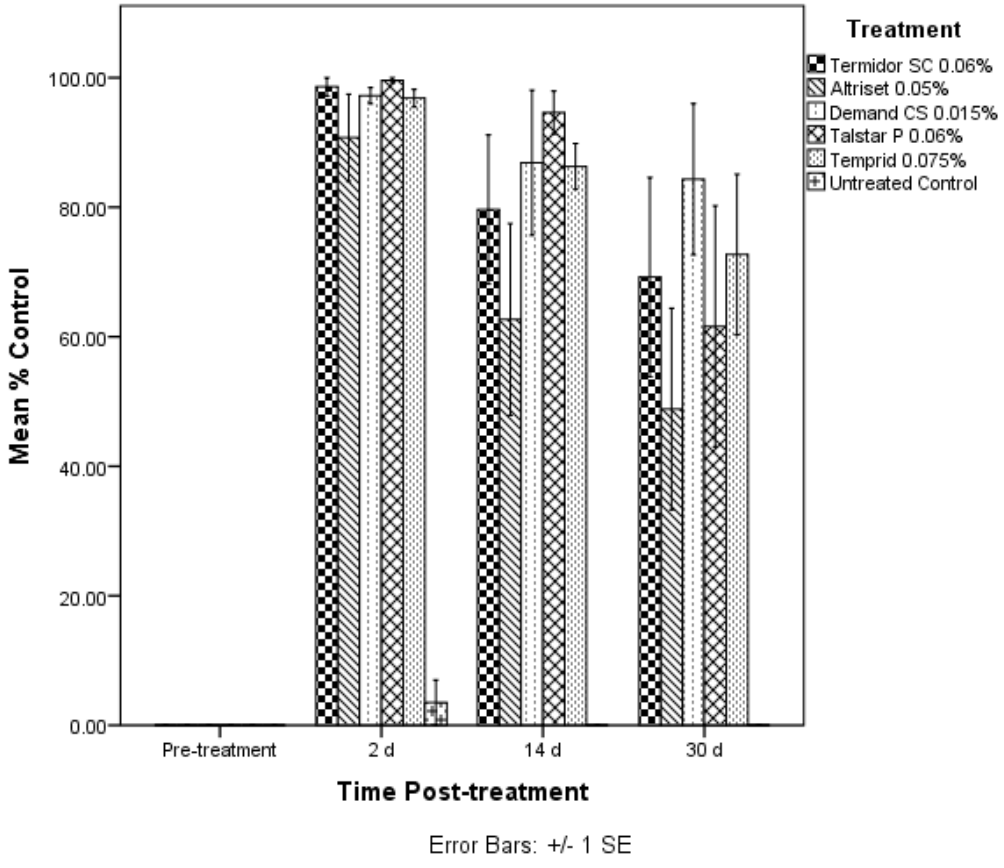

Figure 24. Mean percent control of *B. patagonicus* through time after a treatment with selected pesticide.

Table 13. Mean % control of *B. patagonicus* through time at structures treated with a selected pesticide

		Days Post-treatment		
Treatment	Pre-treatment	2	14	30
Termidor SC 0.06%	0.0 a	98.6 a	79.7 a	69.2 a
Altriset 0.05%	0.0 a	90.7 a	62.7 a	48.8 b
Demand SC 0.015%	0.0 a	97.2 a	86.9 a	84.3 a
Talstar P 0.06%	0.0 a	99.6 a	94.6 a	61.6 a
Temprid 0.075%	0.0 a	96.8 a	86.3 a	72.7 a
Untreated Control	0.0 a	3.5 b	0.0 b	0.0 c

Means followed by the same letter in the same column are not significantly different (p=0.05) per Tukey's Honest Significant Difference Test.

Laboratory Study Baits: There were no significant differences (p=0.05) in the pre-trial weights of the different baits that were in the interior of the arenas and which were available to the ants during the trial (Table 14). There were significant differences (p=0.05) in the post-trial weights of baits exposed to colonoids of *B. patagonicus* (Table 14), and there were significant differences (p=0.05) in the difference between pre and post-trial weights of baits not exposed to ants at the end of the study (Table 14). There were no significant differences (p=0.05) in the pre-trial weights of the different baits that were on the exterior of the arena which were not available to the ants (Table 15). However, there were significant differences (p=0.05) in the post-trial weights of the baits that were not available to the ants (Table 15), and there were significant differences (p=0.05) in the pre and post-trial weights of baits at the end of the study that were not available to the ants (Table 15). There were significant differences (p=0.05) in the pre and post-weight of the weights of the baits that were not available (exterior) and that

were available (interior) to the ants (Table 16). However there were no significant differences in the differences between the exterior and interior weights of the baits (Table 16).

At 1 h post-treatment there was mortality of ants associated with all of the different treatments and the untreated controls (Fig. 25) and, there were significant differences in the mortality of ants exposed to the different treatments and this trend continued throughout the study (Table 17). At the 3 h reading, there was a mean of 31.25% mortality of the ants exposed to the Alpine Gel and 22.25% mortality of the ants exposed to the Advance large granule (Table 17). At the 5 d reading there was a mean of 56.00% mortality of the ants exposed to the Alpine Gel, and the mortality in this treatment was significantly different from all of the other treatments and the untreated control (Table 17). At the 11 d reading there was a mean of 97.00% mortality of the ants exposed to the Terro PCO gel followed by Alpine gel at 86.25%, Advance large granule 50.50% and Siesta granule at 37.50% (Table 17). At the 15 d reading the ants in the Terro PCO gel treatments had a mean % mortality of 98.75% followed by the Alpine gel at 88.25% and the mortality in these two treatments were significantly different ($p=0.05$) from all of the other treatments and the untreated control (Table 17).

Table 14. Mean pre and post-trial weights (grams) and differences of baits pre and post-treatment when exposed to colonoids of *Brachymyrmex patagonicus* through 15 d (interior)

Treatment	Pre-trial weight	Post-trial weight	Difference
Advance (0.71 mm)	0.535±0.170 a	0.359±0.017 a	0.175±0.010 c
Advance (1.0 mm)	0.540±0.030 a	0.356±0.057 a	0.183±0.030 c
Advance (1.4 mm)	0.544±0.027 a	0.371±0.031 a	0.172±0.008 cd
Alpine Ant Gel	0.533±0.015 a	0.019±0.010 c	0.514±0.006 a
Siesta Granule	0.547±0.019 a	0.402±0.018 a	0.145±0.002 de
Terro PCO Gel	0.551±0.017 a	0.261±0.011 b	0.290±0.006 b
Extinguish Plus	0.530±0.028 a	0.400±0.025 a	0.130±0.002 e

Means followed by the same letter in the same column are not significantly different per Tukey's HSD.

Table 15. Mean pre and post-trial weights (grams) and differences of bait weights when exposed to laboratory conditions through 15 d (exterior)

Treatment	Pre-trial weight	Post-trial weight	Difference
Advance (0.71 mm)	0.556±0.027 a	0.388±0.056 a	0.168±0.031 c
Advance (1.0 mm)	0.560±0.033 a	0.384±0.028 a	0.176±0.005 cd
Advance (1.4 mm)	0.529±0.037 a	0.359±0.044 a	0.170±0.002 cd
Alpine Ant Gel	0.531±0.020 a	0.023±0.005 c	0.508±0.015 a
Siesta Granule	0.533±0.038 a	0.389±0.038 a	0.144±0.002 cd
Terro PCO Gel	0.541±0.027 a	0.260±0.022 b	0.281±0.005 b
Extinguish Plus	0.530±0.028 a	0.406±0.025 a	0.124±0.059 d

Means followed by the same letter in the same column are not significantly different per Tukey's HSD.

Table 16. Mean weight differences (grams) of baits when exposed to colonoids of *Brachymyrmex patagonicus* (interior) or laboratory conditions through 15 d (exterior)

Treatment	Difference Exterior	Difference Interior	Difference
Advance (0.71 mm)	0.168±0.031 c	0.175±0.010 c	0.007±0.005 a
Advance (1.0 mm)	0.176±0.005 cd	0.183±0.030 c	0.007±0.002 a
Advance (1.4 mm)	0.170±0.002 cd	0.172±0.008 cd	0.002±0.008 a
Alpine Ant Gel	0.508±0.015 a	0.514±0.006 a	0.006±.006 a
Siesta Granule	0.144±0.002 cd	0.145±0.002 de	0.001±.002 a
Terro PCO Gel	0.281±0.005 b	0.290±0.006 b	0.009±0.006 a
Extinguish Plus	0.124±0.059 d	0.130±0.002 e	0.006±0.002 a

Means followed by the same letter in the same column are not significantly different per Tukey's HSD.

Table 17. Mean % mortality of *Brachymyrmex patagonicus* through time

Treatments	Time Post-treatment						
	1 hr	3 hr	1 d	5 d	7 d	11 d	15 d
Advance (0.71 mm)	5.25± 5.73 b	8.00±8.36 c	11.25±11.67 bc	14.50±12.76 c	18.00±12.51 cd	22.25±15.77 c	25.50±18.73 c
Advance (1.0 mm)	7.50±5.50 b	8.00±6.32 c	11.50±11.38 bc	17.75±19.32 c	19.75±20.54 cd	21.00±22.25 c	25.00±19.91 c
Advance (1.4 mm)	21.50±9.25 a	22.25±9.25 ab	29.00±9.41 ab	43.00±6.48 ab	47.25±8.80 ab	50.50±8.18 b	58.50±13.10 b
Alpine Gel	28.00±5.47 a	31.25±1.70 a	41.25±4.42 a	56.00±4.24 a	63.00±4.16 a	86.25±3.09 a	88.25±1.50 a
Siesta Granule	6.25±3.40 b	12.75±3.30 bc	21.75±3.94 abc	29.50±5.06 bc	32.25±7.08 bcd	37.50±7.41 bc	37.50±7.41 bc
Terro PCO Gel	3.75±2.98 b	7.25±7.36 c	14.25±10.71 bc	29.50±9.39 bc	41.50±10.27 abc	97.00±3.16 a	98.75±2.50 a
Extinguish Granule	3.25±1.25 b	6.75±4.92 c	10.50±6.24 bc	16.00±8.52 c	20.50±6.24 cd	24.25±8.30 bc	24.50±8.54 c
Untreated Control	2.00±1.41 b	3.75±2.87 c	7.25±4.42 c	10.75±5.25 c	12.25±7.36 d	16.25±5.93 c	16.25±5.93 c

Means followed by the same letter in the same column are not significantly (p=0.05) different per Tukey's HSD.

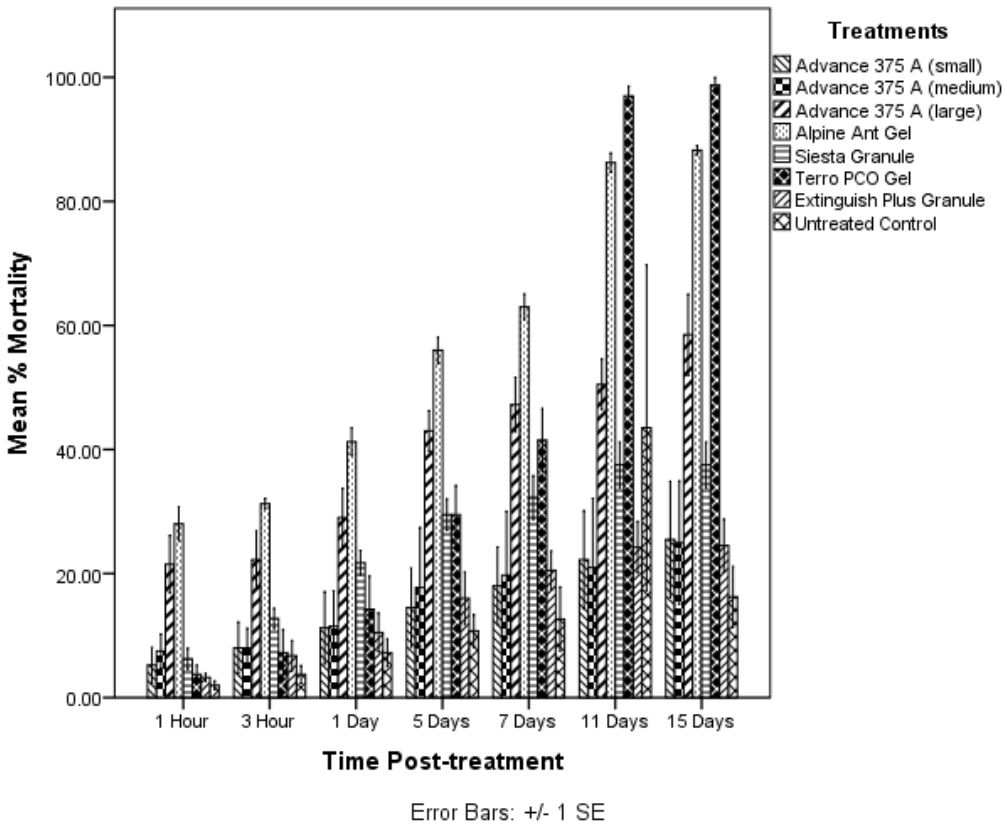

Figure 25. Mean % mortality of *Brachymyrmex patagonicus* against selected baits through time.

Field Study #2: The structures that received the Temprid SC 0.075% treatment had a mean of 2.5 liters of finished solution applied (Table 18). The structures that received the Max Force Quantum Gel 0.03% treatment had a mean of 4.92 grams applied (Table 18), and the structures that received Temprid SC and Max Force Quantum Gel had means of 2.2 liters and 1.43 grams applied, (Table 18).

At the 2 d inspection, structures that received the Temprid SC 0.075% and the Temprid SC 0.075% / Max Force Quantum Gel 0.03% treatments there was 96.00% mean control of the ants, followed by 93.75% mean control at the structures that

received a Max Force Quantum Gel 0.03% treatment (Table 19). At the 30 d inspections the structures that received the Temprid SC 0.075% treatment there was a control of 94.00% control followed by the Max Force Quantum Gel 0.03% treatment with a mean of 91.87% control (Table 19). At the 60 d post-treatment inspection both the Temprid SC 0.075% and the Max Force Quantum Gel 0.03% treatments had a mean percent control of 96.00 followed by the Temprid SC 0.075% / Max Force Qantum Gel 0.03% at 85.12% control of the ants. There were no significant differences (p=0.10) between the treatments through 60 d post-treatment (Table 19), but all of the treatments were significantly different from the untreated controls at the 2-60 d post-treatment inspections (Table 19). At the 90 d inspection the Max Force Quantum Gel 0.03% treatment had 94.50% control and the Temprid SC 0.075% had 88.50% control of the ants (Table 19). The Temprid SC 0.075% / Max Force Quantum Gel 0.03% had 82.75% control of the ants. At the 90 d post-treatment inspection there were significant differences (p=0.10) between the mean percent control of the treatments, and all of the treatments were significantly different (p=0.10) from the untreated controls (Table 19). The ants at the untreated control structures were very active throughout the study (Fig. 26).

Table 18. Mean amount of product applied at each structure

Treatment	Temprid SC	Max Force Quantum
Temprid SC 0.075%	2.50 liters	0.00
Max Force Quantum Gel 0.03%	0.00	4.92 grams
Temprid SC 0.075% and Max Force Quantum Gel 0.03%	2.20 liters	1.43 grams

Table 19. Mean percent control of *Brachymyrmex patagonicus* through time at structures after a treatment with selected pesticides

Treatment	Time Post-treatment					
	Pre-treatment	2 d	14 d	30 d	60 d	90 d
Temprid SC 0.075%	0.0±0.0 a	96.00±5.65 a	91.50±12.90 a	94.00±12.82 a	96.00±9.79 a	88.50±20.77 a
Max Force Quantum Gel 0.03%	0.0±0.0 a	93.75±8.77 a	94.50±10.01 a	91.87±13.09 a	96.00±7.70 a	94.50±7.98 a
Temprid SC 0.075% and Max Force Quantum Gel 0.03%	0.0±0.0 a	96.00±8.55 a	93.00±13.64 a	86.50±13.51 a	85.12±16.98 a	82.75±30.29 b
Untreated Control	0.0±0.0 a	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 b	0.0±0.0 c

Means followed by the same letter in the same column are not significantly different (p=0.10) per Fisher's LSD.

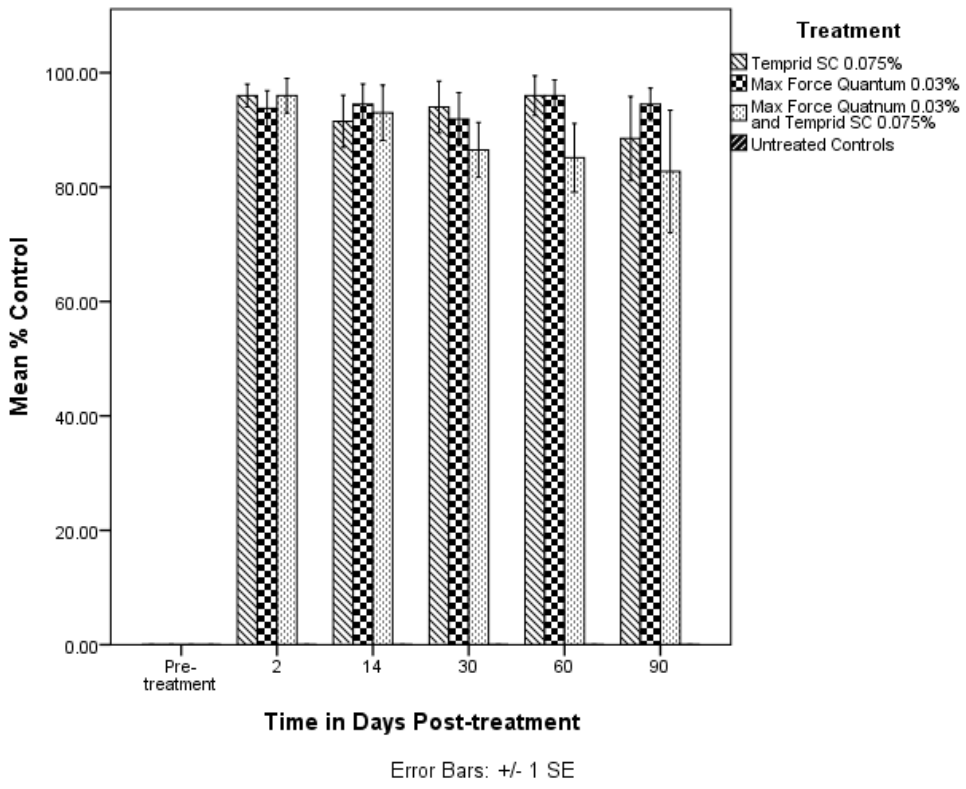

Figure 26. Mean % control of *Brachymyrmex patagonicus* through time after a treatment with selected pesticides.

Discussion

Field Trial #1: In field trial # 1 Altriset had the lowest percent control at all three post-treatment readings. Talstar P had the highest percent control at the 2 and 14 d readings and Demand CS had the highest percent control at the 30 d reading at 84%. The active ingredient in Talstar P is bifenthrin which is a synthetic pyrethroid. The quick knockdown of ants at 2 and 14 d post-treatment is a well-documented result of utilizing bifenthrin on the exterior of structures, but as in the current study as well as in many cases the ant populations tend to rebound (Jiang et al. 2014). The ants in untreated

controls were very active throughout the study. All of the treatments reduced the number of ants at the 2 d post-treatment reading. Ant densities were slightly higher at 30 d than at 14 d post-treatment. Therefore based on these results of this study it would be recommended that Demand CS be utilized as a liquid treatment for control of *B. patagonicus* foragers on the exterior of structures. This confirms a laboratory study performed by Miguelna and Baker (2014) in which they also found Demand CS to have very good efficacy against *B. patagonicus*. This is important because there are few published studies that make any recommendations to the pest management industry on how to control this species with a liquid residual pesticide.

Laboratory Trial: The ants removed more Advance large grit granule than the small and medium grit Advance granule. This is of surprise just based on the small size of *B. patagonicus* foragers. Over all the ants removed more of the Terro PCO gel than any other product in the study followed by the Advance large grit and the Advance small grit. At the 1 hour reading the experimental ant gel had caused more mortality than any of the other treatments, this continued through the 7 d reading. At the 7 d reading the Terro PCO had caused the most more mortality of the ants than any other treatment and this trend continued through the 15 d reading, followed by the experimental ant gel and the Advance large grit bait. The reduction of *B. patagonicus* by the Terro PCO gel bait is quite different to findings by Kotz et al. (1996). Who had reductions of 73% of *M. pharaonis* at 3 d while in the current study reduction of *B. patagonicus* at 5 d was 29.50%. This difference in reduction of these two species by similar boric acid baits is a testament to how difficult it is to control *B. patagonicus* in urban environments.

The gel baits caused more mortality of the ants than the granules, which is consistent with the formulation in which ants have to process a granule to be able to feed the queen and brood. In a gel bait the active ingredient is readily available to the ant and it does not have to be processed by the ant in order to feed others within the colony. There was a correlation between volume of bait removed and mortality, based on the fact that the ants removed more of the Terro PCO than any other bait and there was more mortality of the ants in the Terro PCO replications. Terro PCO gel is a sugar based bait which would correlate with the field study in which the ants preferred sugar based food lures in the winter and spring. There were significant differences in the mortality of the ants associated with the different treatments at different time periods. The untreated controls were very active throughout the study and had minimal mortality.

Field Trial #2: All of the treatments caused a significant decrease in the population of *B. patagonicus* associated with the structures as compared to the untreated controls. The only significant differences ($p=0.10$) in the mean percent control of ants associated with the structures that received different treatments was at 90 days post-treatment in which the number of ants at the structures treated with Max Force Quantum treatment was significantly less than the other treatments. At 90 d post-treatment the Max Force Quantum Gel 0.03% treatment had the fewest mean number of ants. The untreated controls were very active throughout the study.

Future studies should look at the control of *B. patagonicus* with the use of Max Force Quantum and Demand CS as a combination treatment, because those were the two best performing treatments in these studies field studies. In doing so it is important not to

inadvertently spray gel bait placements with the liquid treatment. This will contaminate the gel bait and the ants will not pick up the bait. Although the results of this study indicate that a bait alone would offer long-term control of *B. patagonicus*. I believe based on my 20 years of experience in urban pest management it is important to utilize a two pronged approach when using chemicals to control *B. patagonicus* in that a liquid spray in conjunction with gel bait will maximize control in urban environments. The strategy of utilizing a liquid sprayable along with a bait was also recommended by Klotz et al. (2007) who found that these two formulations used together maximized control. Even though using two different formulations will increase the PMPs cost to treat a structure a call back to an account will be more costly in time, labor, chemical, and reputation. In fact I believe one reason that *B. patagonicus* has become so difficult to control is that pest management professionals rely so heavily on fipronil as a liquid treatment against ants and they do not use baits as often for control (Klotz et al. 2007) on initial treatments for dark rover ants. Fipronil as in the current study did not perform well in Migulena and Baker (2014) against *B. patagonicus*.

Field studies should also be conducted with the use of Demand CS and Terro PCO, because Terro PCO was the best performing gel bait in the laboratory study. I also think that there should be some utilization of a fish based inert matrix added to an active ingredient to develop a gel bait for the control of *B. patagonicus* as well as pancake syrup or pineapple preserve based baits based on the attractiveness of *B. patagonicus* to these food lures in the laboratory and the field trials. Two phenomena that play a role in attractiveness to baits of ants to baits are bait switching and bait shyness. Bait switching

is when ants alternate their food preference by switching between carbohydrates and proteins (Granovsky and Howell 1983, Edwards and Abraham 1990). Bait shyness is described as when a colony feeds on a food source for a prolonged period of time and then stops feeding on that food source (Edwards and Abraham 1990). Therefore, all of these bait matrices would need to be evaluated in the laboratory and then in the field for attractiveness and efficacy before any mass production of the bait were to be performed. As always a complete integrated pest management plan for *B. patagonicus* should include non-chemical management. Education of the public on light management could help control this ant since it swarms in the evening hours and is attracted to lights which could affect nest formation in or near structures (Tamayo 2011), as well as reduction of harborage, and correction of any moisture issues in the structure.

CHAPTER VII

CONCLUSIONS

All of the data gathered within these trials is new and should be brought to the attention of the pest management industry. It is imperative to describe the biology, foraging behavior, diet preferences, and reproduction of ants in complex urban environments to begin to understand how to control them (Carroll and Janzen 1973, Fonseca 2010). More and more studies are implicating that it is important to understand the behavior and diet preferences of a target species in order to develop baits that can control the species utilizing an integrated pest management approach which targets the pest and has minimal effects on beneficial species such as pollinators (Sola et al. 2013).

The data from the reproduction trials is important because it can serve as a baseline of knowledge for future studies that need to be conducted on *B. patagonicus* biology. The optimal temperature for reproduction of *B. patagonicus* in the laboratory was 30˚C and the development time from egg to adult was 33 days. Future studies should focus on that temperature and offer both a carbohydrate and a protein to the ants.

The foraging data confirmed that *B. patagonicus* will readily move the colony to be closer to food and water. Colonoids with queen, brood, and foragers functioned in a more complete manner by finding resources and adjusting the colony location as opposed to colonoids with just workers. This confirms that colony communication between different life stages is important in the everyday functioning of *B. patagonicus* colonies.

The mechanical vector trials confirmed that *B. patagonicus* should be more closely examined as a pest of medical concern. Albeit that in these trials the ants were placed on *E. coli* and did not have a choice to possibly avoid the *E. coli* due to chemical ques. The ants did move the *E. coli* at all three transmission distances.

The food lure trials showed that *B. patagonicus* preferred carbohydrates in the laboratory study. But in the field trials *B. patagonicus* switched from carbohydrates and proteins at different times of the year. This could lead to better IPM practices and a schedule of when utilize which baits during the year. If *B. patagonicus* continues to be difficult to control with current carbohydrate and protein baits, I recommend focusing on developing baits that specifically mimics pineapple, honey spread, and tuna for laboratory trials on efficacy against this ant. All in all this data could lead to the development of bait matrices that can better control this ant in urban environments.

The control trials showed that synthetic pyrethroids had good knockdown of the ants early in the trials and that non-repellent sprayables did not perform as well as repellents. Bait trials both in the laboratory and in the field showed that gel baits should be furthered explored as an option to control this ant in urban environments. In conjuction with the food lure data this work shows that baits should be applied in the afternoon when foraging is heaviest and that PMPs should utilize carbohydrate and protein baits at different times of the year.

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