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An Investigation of the Distribution and Behavior of the Dark Rover Ant, *Brachymyrmex patagonicus* Mayr, in South Carolina

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AN INVESTIGATION OF THE DISTRIBUTION AND BEHAVIOR OF THE DARK ROVER ANT, *BRACHYMYRMEX PATAGONICUS* MAYR, IN SOUTH CAROLINA

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Entomology

by
David Quinn Bowers
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Accepted by:
Dr. Eric Benson, Committee Chair
Dr. Patricia Zungoli
Dr. William Bridges
ABSTRACT

The dark rover ant, *Brachymyrmex patagonicus* Mayr, is a minute (~0.5 mm) dark brown or black formicine ant invasive from Argentina and Paraguay. In the southeastern United States, the dark rover ant is considered a nuisance pest, and is difficult to control. Recently, *B. patagonicus* has expanded its invasive range, especially in the southeastern United States. In South Carolina, this species has become more common in pest control accounts. Despite this, most of the biology and behavior of the dark rover ant remains unknown.

The only official record of *B. patagonicus* in South Carolina is from 2010 in Horry County. Based on personal observation and complaints from pest control operators, it was apparent this species was present in other parts of the state, but its distribution was unknown. Each of the 45 remaining counties in South Carolina were surveyed to investigate the presence of the dark rover ant. Target areas were chosen in each county which received a high volume of traffic such as schools, hospitals, and government buildings, and were sampled. *Brachymyrmex patagonicus* was collected and positively identified in all counties of South Carolina, indicating its invasive range is state wide, and provided 45 new county records. A distribution map was developed using these data, which also includes the first official record in Horry County from 2010.

Reports from pest control operators indicated that *B. patagonicus* became a secondary pest after suppression of the red imported fire ant, *Solenopsis invicta* Buren. A series of four samples were conducted at ten trees on Clemson University Main Campus
to measure the changes in foraging activity of *B. patagonicus* after *S. invicta* suppression using pitfall traps, baits, and tree scans. One sample was conducted to establish a baseline for *B. patagonicus* foraging activity, then half of the trees were treated with Advion® (Syngenta Crop Protection Inc., 410 S Swing Rd, Greensboro, NC) fire ant bait, while the other half were left untreated to serve as a control. Then three more samples were conducted to measure the change in dark rover ant foraging activity. There was no significant difference in the number of *B. patagonicus* captured in pitfall traps before and after *S. invicta* suppression, and there was no significant difference in the number of dark rover ants in pitfalls in the treated area versus the untreated area. Also dark rover ants were no more likely to dominate a bait after the suppression of *S. invicta*. Data from tree scans indicated that the most commonly found co-occurring species with *B. patagonicus* were the red imported fire ant (*S. invicta*), Argentine ant (*Linepithema humile* (Mayr)), black carpenter ant (*Camponotus pennsylvanicus* (De Geer)), and field ants (*Formica* sp.).

It has been widely reported from field observations that despite the fact that *B. patagonicus* co-occurs with other highly invasive or ecologically dominant ant species, it is not met with the same aggression levels as other species. A series of trials were conducted in a laboratory setting to confirm and quantify these interactions observed in the field. *Brachymyrmex patagonius* was paired against *S. invicta*, *L. humile* and *Dorymyrmex bureni* (Trager) to observe their interactions. *Solenopsis invicta* was also paired against *L. humile* and *D. bureni* to serve as a positive control for aggression. Ants were collected in the field and allowed to acclimate before being transferred to an
experimental arena where they were observed for a period of five minutes, and their interactions scored according to a previously established agonism scale. Data indicate that the combinations including *B. patagonicus* versus *L. humile*, *S. invicta*, and *D. bureni* had a mean aggression score that was statistically significantly lower than the combinations including *S. invicta* versus *L. humile* and *D. bureni*. The combinations including dark rover ants versus other ecologically dominant or invasive ants also yielded mean aggression scores that were not significantly different from one another; as did the combinations including *S. invicta* versus *L. humile* and *D. bureni*. The data indicate that *B. patagonicus* is universally met with lower levels of aggression, corroborating field observations. The cause of this interaction remains unknown. Future research should focus on both size and chemical interactions as potential causes for this unique interaction.
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“Go to the ant, thou sluggard; consider her ways, and be wise: Which having no guide, overseer, or ruler, Provideth her meat in the summer, and gathereth her food in the harvest.”

-Proverbs 6:6-9, KJV
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE PAGE</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>x</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>I. LITERATURE REVIEW</td>
<td></td>
</tr>
<tr>
<td>The Importance of Ants in Ecosystems</td>
<td>1</td>
</tr>
<tr>
<td>Ants as Invasive Species</td>
<td>2</td>
</tr>
<tr>
<td>History and Distribution</td>
<td>5</td>
</tr>
<tr>
<td>Biology and Behavior</td>
<td>7</td>
</tr>
<tr>
<td>Pest Status and Control</td>
<td>11</td>
</tr>
<tr>
<td>References Cited</td>
<td>13</td>
</tr>
<tr>
<td>II. THE DISTRIBUTION OF THE DARK ROVER ANT, <em>BRACHYMYSRMEX</em></td>
<td></td>
</tr>
<tr>
<td><em>PATAGONICUS</em> MAYR IN SOUTH CAROLINA BY COUNTY</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>19</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>22</td>
</tr>
<tr>
<td>Results</td>
<td>24</td>
</tr>
<tr>
<td>Discussion</td>
<td>26</td>
</tr>
<tr>
<td>References Cited</td>
<td>28</td>
</tr>
<tr>
<td>III. INVESTIGATION OF THE DARK ROVER ANT’S, <em>BRACHYMYSRMEX</em></td>
<td></td>
</tr>
<tr>
<td><em>PATAGONICUS</em> MAYR, FORAGING ACTIVITY AFTER RED IMPORTED FIRE ANT, <em>SOLENOPSIS INVICTA</em> BUREN, SUPPRESSION</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>31</td>
</tr>
<tr>
<td>Materials and Methods</td>
<td>34</td>
</tr>
<tr>
<td>Results</td>
<td>40</td>
</tr>
<tr>
<td>Discussion</td>
<td>50</td>
</tr>
</tbody>
</table>
Table of Contents (Continued)

References Cited .................................................................................................................. 54

IV. INVESTIGATION OF AGONISTIC INTERACTIONS BETWEEN THE DARK ROVER ANT, *Brachymyrmex patagonicus* Mayr, AND CO-OCCURRING SPECIES

Introduction ......................................................................................................................... 57
Materials and Methods ........................................................................................................ 60
Results ................................................................................................................................. 65
Discussion ........................................................................................................................... 69
References Cited ................................................................................................................. 72

SUMMARY AND RECOMMENDATIONS ................................................................................. 75

References Cited ................................................................................................................. 81

APPENDICES ..................................................................................................................... 82

A: Supporting data for Chapter 2....................................................................................... 83

B: Supporting data for Chapter 3....................................................................................... 86
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>Collection information for each county record of <em>Brachymyrmex patagonicus</em> Mayr in South Carolina, including location, GPS coordinates, date, microhabitat type, and co-occurring ant species</td>
<td>83</td>
</tr>
<tr>
<td>B-1</td>
<td>The number of <em>B. patagonicus</em> Mayr and <em>S. invicta</em> Buren collected in each pitfall trap across four sampling dates</td>
<td>86</td>
</tr>
<tr>
<td>Figure</td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>2.1</td>
<td>South Carolina distribution map by county for <em>Brachymyrmex patagonicus</em> Mayr</td>
<td>24</td>
</tr>
<tr>
<td>3.1</td>
<td>Total number of <em>B. patagonicus</em> Mayr (B.p.), and <em>S. invicta</em> Buren (S.i.) collected in all pitfall traps in both the treated and untreated areas across four sampling dates</td>
<td>41</td>
</tr>
<tr>
<td>3.2</td>
<td>The number of <em>Brachymyrmex patagonicus</em> Mayr (B.p.) in each pitfall trap at each tree in the untreated area across time</td>
<td>44</td>
</tr>
<tr>
<td>3.3</td>
<td>The number of <em>Solenopsis invicta</em> Buren (S.i.) in each Pitfall in the untreated area across time</td>
<td>45</td>
</tr>
<tr>
<td>3.4</td>
<td>Average foraging activities of <em>Brachymyrmex patagonicus</em> Mayr (B.p.) and <em>Solenopsis invicta</em> Buren (S.i.) in both areas over time</td>
<td>46</td>
</tr>
<tr>
<td>3.5</td>
<td>Investigation of the linear relationship between the total number of <em>Brachymyrmex patagonicus</em> Mayr (B.p.) and <em>Solenopsis invicta</em> Buren (S.i.) in pitfall traps</td>
<td>47</td>
</tr>
<tr>
<td>4.1</td>
<td>Diagram for experimental arena used in agonistic trials</td>
<td>62</td>
</tr>
<tr>
<td>4.2</td>
<td>Mean highest agonistic values observed for each combination of ants</td>
<td>67</td>
</tr>
<tr>
<td>B-1</td>
<td>Satellite image of Clemson University from Google Earth® showing both the treated and untreated areas and the distance between them</td>
<td>88</td>
</tr>
</tbody>
</table>
CHAPTER 1.
LITERATURE REVIEW

The Importance of Ants in Ecosystems

The role of ants in ecosystems is often underappreciated. There are approximately 16,000 species of ant worldwide (Bolton World Catalog, 2017). Though this number only accounts for less than one percent of all known insects, it is estimated that there are 10,000 trillion ants alive today and that their total weight is equal to that of all humans on earth (Wilson and Holldobler 1990). Ants are found on every continent except Antarctica, where they fill diverse niches and ecological roles (Wilson and Holldobler 1990). Ants serve important functions such as aerators, undertakers, food sources, pollinators, and consumers of many different types of food sources (McDonald 2012). The important roles that ants fill means that when natural populations of ants are disturbed by outside factors such as invasive species, multiple trophic levels are affected in addition to just ants themselves. For example, Morris et al. (2015) found that coffee branches were colonized more quickly in the absence of the keystone ant species *Azteca sericeasur* Longino. Often the invasive species most damaging to ants are actually other invading ant species (Holway et al. 2002). Invasive ant species often become dominant species in their introduced range, where they are known to impact ant communities by interfering in the foraging activity of other ant species (Carval et al. 2016).
The United States Department of Agriculture defines an invasive species as “an alien species whose introduction does or is likely to cause economic harm or harm to human health.” There are approximately 4,300 invasive species across all taxa in the United States today (Corn et al. 1999). These include four ant species which are considered to be in the top one hundred most invasive species on the planet, the Argentine ant (*Linepithema humile* (Mayr)), red imported fire ant (*Solenopsis invicta* Buren), big-headed ant (*Pheidole megacephala* Fabricius), and little fire ant (*Wasmannia auropunctata* Roger) (Global Invasive Species Database, 2016).

**Ants as Invasive Species**

Because of their nesting behavior, ants are often brought to new areas via human facilitated dispersal, especially to port areas or railways (Lach et al. 2013). This happens most often when ants nest inside materials that are shipped to areas where they are not native. Ephemeral nesting in leaf litter, mulch, and soil increases the chance that invasive ants are accidentally imported. While ants are generally slow dispersers, human interference allows ants to spread faster than natural distribution (Sparks 1999). Ants have a higher reproductive rate than some other insects which aids in colonization of new areas. North America has been highly susceptible to invasive ants from South America. While no native North American ant species are known to establish colonies in other countries, more species of invasive ant originate from South America than from any other continent (McGlynn 1999). North America’s susceptibility is possibly linked to a trend of high import activity, however areas that differ slightly from an ant’s native range are
more susceptible to colonization (Lach et al. 2013). Invasive ants also are more successful in areas of intermediate disruption, such as urban areas, which means more developed areas are more likely to be successfully colonized which can lead to economic impact for certain species (Vonshak and Gordon 2015). King and Tschinkel (2016) reported that exotic ant queens preferentially select for disturbed habitats such as those that have been tilled or have added sand. While native ant queens may show similar preference, they have a harder time establishing in these areas due to the presence of invasive species dominance (King and Tshinkel 2016). Brown et al. (2012) reported that the number of ant species in urban housing developments increased with the age of the development. In addition, foraging territories overlap in areas, suggesting lower competition, which aids in colonization (Brown et al. 2012). Menke et al. (2010) conversely reported that ants may be exempt from faunal homogenization in urban environments because they operate on a much smaller scale than other fauna. Many ants which normally nest in leaf-litter in natural systems are observed in mulch or debris piles in urban areas suggesting there may not be much functional difference between some urban and natural structures and there is some overlap between species in these two areas (Menke et al. 2010).

Ants can be particularly devastating invaders because of their potential ecological impact in an environment. Invasive ants displace both native ant fauna as well as other invertebrate and vertebrate species (Lach et al. 2013). This is accomplished primarily by outcompeting in two major areas: exploitation and interference. In exploitation, invasive ants are more successful at exploiting food and other necessary resources which leads to
the decline of health of native ant colonies (Human and Gordon 1996). Interference competition refers to direct conflict between two species, usually in the form of fighting. In many cases, invasive species are known to have increased interspecific aggression towards native ant fauna than with colonies of their same species (Horn et al. 2013). *Linepithema humile* have been observed to displace native ants at bait stations 60% of the time (Human and Gordon 1996). This loss of ant diversity can have serious implications on the surrounding ecosystem. In areas where invasive species have successfully colonized, ant-dispersed and ant-pollinated plants tend to suffer (Ness and Bronstein 2004). On the other hand, ant-tended hemipterans tend to benefit from invasive ants, which may create further problems for native plants because of over feeding by hemipterans (Ness and Bronstein 2004) (Sharma et al. 2013).

South Carolina has an estimated 200 ant species, of which 120 are currently known, both native and invasive (Davis 2009). In 2007, the dark rover ant was collected in South Carolina for the first time (MacGown et al. 2007). Since then it has been mentioned as an introduced pest, but its distribution throughout the state is unknown (Davis 2009). Anecdotal reports from pest control operators indicate that *B. patagonicus* is likely to become a pest after treatment for red imported fire ants, *S. invicta*. Since 2007, *B. patagonicus* has been collected in at least nine South Carolina counties, indicating the species may be distributed state-wide (Unpublished data). Further survey is needed to confirm the actual range of the dark rover ant in South Carolina.
History and Distribution

Both the genus and species *Brachymyrmex patagonicus* Mayr were first described by Gustav Mayr in 1868 from both reproductives and workers in Argentina (Quiran et al. 2004). The species’ native range also includes Paraguay (Quiran 2007). Since then, the species has spread to several states in the US where it has become an invasive pest (MacGown et al. 2010). The species was described from workers and males in Rio Negro, Argentina which were all light colored. Santschi described *Brachymyrmex patagonicus* var. *atratulus* from dark colored workers in Alfarcito, Jujuy (Quiran et al. 2004). However, specimens from the Naturhistorisches Museam Wien in Austria, which were also collected and identified by Gustav Mayr from Chile and Argentina were also dark in coloration and were identified as *Brachymyrmex patagonicus*. Therefore coloration is not sufficient to warrant the separation of *Brachymyrmex patagonicus* and *Brachymyrmex patagonicus* var. *atratulus* and the two are considered to be synonymous (Quiran et al. 2004).

The dark rover ant was first reported in the United States in 1978 from St. Tammany Parish, Louisiana (Wheeler and Wheeler, 1978). At the time, it was incorrectly identified as *Brachymyrmex musculus* Forel, and was collected from a single colony living in saw dust under a recently cut oak tree (Wheeler and Wheeler, 1978). Unpublished reports of *B. patagonicus* in Florida were made in 1976, but subsequent lists of ant fauna for the state did not include it (MacGown et al. 2007) (Deyrup 2003). *Brachymyrmex musculus* was reported again in 2000 in Florida (Deyrup et al. 2000).
Various reports of different ants from the genus *Brachymyrmex* are mentioned throughout the southeast as household pests and it is thought that these were all probably the same species. Specimens marked as *B. musculus* from Texas, Louisiana, Georgia, Alabama, Florida, and Mississippi were collected and observed against specimens from the Louisiana State University Arthropod Collection that were previously identified as *Brachymyrmex obscurior* Forel and *B. patagonicus*. All were found to be the same species (MacGown et al. 2007). The original description of *B. patagonicus* by Gustav Mayr in 1868, was not helpful in terms of identification, but a redescription of the genus by Quiran et al. (2004) was highly detailed and included all castes of *B. patagonicus*, making it possible to confirm that all of these specimens in the Southeastern US were *B. patagonicus*.

One year after its first report in the United States in St. Tammany Parish, Louisiana (Wheeler and Wheeler 1978), *B. patagonicus* was reported in Marshall County, Mississippi in 1977 (MacGown et al. 2007). Since then it has become common in all US Gulf States. As of 2007, it has been reported from 27 counties in Georgia, 23 counties in Florida, 27 counties in Alabama, 31 counties in Mississippi, 15 parishes in Louisssiana, 2 counties in Arkansas, and 2 counties in Texas though it is commonly referred to as *B. musculus* in these reports (MacGown et al. 2007). MacGown et al. (2007) makes note that *B. patagonicus* is found in Chatham County, Georgia, which borders South Carolina and that it “probably occurs there as well” but finds no official report of the species from the state. This report also references unpublished reports of *B. patagonicus* at a hotel in Tucson, Arizona.
In 2010, *B. patagonicus* was reported from South Carolina in Horry County (MacGown et al. 2010). In 2011, *B. patagonicus* was reported from one collection site in Riverside County, and multiple sites in Orange County, in California (Martinez et al. 2011). While sampling for *S. invicta*, dark rover ants were reported in four additional counties in Texas (McDonald et al. 2016). In 2015, dark rover ants were discovered on the campus of North Carolina State University (Guénard et al. 2012). In 2017, *B. patagonicus* was collected near Memphis, TN (Hill 2017). As of 2017, *B. patagonicus* is known to occur in Alabama, Arkansas, Arizona, California, Florida, Georgia, Louisiana, Mississippi, Nevada, North Carolina, South Carolina, Tennessee, and Texas (MacGown 2008) (Guénard et al. 2012) (Hill 2017). The spread of the dark rover ant is facilitated by both its small nest size making it easy to be transported by human activity, and its association with other highly invasive and aggressive ants which may offer it protection in established areas (MacGown et al. 2010). The potential range of *B. patagonicus* is thought to be as far north as Tennessee (MacGown et al. 2010). In South Carolina, the species has only been officially reported from Horry County (MacGown et al. 2010). Further investigation is required to determine the actual distribution of the dark rover ant in the state.

**Biology and Behavior**

*Brachymyrmex patagonicus* is a minute (~0.5mm) soft-bodied formicine ant that ranges in color from black to dark-brown. Ants of the genus *Brachymyrmex* are characterized by nine-segmented antennae (MacGown et al. 2007) (MacGown 2008).
Workers of this species are most often confused with *B. obscurior*, but can be identified by the larger eye size, which is approximately equal to the malar length, and sparse pubescence of the first gastral tergite (MacGown et al. 2010) (MacGown 2008). The dorsum of the promesonotum also possesses sparse pubescence. Three minute ocelli are present which form an isosceles triangle (QUIRAN et al. 2004) (MacGown et al. 2010). Queens are reddish-brown, and are more pubescent than workers across the whole body. Males have 10-segmented antennae and are bicolored, with a black head and tan-colored body. They have reduced pubescence which gives them a more shiny appearance (MacGown et al. 2010).

Dark rover ant nests often can be found near the bases of plants, including hardwood trees, as well as in mulch, leaf litter, trash, and human structures such as wall voids (MacGown et al. 2010) (Dash 2004). The actual entrance point into the soil may be difficult to locate since there is no mound to indicate the location. From personal observation, in South Carolina, they often are be found near nests of other invasive ants such as *S. invicta* and *L. humile*. In their native range, colonies are most abundant in grassland, but also are found in shrub-land biomes (Calcaterra et al. 2016). Their prevalence and invasive status in urban areas is speculated to be the result of physiological adaptations which suit them for moderately disturbed areas, rather than the ability to gather resources more efficiently (Calcaterra et al. 2016).

The diet of *B. patagonicus* consists mostly from root feeding hemipterans (Dash 2004). However, in their introduced range they have been observed foraging on the trunks of hardwood trees and ascending into the canopy. *Brachymyrmex patagonicus* has
been observed tending *Aphis gossypii* Glover (Aphididae) and *Diaphorina citri* Kuwayama (Psyllidae) (Navarette et al. 2013). Dark rover ants prefer sugary carbohydrates in the winter and spring, and protein in the summer and fall (Keefer 2016). Keefer (2016) observed that *B. patagonicus* will actively move colonies to be nearer to food sources. Thus if a species of ant is entering a home for a food source, and then are eliminated, dark rover ants could potentially move its colony to be closer to this new food source, which could account for reports of dark rover ants becoming an urban pest after areas have been treated for other ant problems. In their native range, *B. patagonicus* was shown to have high potential to maintain numerical dominance at baits, meaning their numbers do not decrease at baits as quickly as other co-occurring species. (Carval et al. 2016).

Dark rover ants do have nuptial flights which occur at night in the summer, where alates are attracted to lights (Keefer 2016). Miguelina and Baker (2014) observed alate swarms from February to October in Arizona. During this time, they are likely to enter homes, especially if they are nesting in a wall void or under a foundational slab (MacGown et al. 2010). In Puerto Rico, *Brachymyrmex* foraging activity increased by 55% during the wet season (June-August) which lines up with the onset of nuptial flights and suggests the success of this species is linked to water availability (Brown et al. 2012). Brood development is optimized at 30°C and takes around 33 days to complete at this temperature (Keefer 2016). Eggs which are laid by workers in the absence of a queen will result in winged males (Miguelena and Baker 2014).
Dark rover ants often form colonies very close to one another, and show a high level of tolerance for non-nestmates (MacGown et al. 2007). However, in a lab setting aggression was observed between workers of different colonies 60% of the time (Miguelena and Baker 2014). Unlike other invasive ants, they do not form true supercolonies (Miguelena and Baker 2014). They have been observed establishing permanent satellite nests near food sources with both eggs and larvae in less than 24 hours (Miguelena and Baker 2014). Dark rover ants are generally thought to be monogynous, but it is hypothesized that there may be a high rate of cooperative nest founding since queens will aggregate at lights (Miguelena and Baker 2014). However this has not been observed in the field.

Dark rover ants often are found both nesting and foraging next to ecologically dominant ants, especially invasives including *S. invicta, S. richteri, L. humile,* and *Nylanderia fulva* (Mayr) while exhibiting no aggression (MacGown et al. 2007) (Keefer 2016). The reason for this lack of aggression is unknown but it is hypothesized to be chemical, such as a chemical mask which prevents them from being positively identified (MacGown et al. 2007). Aggression levels between dark rover ants and co-occurring ants have not been formally investigated. There also are reports of callbacks for dark rover ants after suppression of red imported fire ants from pest control operators (Dash 2004). However, research is needed to determine whether this is the result of immigration due to availability of resources, or if existing colonies are creating satellite nests near newly available food sources. More research is needed to quantify population increases after red imported fire ant suppression.
Pest Status and Control

Dark rover ants are considered to be a nuisance pest (MacGown et al. 2007). There are no records of dark rover ants biting and they cannot sting. There are no records of them transmitting diseases; however they have been shown to have the potential to carry *E. coli* for short distances (Keefer 2016). Occasionally they will enter structures in search of food, especially if nesting within walls or under the foundation. They also will swarm lights during nuptial flights, or they may enter homes during this time if they are nested within the walls (Miguelena and Baker 2014). There are unconfirmed reports of this species entering hospitals where they are difficult to control (Keefer 2016). Moisture issues and fungal outbreaks also are associated with dark rover ant infestations (Keefer 2016).

Dark rover ants have been found tending the invasive Asian citrus psyllid, *Diaphorina citri* Kuwayama which is a contributor to economic loss in the citrus industry (Navarrette et al. 2013). In addition, *B. patagonicus* has been shown to displace *P. megacephala*, another major invasive ant, in citrus orchards (Navarrette et al. 2013). These findings suggest that dark rover ants may become a more serious agricultural pest in the future.

When dark rover ants do become a nuisance they often are difficult to control and result in callbacks if they are not properly suppressed initially (Dash 2004). Dark rover ant nests are particularly challenging to locate because the ants form seemingly disorganized trails that are difficult to track, and have small nests which are not apparent. They also often nest at the base of plants which can complicate treatment. This makes
direct nest treatment unfavorable. Baits also may be ineffective because dark rover ants will establish many independent colonies in close proximity to one another without aggression, and because they will readily establish satellite nests near food sources (Miguelena and Baker 2014). A single colony will establish a satellite nest near the bait and dominate it. Thus only one colony may be exposed to the treatment, leaving others to expand after treatment. Despite this, gel baits gave the highest mortality rate in a laboratory setting (Keefer 2016). Demand® liquid spray (Syngenta Crop Protection, Greensboro, NC, USA) CS 0.015% also was effective for exterior infestations (Keefer 2016). Max Force Quantum Gel (Bayer, Leverkusen, Germany) 0.03% was shown to have a 94.5% control rate after 90 days (Keefer 2016). Intice® (Rockwell Labs, North Kansas City, Missouri, USA) ant bait provided a higher mortality rate than Terro Ant Bait® (Terro, Lititz, Pennsylvania, USA) after 1 week (Miguelena and Baker 2014).

Miguelena and Baker (2014) also investigated Termidor®SC (BASF, Ludwigshafen, Germany) (fipronil), Demand®CS (lambda-cyhalothrin), and Arilon® (Syngenta Crop Protection, Greensboro, NC, USA) (indoxacarb). Termidor®SC, was ineffective, and did not significantly differ from no treatment, while indoxacarb was most effective, having a mortality rate of 95% (Miguelena and Baker 2014). Application of pesticides on porous surfaces greatly increases dark rover ant survival (Miguelena and Baker 2014).

Because of the difficulties with chemical control, cultural and non-chemical control is recommended when treating dark rover ants (Keefer 2016). In arid environments such as the American Southwest, removal of moisture can be effective in reducing numbers (Miguelena and Baker 2014). Proper upkeep and rotation of plants, as
well as aphid control can help to reduce dark rover ant populations outside of structures (Hedges 2010).

Because dark rover ant infestations can be difficult to treat, it is important to understand how infestations may be prevented. The development of a map of \textit{B. patagonicus’} range in South Carolina will increase awareness of its presence, resulting in targeted treatment when this species becomes a pest problem. Successful control of this species and a greater awareness of its presence will aid to prevent spread to new areas. Furthermore, understanding the relationship between dark rover ants and other invasive ants may insure that treatment for other pest ants does not cause a secondary infestation of dark rover ants. Understanding agonistic interactions between these ants could provide useful information for control of invasive species in the future, and may better aid in understanding conditions which allow for successful colonization by future invasive pests.

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CHAPTER 2.

THE DISTRIBUTION OF THE DARK ROVER ANT, *BRACHYMYRMEX PATAGONICUS* MAYR IN SOUTH CAROLINA BY COUNTY

*Brachymyrmex patagonicus* Mayr was first reported in the United States from St. Tammany Parish, Louisiana in 1978 (Wheeler and Wheeler 1978). A single colony was found nesting in a saw dust pile from a recently cut oak tree. At the time they were misidentified as *Brachymyrmex musculus* Forel, and this error was often repeated in subsequent distribution reports throughout the southeast, making it difficult to track this species’ spread (MacGown et al. 2007). Since then, the dark rover ant has become common in all US Gulf States and much of the southeast (MacGown 2008). They also occur in the western US in Arizona (MacGown et al. 2010), Nevada (MacGown 2008), California (Martinez et al. 2011), and Texas (McDonald et al. 2016). Most recently, it has been documented in North Carolina (Guénard et al. 2012) and Tennessee (Hill 2017). A 2008 report mentions since *B. patagonicus* occurs in Chatham County, Georgia, which borders South Carolina, it most likely occurs in South Carolina as well (MacGown 2008).

In 2010, the dark rover ant was reported from Horry County, South Carolina from a single site (MacGown et al. 2010). Horry County is located on the coast, in the northeastern corner of the state. This is still the only record of this species in the state, although some pest management professionals mentioned they believed they had
observed the dark rover ant in various parts of South Carolina after having it shown to them.

In their native range of Argentina and Paraguay, dark rover ants are reported most often in grassland and shrub-land habitats, which may contribute to their success in urban environments which are less forested (Calcaterra et al. 2016). Nests often are located in soil or rotting wood at the base of plants including trees (Keefer 2016). In urban areas, nests can be found in potted plants, mulch, leaf litter, trash, or wall voids where they can be a nuisance (Dash 2004). Dark rover ant nest areas are generally spatially undefined, and difficult to locate, which can make tracking colonies difficult. The colony entrance hole is small, best described as resembling the hole left after inserting a toothpick into the ground. The colony size is small, containing a few hundred to a thousand workers and a single queen (Keefer 2016). There are many reports of *B. patagonicus* founding nests in close proximity to nesting sites of other aggressive and invasive ants (MacGown 2008) (Keefer 2016). It is also common for multiple colonies of *B. patagonius* to exist close to each other with apparent high tolerance for non-nestmates (MacGown et al. 2010).

Dark rover ant nuptial flights usually occur at night during the summer months, where they are often attracted to lights (Keefer 2016) (MacGown et al. 2010). In Arizona, nuptial flights have been observed as early as February and as late as October (Miguilena and Baker 2014). Increases in foraging activity of up to 55% have been observed from June to August, which is when rainfall is generally at its highest, suggesting that *B. patagonicus* success is related to water availability (Brown et al. 2012). Because of this increase in foraging activity, the summer is the optimal time to survey for dark rover ants.
Dark rover ants do not bite or sting, and are considered a nuisance species around structures. During nuptial flights, they may be attracted to human structures by lights, where they may enter in large numbers (MacGown et al. 2010). Occasionally, dark rover ants will enter human structures to forage for food, and can be difficult to control. Dark rover ants are especially likely to enter human structures if they are nesting within wall voids (Miguelena and Baker 2014). During personal communication with pest management professionals, there were reports of dark rover ants invading hospitals in South Carolina as well. In Florida, a mutualistic interaction between *B. patagonicus* and the Asian citrus psyllid (*Diaphorina citri* Kuwayama), an insect that is responsible for significant economic damage in the citrus industry, has been observed (Navarrette et al. 2013). Similar mutualistic interactions between ants and honeydew-secreting hemipterans have caused significant damage to agriculture in the past (Zenn er de Polannia 1990).

There have been reports that *B. patagonicus* may become a secondary pest after an area is treated for red imported fire ants (*Solenopsis invicta* Buren) (Dash 2004). When dark rover ants do become a problem, they are difficult to treat because they will readily establish satellite colonies near baits, rather than bring the bait back to the colony (Miguelena and Baker 2014).

Because of the rising pest activity of the dark rover ant in South Carolina, it is important to understand the distribution of this species in the state. Although it has been suspected that the dark rover ant has established in South Carolina, there is still currently only a single published record of this ant in the state (MacGown 2008) (MacGown et al. 2010).
This study had two objectives: 1) to identify the distribution of the dark rover ant in South Carolina by county and 2) to use this information to develop a distribution map for this species which may be used by pest management professionals to determine if the dark rover ant is present in their area. I hypothesized that the dark rover ant is widespread in South Carolina and can be found in all forty-six counties.

**Materials and Methods**

During the spring and summer of 2016-2017 a survey of South Carolina counties was conducted to determine the presence of *B. patagonicus* in the state. All 46 counties of South Carolina were surveyed for simple presence/absence of *B. patagonicus* within the county. Because the presence of one ant caste implies the presence of a full colony, the collection of any *B. patagonicus* castes was considered enough to determine the species present in that county. While the survey focused on each county individually, the overall goal was to establish a county distribution map for South Carolina.

Surveying an entire county evenly was not feasible, so targeted sites were chosen within each county based on their likelihood to harbor invasive ants. Because dark rover ants are often spread through human mediated dispersal (Keefer 2016), sites were chosen that received a high volume of human activity, especially from many different geographic areas. Because the dark rover ant is thought to be better adapted for disturbed areas (Calcaterra et al. 2016), all target sites were in urban environments. Other potential sites such as state parks and national forests were not chosen. Examples of chosen target sites
included: government buildings, universities, hospitals, town squares, main streets, truck stops, gas stations, fast food restaurants, outlet malls, and city parks.

Because dark rover ants tend hemipterans for their honeydew, the trunks of hardwood trees were often sampled for foraging trails. Ants were collected via an aspirator and transferred to a vial containing 80% ethanol. If there were other ant species foraging around dark rover ants, they were also collected via aspirator and transferred to a vial containing 80% ethanol. The type of tree which the ants were collected from was noted as well.

Before the inspection of a county, several target sites were planned on a map for sampling in case dark rover ants were not collected immediately. These target sites were visited in order of convenience and were not rated in according their likelihood to contain dark rover ants. If additional locations were discovered during the course of sampling that appeared suitable, they were also visited. When dark rover ants were successfully collected at any given target site, detailed information about the collection site was logged. Data collected included county name, nearest city, specific location (Name of business, park, school, etc.), GPS coordinates, date, collector’s name, lowest relevant taxonomic classification of co-occurring ants (if collected), and type of tree ants were collected on if applicable. GPS coordinates were logged using Google Earth™.

Collected ants were taken back to the Clemson University Urban Entomology lab and were examined under a microscope to confirm field identifications. All *Brachymyrmex* were identified to species to confirm that they were *Brachymyrmex patagonicus*. Non-target (not *Brachymyrmex* spp.) ants were identified to the lowest
relevant taxonomic level. Major pest ants were identified to the species level, while ants which are normally not pests were identified to the genus level. All ants were identified according to (MacGown 2014). All dark rover ants collected were vouchered in the Clemson University Arthropod Collection. A distribution map (Figure 2.1) for *B. patagonicus* was developed using these data.

**Results**

![Figure 2.1 South Carolina Distribution Map by County for Brachymyrmex patagonicus Mayr](image)

*Brachymyrmex patagonicus* was collected in 46 of 46 South Carolina counties, indicating that the dark rover ant has a statewide distribution and is established in all
areas of the state. Workers were most commonly collected, with only a single male alate being collected in Pickens County. Dark rover ants were collected in both metropolitan and rural areas of the state, and there was no difference in difficulty in finding dark rover ants between the two. The details of each collection site, including location, GPS coordinates, date, microhabitat, and co-occurring ants are reported in table A-1.

Dark rover ants were most commonly collected at restaurants and businesses for a total of 13 times, but were also collected at government buildings 11 times, highways and rest areas 5 times, Universities 4 times, parks and tourist attractions 4 times. Dark rover ants were collected less commonly at churches, street fronts, and houses in residential areas for a total of 9 times. Dark rover ants were often collected foraging on trees, for a total of 32 times. Dark rover ants were most commonly collected from oak trees, for a total of 14 times, but were also collected on a variety of other hardwood trees as well. Dark rover ants were only collected on trees if they had green leaves at the time. When no trees with green foliage were present, dark rover ants were collected on sidewalks and parking lot curbs for a total of 10 times. Dark rover ants were less commonly collected from leaf litter or sandy patches for a total of 3 times. In a single instance, dark rover ants were collected from a decorative potted plant. Dark rover ants were rarely found foraging on coniferous trees. In Hampton county, *B. patagonicus* was collected from a palmetto tree. In most instances, *B. patagonicus* was found co-occurring and in some instances co-foraging with other ants, both native and invasive. The red imported fire ant (*S. invicta*) was the most commonly observed co-foraging ant species for a total of 12 times. Argentine ants (*Linepithema humile* (Mayr)) was the second most common species, with
a total of 3 times. Pyramid ants (*Dorymyrmex bureni* (Trager)) and acrobat ants (*Crematogaster* sp.) were each collected 2 times, and carpenter ants (*Camponotus* sp.) and big headed ants (*Pheidole* sp.) were each collected a single instance.

**Discussion**

In most instances, *B. patagonicus* was collected at the first target site visited, often from the first tree observed. In multiple instances, ants were collected before a planned target site was reached, from a gas station, interstate rest area, or restaurant. Dark rover ants were collected in all South Carolina counties in a variety of habitats of different levels of urbanization, and at multiple target sites, and multiple microhabitats within target sites, indicating that dark rover ants had colonized the entire state for many years. The lack of county collection records for this species is reflective of the lack of research in this area and does not indicate that this species has recently arrived to the state. Although the first record of *B. patagonicus* is from 2010, it is impossible to know when this species first arrived, or how established the species was at the time. Anecdotal reports of *B. patagonicus* as a pest problem have recently increased in the state. In personal communication, pest control operators have described multiple instances of unknown ant infestations that best fit descriptions of *B. patagonicus*. However, this is not likely evidence of expanded range or increasing populations. It is instead more likely the result of increased awareness of the presence of this species in the state as more research is conducted and reported. In most instances where the dark rover ant was reported as a
pest problem, pest control operators expressed that it had been difficult to identify which had made it difficult to treat. The dark rover ant has conspicuous key characters which make it easily identifiable under a microscope (nine-segmented antennae, sparse and erect pubescence on first gastral tergite, and a single node), but pest control operators may have a difficult time distinguishing this species from other very small species in the field, such as the little black ant, *Monomorium minimum* (Buckley). For pest control operators to reliably identify this ant in the field, this species must be included in future training material. Further research is needed to determine the extent of *B. patagonicus* as a pest problem in the state, and training materials and keys to pest ants aimed at pest control operators must be updated.

Dark rover ants are most commonly found foraging on trees during mid-spring to mid-fall, likely because of their known association with both root-feeding and foliage-feeding hemipterans which they tend for honeydew secretions. It is likely that dark rover ants were rarely observed on coniferous trees because hemipterans may not feed on their leaves and so ants are not attracted to them. Dark rover ants also commonly were observed nesting at the bases of the same vegetation they foraged on, indicating the presence of vegetation may be a useful tool for predicting the presence of this species, and may aid pest control operators to better target treatments.
References Cited


CHAPTER 3.

INVESTIGATION OF DARK ROVER ANT, *BRACHYMRYMEX PATAGONICUS* MAYR, FORAGING ACTIVITY AFTER RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA* BUREN, SUPPRESSION

The dark rover ant, *Brachymyrmex patagonicus* Mayr, is a very small (~0.5 mm) dark brown or black formicine ant native to Argentina and Paraguay. It is an emerging pest species in the United States. It was first discovered in the United States in St. Tammany Parish, Louisiana in 1976 (Wheeler and Wheeler 1976). Since then, it has expanded its invasive range and is established in nearly all areas of the Gulf Coast (MacGown et al. 2007). Most recently it has been discovered in North Carolina and Tennessee, providing evidence that its range is still expanding. (Guénard et al. 2012) (Hill 2017).

Dark rover ants nest in small colonies (~1000 workers) in the soil often at the base of plants, especially hardwood trees or bushes, but will also nest in loose matter such as mulch, leaf litter, or trash (MacGown et al. 2010) (Dash 2004). Occasionally, they will nest inside of wall voids, where they can become a nuisance pest (MacGown et al. 2010). The entrance point to a colony is often difficult to locate because there is no mound and the entrance hole is often a single tiny hole in the soil. Dark rover ants thrive in moderately disturbed habitats, such as urban environments. This prevalence in urban environments is likely the result of a physiological adaptive
predisposition and not because of an ability to outcompete other species in gathering resources (Calcaterra et al. 2016).

The diet of *B. patagonicus* is thought to consist mostly of honeydew from root-feeding hemipterans (Dash 2004). In their invasive range, they also are often observed foraging on tree trunks (especially hardwood), ascending into the canopy where they are reported to tend foliage-feeding hemipterans, including the economically important Asian citrus psyllid, *Diaphorina citri* Kuwayama (Navarette et al. 2013). Dark rover ant food preference changes seasonally, with sugary carbohydrates preferred in the winter and spring, and protein preferred in the summer and fall (Keefer 2016). *Brachymyrmex patagonicus* has been shown to have a high potential to maintain numerical dominance at baiting sites, with populations at baits remaining constant for a longer time period than co-occurring species (Carval et al. 2016). This species also has been observed to actively move colonies to be closer to food sources (Keefer 2016). Unlike other ant species which forage and bring food back to the colony, dark rover ants establish satellite nests near food sources, complete with larvae and brood, within 24 hours (Miguelena and Baker 2014).

Dark rover ants often are observed nesting and foraging near other invasive, aggressive, and ecologically dominant ant species (MacGown et al. 2007). Common co-occurring ant species include *Solenopsis invicta* Buren, *Solenopsis richteri* Forel, *Linepithema humile* (Mayr), and *Nylanderia fulva* (Mayr) (MacGown et al. 2007). *Nylanderia fulva* is not present in South Carolina at the time of this writing. These ants are all known to be highly aggressive, especially towards one another, however
B. patagonicus is not met with the same level of aggression expected between competing ant species (Keefer 2016). The cause of this interaction is unknown but it has been hypothesized to either be the result of chemical interactions (MacGown et al. 2007), or that it is due to size differential (Miguelena and Baker 2014). This behavior may have implications for aiding B. patagonicus in expanding its invasive range if nesting inside of the foraging territory of aggressive ants offers them unique protection when founding new colonies.

Anecdotal reports from pest control operators in South Carolina mention that B. patagonicus will sometimes become a secondary pest after other major pest ants have been suppressed, especially S. invicta. These reports have not been confirmed, and it is unclear whether this interaction is the result of increased foraging activity after resources become newly available, or the result of immigration by new colonies. Dark rover ants do not bite or sting, but are considered a nuisance pest (MacGown et al. 2007). When B. patagonicus does become a pest, they are difficult to control and often result in callbacks (Keefer 2016) (Dash 2004). Baiting for B. patagonicus may be ineffective due to their behavior of establishing satellite colonies near bait, resulting in baits never being brought back to the original colony (Miguelena and Baker 2014). Despite this, gel baits are considered the most effective method of control for B. patagonicus, but should be combined with non-chemical control measures such as moisture reduction and aphid control (Keefer 2016) (Miguelena and Baker 2014).
This experiment had three objectives: 1) to determine if there is an increase in foraging activity by *B. patagonicus* after suppression of *S. invicta*, 2) to determine whether or not *B. patagonicus* will become the dominant forager on baits after the suppression of *S. invicta*, and 3) to establish a list of common co-occurring ant fauna with *B. patagonicus*, both native and invasive. I hypothesized that *B. patagonicus* foraging activity will increase after *S. invicta* suppression, but that *B. patagonicus* will not become the dominant forager after *S. invicta* is suppressed.

**Materials and Methods**

**Experimental Overview**

Ten trees in two areas were chosen on Clemson University Main Campus (34.6761° N, 82.8364° W). Each area contained five (5) trees. One area was chosen to receive treatment for red imported fire ants; the other received no treatment for the duration of the study to serve as a control. Each of the ten trees was sampled using three methods to investigate the foraging behaviors of dark rover ants in relation to red imported fire ants. Pitfall trap sampling was used to quantify the foraging activity of both species. Bait sampling was used to determine which species was the dominant forager at each tree. Tree scan sampling was used both to confirm the presence of *B. patagonicus* and *S. invicta* at each tree, as well as to establish a list of co-occurring ant species (both native and invasive) in urban environments.

On July 25, 2017, each tree in both sections was sampled using pitfalls, baits, and tree scans to establish a baseline for ant foraging activity. On July 31, 2017, the five
treatment trees were baited with Advion® fire ant bait (Syngenta, 410 S Swing Rd, Greensboro, NC 27409, indoxacarb 0.045%) to suppress *S. invicta* in the area. On August 9, 2017, each tree in both areas was sampled again using the same methodology as before to quantify *B. patagonicus* foraging activity after *S. invicta* suppression.

On August 14, 2017, the treatment area was baited with Advion® fire ant bait again using the same methodology as before to insure that *S. invicta* remained suppressed. On August 25, 2017, and September 8, 2017, all trees in both areas were sampled again using identical methodology as before. Before these two sampling dates, the treated area was not baited using Advion® fire ant bait because fire ants remained suppressed.

**Tree Selection**

Two separate areas of Clemson University Main Campus were chosen which contained trees that would be suitable for this study. The two areas were separated by approximately 100m such that there was enough space that there would be no overlap in ant populations between them. A satellite image from Google Earth® of Clemson University showing both areas from this study is reported in figure B-1 (Google Earth 2018).

Area 1 was the McGinty Mall, serves as a courtyard for Poole, Barre, McAdams, and Lehotsky Halls. The area has an interlocking grid of sidewalks, but the majority of the surface area was exposed ground cover containing many trees. This area was chosen to be the treated area.
Area 2, a green-space which lies just outside the Cooper Library contained less sidewalk area than Area 1 and had more grass as well. The differences between Area 1 and Area 2 were not considered to potentially impact dark rover ant activity because the specific microhabitats at each tree were investigated, not the areas as a whole. Both areas were considered to have approximately the same level of ant foraging activity.

Five trees were chosen in each area to be investigated throughout the study in every trial. All selected trees fit the following criteria: 1) *B. patagonicus* must have been observed foraging alongside *S. invicta* on the tree. 2) All were oak trees (*Quercus* sp.); 3) trees had sufficient exposed ground area around their base such that ants were nesting around the tree. Trees within each area were examined to determine whether or not they fit this criteria. After a list of all potential trees within each area was compiled, the five trees with the most observed *B. patagonicus* and *S. invicta* workers were chosen.

**Methodology for Treatment.** Advion® Fire Ant Bait was loaded into an automatic granule-dispenser, the Scotts Wizz hand held spreader (Scott’s Miracle-Gro Company, 14111 Scottslawn Rd, Marysville, OH) and was distributed across all areas of exposed ground within Area 1. Application was made by applying the bait around the perimeter of each section of exposed ground with the spreader held out in front, walking at a steady pace. Then the section was crossed diagonally twice (forming an “X” shape) to insure that the interior was treated thoroughly. If the section was large enough to necessitate it, the area would be crossed again longitudinally. Bait was applied liberally because this
study required that *S. invicta* be completely suppressed within the area, and did not focus on the efficacy of the bait at recommended application rates.

**Methodology for Pitfall Trap Sampling.** Pitfall traps were installed approximately 24 hours before trees were sampled. A battery-powered drill with a 1” drill bit was used to create a hole in the soil at the base of each tree, and a standard size aspirator vial was inserted. The aspirator vial was filled with antifreeze (propylene glycol) and allowed to run for approximately 24 hours. At the end of 24 hours traps were collected and capped. Caps were labeled with the date and tree number. The position of each trap was chosen based on the permeability of the soil but all traps were set within 4 inches of where the base of the tree contacted the ground. The position of each trap was held constant throughout the entire study. All collected ants were identified to the lowest relevant taxonomic classification (pest ants were identified to species while non-pest ants were identified to Genus) using a dissecting microscope and the identification keys, of MacGown (2014). The number of individuals of each species in each pitfall trap was logged.

**Methodology for Bait Sampling.** A bait was used to assess which ant species was the dominant foraging species at each tree. Stolen baits by the large population of eastern grey squirrels (*Sciurus carolinensis* Gmelin) were a recurring problem during preliminary trials. A device was created which would serve to both prevent squirrel interference and to standardize the area in which ants could be considered to have responded to the bait. The device consisted of a small Petri dish which had several holes drilled into the sides, and a single hole on the top and bottom, which would allow ants of any size to enter.
Several chunks of a Keebler® (677 Larch Avenue, Elmhurst, IL 60126) brand pecan sandy cookie (around ¼ of a cookie) were placed inside of the device. A single roofing nail was inserted through the top hole, down through the bottom hole, and into the soil, to prevent the device from being moved. Because squirrels could potentially still break the Petri dishes, a rectangular cover was created for each device using metal wire mesh and was fixed to the ground using roofing nails in order to prevent squirrels from accessing the device.

After a bait was placed at each tree, all other sampling was then completed, allowing bait access for approximately 1 hour. After an hour elapsed, the cover was removed from each device and the entire device was placed into a Ziploc® (S.C. Johnson, 1525 Howe Street, Racine, WI 53403) bag, and the bag was labeled with the date and tree number. Bags were then placed into a freezer for future inspection. The contents of each bag were thoroughly inspected to verify that all ants inside were the same species. Ants were identified to the lowest relevant taxonomic classification, meaning pest ants were identified to species while non-pests were identified to genus, using the identification keys by MacGown (2014). The number of ants at each bait was also logged. If multiple species were present at one bait, the ant species with the most individuals was considered the dominant species at that bait, but the exact number of each species was logged.

**Methodology for Tree Scan Sampling.** Tree scan sampling was used to determine a list of co-occurring ant species with *B. patagonicus*. Tree scanning consisted of a team of two people standing on opposite sides of a tree and carefully examining the tree for exactly five
minutes. A few ants of each species observed foraging on each tree were collected via aspirator. When an ant species was observed for the first time, it was collected, and that species was not subsequently collected if observed again. If there was a possibility that a foraging ant belonged to a species not yet collected, then it was collected to insure that all foraging species were recorded. If a foraging ant was observed which belonged to a species already collected, then it was not collected because the goal of this sampling method was only to determine which species were foraging on each tree.

At the end of the sampling period, the collected ants of each observer were combined into a single vial filled with 80% ethanol. All collected ants were identified to the lowest relevant taxonomic classification (genus or species depending on if multiple species are usually grouped under one common name for pest control purposes) using the identification keys of MacGown (2014).

All collected ant specimens in this study were vouchered in the Clemson University Arthropod Collection, Clemson, South Carolina.

**Methodology for Statistical Analyses.** The study design consisted of a treatment design and an experiment design. The treatment design was a one factor design (treated trees vs. untreated trees) with repeated measures. The repeated measures consisted of a pre-treatment time period (one sampling date for *B. patagonicus* and *S. invicta*) and a post-treatment time period (three sampling dates for *B. patagonicus* and *S. invicta*). The experiment design was a completely randomized design with 5 treated and 5 untreated trees. The analyses were completed in two steps. First, a series of graphical approaches were used to determine changes across time periods for both *B. patagonicus* and *S.*
*invicta* in treated trees and untreated trees. A scatterplot was then used to determine the overall relationship between *B. patagonicus* and *S. invicta* foraging activity. A linear regression analysis was performed to determine if there was a significant linear relationship. A paired T-test was used to determine statistical differences between means in the pre-treatment and post-treatment periods.

**Results**

**Pitfall Traps.** An analysis of the total number of dark rover ants and red imported fire ants at all trees in each treatment area (figure 3.1) shows that in the treated area, red imported fire ant numbers fell to zero and remained there for the duration of the study, showing that they were successfully suppressed. In the untreated section, red imported fire ant numbers increased significantly on the second treatment before falling again and remaining relatively stable for the duration of the study. Dark rover ant numbers were low in the treated area for the duration of the study, but increased slightly for the third sampling date before falling again. In the untreated section dark rover ant numbers followed the same overall trend, but with a higher overall number of ants. In both the treated and untreated areas, a slight increase in dark rover ants in pitfalls can be observed after a decline in red imported fire ant numbers, however, the change in ants is too small to be significant.
Figure 3.1 Total Number of *B. patagonicus* Mayr (B.p.), and *S. invicta* Buren (S.i.) collected in all pitfall traps in both the treated and untreated areas across four sampling dates. The number of B.p. in pitfall traps at each tree in the treated area across time are also reported. No increase in B.p. activity is detected in the absence of S.i. in the treated area.

Analysis of the number of dark rover ants in each pitfall at each tree in the treated area (figure 3.1) shows the overall number of ants found in each pitfall was very low. For trees 1, 2, and 3 no dark rover ants were ever collected in a pitfall. The increase detected in dark rover ant numbers during the third sampling date were only detected at trees 4 and 5. This increase was the highest at tree 4, between the second and third sampling dates, where the increase was only 0 to 2 ants collected. At tree 5, dark rover ants increased from 0 to 1 ant. The number of *B. patagonicus* collected in each pitfall trap in the treated area are reported in table B-1.
Analysis of individual pitfalls at trees for *S. invicta* in the treated area showed *S. invicta* remained suppressed for the duration of the study at each tree and no *S. invicta* were collected. The number of *S. invicta* collected in each pitfall trap are reported in table B-1. Analysis of the number of dark rover ants in each pitfall at each tree in the untreated area (area 2) (figure 3.2) showed that dark rover ant activity followed the same trend as in the treated area, where the number of ants in pitfalls started at a moderately high level, fell down to near zero on the second sampling date, increased on the third sampling date, before decreasing to near zero again on the final sampling date. However, in the untreated area, a higher number of dark rover ants in each pitfall at each tree was detected. In the untreated area, only 8 *B. patagonicus* were collected in pitfalls throughout the study, while in the untreated area 51 *B. patagonicus* were collected. Trees 6 and 7 had the highest increase in *B. patagonicus* collected between sampling date 2 and 3. Tree 6 increased from 1 *B. patagonicus* on sampling date 2 to 10 on sampling date 3, and tree 7 increased from 0 to 11 *B. patagonicus*. The number of *B. patagonicus* collected in each pitfall trap in the untreated area are reported in table B-1. Analysis of the number of red imported fire ants detected in each pitfall at each tree in the untreated area (figure 3.3) revealed that the number of collected *S. invicta* increased from 36 to 93 between the first and second sampling date, before decreasing to 19 on the third sampling date and 13 on the final sampling date. The increase of *S. invicta* on the second sampling date is due to tree 6, where 92 of the 93 ants were collected. A single *S. invicta* was collected at tree 10. Without including tree 6 in the analysis, these data followed the same trend as dark
rover ants in the untreated area, but with much less fluctuation between sampling dates.

The number of *S. invicta* collected in each pitfall trap are reported in table B-1.
Figure 3.2. The number of Brachymyrmex patagonicus Mayr (B.p.) collected in each pitfall trap at each tree in the untreated area across time. The total number of B.p. in each pitfall trap (one pitfall trap per tree) was calculated for the untreated area (Trees 6-10) across four sampling dates. The observable changes in B.p. foraging activity over time were the same as in the treated area.
Figure 3.3. The number of Solenopsis invicta Buren (S.i.) in each pitfall trap in the untreated area across time. The total number of S.i. collected in each pitfall trap at each tree (one pitfall trap per tree) was calculated for the untreated area (Trees 6-10) for four sampling dates.

Data were further analyzed by converting the sampling dates into two categories; pre-treatment and post-treatment. The pre-treatment period consisted of a single sampling date (7.25.17), while the post-treatment period consisted of the three sampling dates which took place after initial treatment with Advion® (8.9.17, 8.25.17, and 9.8.17). The mean number of each species of ant collected in pitfall traps was calculated for the treated and untreated area. This analysis (figure 3.4) shows that for red imported fire ants, the number of ants detected by pitfall traps in the treated area decreased after treatment from 88 to 0, while the number of ants detected in the untreated area increased slightly over time from 36 to 41.7. For dark rover ants, the number of ants detected by pitfalls decreased slightly in both the treated and untreated areas. In the treated area the
mean number of *B. patagonicus* collected in pitfall traps decreased from 3 to 2. In the untreated area the mean number of *B. patagonicus* collected in pitfall traps decreased from 20 to 10.3. Because there was a decrease in the number of dark rover ants over time in both the treated and untreated areas, the data does not seem to support the idea that dark rover ant foraging activity increases after red imported fire ants are suppressed. A paired T-test showed there was no significant difference in the mean number of dark rover ants found in pitfall traps in trees 1-5 between pre-treatment and post-treatment (F=1.888, p=0.24).

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**Figure 3.4.** Average foraging activities of *Brachymyrmex patagonicus* (B.p.) and *Solenopsis invicta* (S.i.) over time. The mean number of B.p. and S.i. in pitfall traps at all trees in the treated (Trees 1-5) and untreated (Trees 6-10) were calculated for a pre-treatment period (one sampling date), and a post-treatment period (three sampling dates) for S.i. suppression. The data was analyzed using a T-test. No significant difference between the mean number of B.p. in pitfall traps during pre-treatment and post-treatment was determined (p=0.24).
Analysis of the linear relationship between total number of dark rover ants and red imported fire ants collected at each pitfall trap across all dates (figure 3.5) indicates no linear relationship between the foraging activity of the dark rover ant and the red imported fire ant. A simple linear regression showed there was no linear relationship between the number of dark rover ants and red imported fire ants collected in pitfalls at all trees (F=0.28, p=0.24).

![Graph](image)

**Figure 3.5.** Investigation of the Linear Relationship between the Total Number of Brachymyrmex patagonicus Mayr (B.p.) and Solenopsis invicta Buren (S.i.) in pitfall traps. The total number of B.p. and S.i. in each pitfall trap across all sampling dates was calculated for all 10 trees. The data were analyzed using simple linear regression and no significant linear relationship was found. (F=.28, p=.24).

**Baits.** On sampling date 1 (7.25.17), prior to treatment with Advion®, *S. invicta* was the dominant forager at all 10 trees. An average of 83.2 *S. invicta* workers responded to each bait in area 1, and an average of 59 *S. invicta* responded to each bait in area 2. At a single
tree (tree 6), 3 *B. patagonicus* workers were collected on the bait co-foraging with *S. invicta*.

On sampling date 2 (8.9.17), no ants were collected on baits at trees 1-5, which confirms that *S. invicta* was suppressed in area 1. In the untreated area (area 2), *S. invicta* remained the dominant forager at trees 6-10. An average of 283.8 *S. invicta* workers responded to bait at trees 6-10. This increase in foraging workers at bait from sampling date 1 to sampling date 2 was in contrast to data from the pitfall data from the same sampling date, where the number of collected *S. invicta* workers decreased. At tree 10, a single *B. patagonicus* worker was collected co-foraging on the bait with *S. invicta*.

On sampling date 3 (8.25.17), no foraging ants were collected on baits at trees 1-5, which demonstrates that *S. invicta* remained suppressed in the treated area. In the untreated area, *S. invicta* was the dominant forager at trees 6 and 7 with 300 and 161 workers collected respectively. At tree 8, no ants were collected responding to the bait. At tree 9 the little black ant (*Monomorium minimum* (Buckley)) was the dominant forager with 46 workers collected. At tree 10 a single *B. patagonicus* worker was the only ant collected. In this instance, because only a single *B. patagonicus* worker was collected, *B. patagonicus* was not considered to have dominated the bait.

On the final sampling date (9.8.17), no foraging ants were collected at baits at trees 1, 3, 4, or 5. At tree 2, a single *Nylanderia* sp. Worker was collected on the bait. Because it was a single worker, *Nylanderia* sp. was not considered to have dominated the bait. These results show that *S. invicta* remained suppressed in area 1. At area 2, *S.
*Solenopsis invicta* was the dominant forager at trees 6-9 with an average of 167.25 ants responding to each bait. At tree 10, no ants were collected responding to the bait.

The results of this study found no evidence that *B. patagonicus* could become the dominant forager after the suppression of *S. invicta*. *Brachymyrmex patagonicus* was not found to be more likely to respond to a bait when *S. invicta* had been suppressed, and there was no significant difference in the number of responding *B. patagonicus* workers to a bait when *S. invicta* was present than when they had been suppressed.

**Tree Scans.** *Brachymyrmex patagonicus* was collected during tree scans at every tree in both areas on all sampling dates, except on sampling date 3 (8.25.17) at tree 8 in the untreated area. The presence of *B. patagonicus* at this tree on sampling date 3 was still confirmed by pitfall data for the same sampling date. The presence of *S. invicta* in area 1 prior to treatment with Advion ® fire ant bait was confirmed at all 10 trees on sampling date 1 (7.25.17). No *S. invicta* were collected during tree scans at trees 1-5 for the duration of the study, which confirms previous results that *S. invicta* was suppressed in area 1 for the duration of the study. On sampling date 2 (8.9.17), *S. invicta* was collected at trees 6-10, confirming the species’ presence at all trees in area 2 for that date. On sampling date 3 (8.25.17), *S. invicta* was collected at trees 6, 7, 9, and 10 but was absent at tree 8. *Solenopsis invicta* was confirmed to be present at tree 8 on this sampling date from pitfall sampling. On sampling date 4 (9.8.17), *S. invicta* was collected at trees 6-9, but was absent from tree 10. *Solenopsis invicta* was also absent at tree 10 on this sampling date in pitfall samples as well.
Solenopsis invicta was the most common co-occurring ant collected with B. patagonicus and was collected and was collected 23 times from all 10 trees. Formica pallidefulva Latreille was the second most commonly collected co-occurring ant with B. patagonicus and was collected a total of 34 times, from 10 trees. Camponotus pennsylvanicus (De Geer) was the third most commonly collected, and was collected a total of 26 times from 9 trees. Less frequently collected were Linepithema humile (Mayr), which was collected 4 times from tree 6 only, Crematogaster sp., which was collected 1 time from tree 6 only, Nylanderia sp., which was collected 5 times from 4 trees, Brachyponera chinensis (Emery), which was collected 3 times from tree 3 only, Monomorium minimum Mayr, which was collected 1 time from tree 9 only, and Pseudomyrmex sp., which was collected 1 time from tree 6 only.

**Discussion**

There was a general downtrend in the amount of ants found in each pitfall from sampling date 1 (7.25.17) to sampling date 2 (8.9.17). However, a significant increase in *S. invicta* activity at baits was observed in trees 6-10 from sampling date 1 (7.25.17) to sampling date 2 (8.9.17). This increase was not observed in trees 1-5 because *S. invicta* was suppressed with Advion® fire ant bait there a week prior. The decrease in activity in pitfalls from sampling date 1 to sampling date 2 was most likely the result of weather. Because pitfall traps run for 24 hours and baits only run for 1 hour, pitfall traps are exposed to weather conditions that baits are not. In the early morning hours of 8.9.17
there was rain for several hours that likely resulted in a reduction in the number of
foraging ants at the time. Pitfall traps were outfitted with rain covers, but rainfall levels
were enough to cause runoff that resulted in some pitfall traps being overflowed. Ants
were still collected from these pitfalls, but it is impossible to know if some ants were lost
due to overflow.

No significant difference was found between the number of *B. patagonicus* in
pitfall traps before and after *S. invicta* suppression. However, anecdotal reports from pest
control operators of *B. patagonicus* becoming a secondary pest after fire ant treatment
remain. While performing the experiment, an apparent increase in *B. patagonicus*
foraging activity was observed personally, but this increase was not captured in the pitfall
data. This is most likely due to two major reasons: Much of the increase in foraging was
observed on the roots of large oak trees, where the pitfall traps cannot sample, and the
design of the pitfall traps may have inefficient for sampling dark rover ants. From
personal observation, dark rover ants seemed to have a better ability to grip to surfaces
than some other ant species encountered during this study, which seemingly may have
decreased the chance that a dark rover ant actually falls into a pitfall trap when
encountered. When trying to remove dark rover ants from aspirator vials, they were much
more difficult to remove than other species. Because dark rover ants are smaller and
slower than other ant species, they may be less likely to run over the edge into a pitfall
trap. Dark rover ants also trended towards foraging on trees meaning increases in
foraging activity might not have been measureable with pitfall traps placed on the
ground. In addition, dark rover ants have a smaller colony size and therefore fewer
foraging workers out at any given time. This means that dark rover ants may be less likely to encounter pitfall traps at all in comparison to red imported fire ants. Future work in attempted to quantify foraging activity for *B. patagonicus* should include multiple pitfall traps at each site in order to more thoroughly sample for dark rover ants.

The original intent of the using the Petri dish apparatus for the bait samples was to be able to accurately quantify how many ants were present at a bait after an elapsed period of time. These data could be used to establish which species was the dominant forager at the each bait, and to determine if there was an increase in foraging activity over time for dark rover ants after red imported fire ant suppression. The device worked as intended and did identify the dominant foraging species at each bait (if there was a dominant foraging species), but there was never any significant amount of ant activity at any bait after *S. invicta* suppression in the treated area. Based on this, there is no evidence from bait sample data to support the idea that dark rover ants increase their foraging activity after the suppression of *S. invicta*. However, it is possible that the experimental design is responsible for affecting the data.

From personal subsequent observation, dark rover ants may take longer to appear on a bait and take longer to recruit other workers to a food source as well. There was an instance of a single dark rover ant being collected on a bait after *S. invicta* was suppressed at that site. It is possible that the bait was only being first discovered and would have been dominated by *B. patagonicus* given more time. While one hour may have been enough for ant species with large colony sizes to locate and dominate a bait, dark rover ants may require more time, which should be considered for future work. In
addition, most of the observed foraging by *B. patagonicus* was on the roots and trunks of trees, where workers were often headed into the canopy where they presumably are tending hemipterans for honeydew secretions. Given this, it is possible that an increase in foraging activity by dark rover ants could be mostly targeted towards the trees and therefore would not be detectable by a bait on the ground as strongly. In a human structure, where trees and hemipteran honeydew secretions are not available, dark rover ants may be more likely to direct their foraging towards food sources where an increase in activity is more apparent.

There were two instances of dark rover ants being collected co-foraging on a bait alongside *S. invicta*. This supports field observations from others that dark rover ants will forage around other invasive species (especially *S. invicta*) and are not met with aggression while doing so. This unique interaction could allow *B. patagonicus* to increase its invasive range if it does not face interference competition and may suggest that weather is the main factor limiting its potential invasive range. This could be especially true for urban areas where invasive ants are often the dominant species.

Of the most commonly collected co-foraging ants during tree scan sampling, three of the species collected, *Solenopsis invicta*, *Linepithema humile*, and *Brachyponera chinensis*, are invasive species which are economically or ecologically important. While dark rover ants are currently considered a nuisance pest to humans (MacGown 2008), their ecological impact on the environment remains poorly understood. However, if this species is successful in co-habitating with other ecologically important species, it may
have underlying negative ecological effects that have yet to be detected. Future research should investigate these interactions.

**References Cited**


The dark rover ant (*Brachymyrmex patagonicus* Mayr) is a minute (~0.5 mm), black or dark brown formicine ant from Argentina and Paraguay that is an emerging pest species in the United States. It was first discovered in St. Tammany Parish, Louisiana in 1976 where a single colony was found in a saw dust pile (Wheeler and Wheeler 1978). Since then it has spread to almost all areas of the Gulf Coast where it has become a nuisance pest (MacGown et al. 2007). Most recently, it has been discovered in North Carolina and Tennessee, indicating that its northern range in the United States is still expanding (Guénard et al. 2012) (Hill 2017).

Dark rover ants usually nest in soil at the base of plants including trees and bushes in small colonies of around 1000 workers (MacGown et al. 2010). However, they also will nest in loose material such as leaf litter, trash, and mulch which may aid in their spread (Dash 2004). Occasionally, *B. patagonicus* will also nest in wall voids of structures where they may become nuisance pests (MacGown et al. 2010). The entrance hole to a nest is inconspicuous with no mound and is characterized usually only by a single hole which may resemble a pin prick in the soil. Because dark rover ants usually have weak trailing behavior, nests are often difficult to locate and can complicate treatment. Like other invasive species, dark rover ants tend to thrive in moderately disturbed habitats such as urban environments (Vonshak and Gordon 2015). Dark rover ants are specifically thought to be successful in urban environments because of physiological adaptations.
which suit the species for these environments, and not because they are better at outcompeting other ant species at gathering resources (Calcaterra et al. 2016). Dark rover ants often are observed nesting near other invasive or highly aggressive ant species, however they do not appear to experience aggressive behavior from these species (MacGown et al. 2007). This unique interaction is thought to be the result of chemical protection, but may also be related to their small size (MacGown et al. 2007). The agonistic interactions of B. patagonicus and co-occurring invasive ants has not been formally investigated.

The diet of B. patagonicus is thought to consist mostly of honeydew from root-feeding hemipterans (Dash 2004). In their invasive range they often are observed foraging on the trunks of hardwood trees, ascending into the canopy where they have been reported to collect honeydew secretions from foliage-feeding hemipterans. In Florida, B. patagonicus has been observed tending the economically important citrus pest, the Asian citrus psyllid, Diaphorina citri Kuwayama (Navarette et al. 2013). Food preference changes with the seasons, with sugary carbohydrates being preferred in the winter and spring, and proteins being preferred in the fall and summer (Keefer 2016). Dark rover ants have been observed moving colonies closer to food sources and also will establish new satellite nests, with brood, near food sources instead of bringing resources back to an existing colony (Keefer 2016) (Miguelena and Baker 2014). This behavior can make control with baits more difficult.

Dark rover ants are often observed foraging in the same areas as other invasive or ecologically dominant ant species (MacGown et al. 2007). Common co-occurring species
include *Solenopsis invicta* Buren, *Solenopsis richteri* Forel, *Linepithema humile* (Mayr), and *Nylanderia fulva* (Mayr), however *N. fulva* is not present in South Carolina at the time of this writing (MacGown et al. 2007). Currently, the ecological impact of *B. patagonicus* in its invasive range is poorly understood, but a lack of interspecific aggression could increase its impact. Because *S. invicta* and other species are generally hostile to most co-occurring species, a lack of displayed aggression towards *B. patagonicus* could give this species a unique type of protection from competing species. This also may allow *B. patagonicus* to more spread more quickly in its invasive range by increasing the likelihood of successful colonization in areas where other invasive ants are already present. There is also some evidence that this behavior may result in *B. patagonicus* emerging as a secondary pest in areas where *S. invicta* has recently been suppressed (Dash 2004). Increases in dark rover ant populations after red imported fire ant suppression could be explained by dark rover ants exploiting new resources that they would not normally attempt to compete for when red-imported fire ants were present. More research is needed to better understand how this unique lack of aggression between invasive species may contribute to the pest status of this ant and the increase of its invasive range.

This experiment had two main objectives: 1) to investigate whether or not *B. patagonicus* will be met with the same lack of aggression from co-occurring ants in laboratory trials that is observed in the field, and 2) to quantify the level of aggression *B. patagonicus* receives from co-occurring ant species relative to other interactions between invasive ant species. I hypothesized that *B. patagonicus* will be met with the same lack
of aggression from co-occurring ant species in the laboratory that is observed in the field, and that the level aggression between *B. patagonicus* and co-occurring ant species will be statistically significantly lower than that of interactions between other species.

**Materials and Methods**

Three species of invasive or ecologically dominant ants in South Carolina were selected to evaluate their agonistic interactions with *B. patagonicus* in an experimental setting to better understand how these interactions may affect their spread and pest status. The Argentine ant, *L. humile*, and the red imported fire ant, *S. invicta* were included because these species are the two most commonly observed invasive pest ants to co-occur with *B. patagonicus*. The pyramid ant, *D. bureni*, which is native to the United States, was also included because of its ecological dominance, frequency of co-occurrence with *B. patagonicus*, and because it is often observed exhibiting aggression towards *S. invicta*, making this interaction a good candidate for a control for aggressive interactions (MacGown 2014).

Six unique combinations or pairings of ants were selected for observation and their interactions were scored according to a previously established agonism index (Suarez et al. 1999). Dark rover ants were paired against Argentine ants, red-imported fire ants, and pyramid ants because these interactions have been observed in the field with no aggressive interaction. Dark rover ants were also paired against dark rover ants of different colonies to investigate if dark rover ants receive more or less aggression from their own species than they do from other co-occurring ecologically dominant ants.
Five colonies of each ant species were identified on Clemson University Main Campus and the Clemson University Cherry Farm Insectary using the identification keys of MacGown (2014), and their GPS coordinates were logged. A labeled flag was placed at the location of each to insure that the same colonies were used throughout the study. Participating colonies were separated by sufficient distance to insure that there was no overlap and the colonies were indeed different. The distance between colonies was different depending on each species’ nesting behavior. Argentine ants and red imported fire ant colonies were separated by at 100 meters. Dark rover ant colonies are comparatively small and were considered to be different if they occurred at the base of different trees. Pyramid ant colonies were considered to be different if there was a separate entrance hole, since D. bureni will nest in close proximity to one another. Red imported fire ant nests were not investigated to see if they were monogynous or polygynous.

**Study Design.** On the day of the study, five worker ants from each participating colony were collected via aspirator and were stored in an aspirator vial for a period of one hour, allowing them to acclimate. After one hour, two vials containing five ants each were placed into a large Petri dish with a lid serving as the experimental arena. One vial containing five workers had the lid removed so that the ants could walk around freely inside of the Petri dish. The other vial did not have its lid removed so that the ants inside were still confined to the vial (figure 4.1). The five representative worker ants of each
colony were then allowed to acclimate for an additional period of one minute.

![Diagram for experimental arena used in agonistic trials. One sealed vial, and one open vial containing 5 worker ants were placed into the arena and were allowed to acclimate for one minute. At the end of a one minute acclimation period, the second vial was opened allowing ants to explore freely and interact.](image)

**Figure 4.1.** Diagram for experimental arena used in agonistic trials. One sealed vial, and one open vial containing 5 worker ants were placed into the arena and were allowed to acclimate for one minute. At the end of a one minute acclimation period, the second vial was opened allowing ants to explore freely and interact.

At the end of the one minute acclimation period, the lid of the second vial containing ants was removed and the ants were allowed to explore the experimental arena and interact with one another. Their activity and interactions were observed for a period of five minutes. During this time, interactions between colonies were scored according to an established agonism index, and the highest level of aggression observed was recorded (Suarez et al. 1999). The scale assigned numerical values to specific behaviors observed.
between ants ranging from a zero (0) to a four (4), with a four being the highest possible value. The definition of each value and its explanation are as follows:

**Ignore (0)** - a value of zero is defined by an apparent ignorance of one another between two ant colonies. In this circumstance, the two ant colonies never interact with one another at all and do not seem to either notice one another. This interaction is not considered aggressive or agonistic.

**Some touching (1)** - a value of one is characterized by some interaction between two colonies that results in no aggressive behavior. Unlike before, the two ant colonies do notice one another and approach one another, but the interactions are neutral and do not result in aggressive behavior, with the ants seemingly not disturbed by one another’s presence. This is usually observed in the form of antennulation. This interaction is not considered aggressive or agonistic.

**Active avoidance (2)** - a value of two is defined by an apparent attempt between two ants to remove themselves from one another. In this situation, two ants notice one another and approach one another, but after initial contact the two ants quickly separate in such a way that it is apparently intentional. This behavior is not considered to be aggressive or agonistic.

**Some aggression (3)** - a value of three is defined by some display of aggression between two ants. In this situation two ants notice and approach one another, but after initial contact, the ants display some sort of aggressive behavior towards one another that does not result in direct conflict. This is usually characterized by defensive posture such
as open mandibles, presenting of the stinger, an aggressive charge forward without contact. This behavior is considered aggressive or agonistic.

Direct fighting (4) - a value of four is defined by direct aggressive contact between two ants. In this situation two ants notice and approach one another, but after initial contact the two ants engage in direct physical conflict. This value is usually characterized by biting or stinging, and may result in the death of one or both ants involved. This behavior is considered aggressive or agonistic and is the highest agonistic value which can be achieved.

Each combination or pairing of species was repeated five times, using five different colonies such that the interactions of two specific colonies were never observed more than once. For example, the interactions of Argentine ants and red-imported fire ants were observed a total of five times, but each time this pairing was repeated, a unique combination of Argentine ants and red imported fire ants was used. Ants used in this study were killed by being placed into a vial containing 80% ethanol and were vouchered in the Clemson University Arthropod Collection, Clemson, South Carolina.

Methodology for Statistical Analysis. The study consisted of a treatment design based on the combinations of ant species and a completely randomized experiment design with the five agonistic trials serving as the replications. The analyses were completed in two steps. First a graphical approach was used to determine differences in mean aggression scores for different combinations of ants. Second a one-way ANOVA was performed to determine significant differences between mean aggression scores for different combinations of ant species. A Fisher’s protected least significant differences test was
performed to determine which species combinations had means that were significantly different from one another.

**Results**

The mean score for the highest level of aggression observed for each interaction is reported in Figure 4.2. All four combinations of ants including *B. patagonicus* had a lower mean score on the agonism scale than those which did not include *B. patagonicus*. Argentine ants versus dark rover ants was the combination with the lowest mean score on the agonism scale, with all five treatments scoring a zero, meaning that Argentine ants and dark rover ants never gave any indication that they noticed one another. The next highest scored interaction was dark rover ants versus red imported fire ants, with a mean score of 0.06 on the agonism scale. In this instance, 4 of the 5 treatments of this pairing scored a 0, while a single pairing scored a 3, which is considered an aggressive interaction on the scale. The next highest scored interaction was dark rover ants versus pyramid ants, with a mean score of 0.8 on the agonism scale. Three of the five treatments scored a 0, one scored a 1, which is not considered an aggressive interaction, and one scored a 3, which is considered an aggressive interaction on the agonism scale. The next highest scored interaction was dark rover ants versus non-nestmate dark rover ants, with a mean score of 1.8. One of the five treatments scored a 1, and the remaining four scored a 2. Neither of the two values are considered aggressive on the agonism scale. Although the mean is higher for this combination, the values differ from the previous two in that no aggressive interactions were observed in any treatment.
The remaining two combinations did not include *B. patagonicus*. Of these two combinations, red imported fire ants versus pyramid ants was the lowest with a mean score of 2.2. In this combination four out of the five treatments scored a 2, which is not considered aggressive, and one treatment scored a 3, which is considered an aggressive interaction. The highest scored combination was red imported fire ants versus Argentine ants, which had a mean score of 3. One treatment scored a 2, which is not considered aggressive, three treatments scored a 3, and one scored a 4, which are both considered aggressive interactions, and a score of 4 is the highest possible score possible on the agonism scale.
Figure 4.2. Mean highest agonistic value observed for each combination of ants. The mean observed aggression score for each combination of ants including Brachymyrmex patagonicus (B.p.), Solenopsis invicta (S.i.), Linepithema humile (L.h.), and Pyramid ants (D.b.) was calculated from five trials. The highest and lowest agonistic values are also reported. The data were analyzed using a one-way ANOVA. It was found that some mean aggression scores were significantly different (F=8.62, p=<.0001).

A one way analysis of variance indicated some mean scores for combinations were significantly different from one another (F=8.62, p=<.0001). In addition, a Fisher’s least protected significant differences test determined the three combinations of ants which included dark rover ants versus a different species (L. humile, S. invicta, and D. bureni) were all significantly different from the two combinations which did not contain dark rover ants. This supports the hypothesis that dark rover ants face a lower level of aggression from co-occurring ants than other species. The two combinations which did not include dark rover ants were found to be statistically similar, and the three
combinations which included *B. patagonicus* versus different ant species were also found to be statistically similar to one another. This indicates that levels of aggression are statistically significantly different when dark rover ants are included in a combination, than when they are not. The combination which consisted of *B. patagonicus* versus non-nestmate *B. patagonicus* was found to be statistically similar to the combination containing *S. invicta* versus *D. bureni*. This may indicate that dark rover ants face levels of aggression from other *B. patagonicus* that are more typical than they do from different species.

The means of individual combinations of ants were compared against combinations which shared one species of ant in common to further investigate whether or not *B. patagonicus* face different levels of aggression from co-occurring ant species, than they do. The mean aggression scores for the interaction of *S. invicta* versus *L. humile* was found to be significantly different from the mean aggression score for the interactions of *L. humile* versus *B. patagonicus* (p=<.0001), and from *S. invicta* versus *B. patagonicus* (p=.0002). The mean aggression score for the interaction of *S. invicta* versus *D. bureni* was also found to be significantly different than the mean aggression score for the interaction of *S. invicta* versus *B. patagonicus* (p=.0069), and *D. bureni* versus *B. patagonicus* (p=.0162). These findings further provide evidence that support the hypothesis that dark rover ants face lower levels of aggression from co-occurring ant species than those co-occurring ant species face from one another, and that dark rover ants face higher levels of aggression from non-nestmates of the same species than they do from co-occurring species.
Discussion

The mean score of aggression for every combination of ants that included dark rover ants versus another ecologically dominant species was significantly lower than any combination that did not include dark rover ants. These data support reports from the field that dark rover ants are not met with aggression by some co-occurring invasive and ecologically dominant ant species. However, it was previously not known whether or not this unique interaction would still be observed in a laboratory setting where the ants were forced into a smaller area, which is foreign to them, after being removed from their environment. The fact that this interaction was observable in a laboratory setting indicates that the interaction is the result of some other factor that does not appear to be related to available space.

There is still some evidence that in rare circumstances dark rover ants may face aggression from co-occurring species. In the combinations, dark rover ants versus red imported fire ants and dark rover ants versus pyramid ants, there was a single trial in each that scored a 3 on the agonism scale, which is considered an aggressive interaction. Additionally, during the pre-trial testing phase there was a single instance of a dark rover ant attacking a red imported fire ant which resulted in the death of the dark rover ant. This indicates that there are situations in which whatever mechanism causes lack of aggression can fail and aggression can occur. Before beginning this study I had previously hypothesized that competition over available resources, such as food, may cause this interaction to break down. However, during field trials of different studies, I have observed dark rover ants and red imported fire ants both recruit to a cookie bait, and
both Argentine ants and dark rover ants recruit to a gel bait, with no aggressive interactions observed in either cases. These observations would seem to indicate that available resources are not a significant factor in their interactions.

It was also noted that the mean score of aggression for the combination of dark rover ants versus non-nestmate dark rover ants was higher than the combinations including dark rover ants versus other species. This is likely due to the nature of the scale used and the expected interactions of ants of the same species. Every trial including dark rover ants versus Argentine ants scored a 0 on the agonism scale, while most interactions in the trials including dark rover ants versus non-nestmate dark rover ants scored a 2. It should be expected that two ants of the same species would have to interact at some level in order to establish whether or not they are nestmates. It would not be accurate to say that dark rover ants face a higher level of aggression from non-nestmate dark rover ants than they do from Argentine ants, because no interaction was observed that would actually be considered an aggressive interaction in either of these combinations. It should be expected that ants of the same species interact more than ants of different species. Additionally, it was found that the combination containing dark rover ants versus non-nestmate dark rover ants was not significantly different from the combination containing red imported fire ants versus pyramid ants. This would seem to indicate that dark rover ants face similar levels of aggression from non-nestmate dark rover ants than other species may exhibit towards one another. However there were no instances of an interaction between dark rover ants that could be considered aggressive (3 or higher), but there were instances of this between red imported fire ants and pyramid ants. Because the
mean scores of aggression are being compared, it is possible for one combination of ants to contain interactions which are considered aggressive, and another combination not have any values considered aggressive, while the two combinations have similar means. However, these results do show that all combinations including dark rover ants versus another species, were lower and significantly different from all combinations which did not include dark rover ants, which supports my hypothesis that dark rover ants are met with lower levels of aggression from co-occurring ants than other species.

The results of this study indicate that dark rover ants are detected by other species because they interact, but this does mean that size is not still a factor. Further research could determine whether or not size is important. This study could be repeated with the inclusion of another very small ant species such as the little black ant, *Monomorium minimum* (Buckley), and some very large species of ants. This would allow researchers to determine if another very small species of ants faced similar level of aggression from larger ants, and to see if aggression levels between ants follow a trend where the greater the size differential between them, the lower the levels of aggression observed.

While the mechanism remains unknown, dark rover ants are facing lower levels of aggression in the field than other ants. The dark rover ant is an invasive species which has been expanding its range, especially in the southeastern United States, and this lack of aggression may be responsible in aiding its spread. A small colony size and lack of aggression may provide them with the opportunity needed to spread quickly. Other invasive ant species such as the red imported fire ant and the Argentine ant are known to be both very aggressive and territorial. If dark rover ants could establish themselves
inside of the protected territory of one of these species, but not face aggression from
them, then this would offer their colonies protection as they established themselves. This
would also mean that the only real factor that would affect their growth would be their
ability to gather resources. It has already been established that dark rover ants do not
thrive in urban environments because of their ability to outcompete other species in
resource allocation, but because of physiological adaptations that suit them to these
environments (Calcaterra et al. 2016). This indicates that competition for resources also
would likely not limit their ability to spread, making the protected territories of other
species an even more favorable place to establish new colonies. If two species are not
actively physically fighting one another, and are not competing for resources, then the
two species can exist in the same available space, helping to increase the dark rover ant’s
invasive range. This could also potentially account for anecdotal reports from pest control
operators in the southeastern United States of dark rover ants becoming a secondary pest
after the suppression of red imported fire ants.

References Cited

Calcaterra, L., S. Cabrera, and J. Briano. 2016. Local co-occurrence of several
highly invasive ants in their native range: are they all ecologically dominant

Dash, S. T. 2004. "Species diversity and biogeography of ants (Hymenoptera:
Formicidae) in Louisiana with notes on their ecology". LSU Master's Theses.


The dark rover ant, *Brachymyrmex patagonicus* Mayr, is a small (~0.5 mm) dark brown or black formicine ant that is invasive from Argentine and Paraguay. It was first discovered in the United States in St. Tammany Parish, Louisiana in 1978 (Wheeler and Wheeler 1978). It has been expanding its invasive range since then and can now be found in 13 states, with the most recent discoveries being Tennessee and North Carolina (Guénard et al. 2012) (Hill 2017). The dark rover ant is considered a nuisance pest where it occasionally enters homes, but is difficult to treat when it does become a problem (MacGown et al. 2007). Much of the dark rover ant’s biology, and behavior in South Carolina remain unknown.

The first study in this research had two main objectives: 1) to survey all 46 counties of South Carolina in order to determine the invasive range of the dark rover ant in the state and 2) to use this information to develop a distribution map for *B. patagonicus* which could be used to help pest control operators determine if infestations by *B. patagonicus* could be expected in their area. Previously, there was a single record of the dark rover ant in South Carolina from Horry County (MacGown et al. 2010). Because dark rover ants were being observed personally in Pickens County, which lies on the opposite side of the state, and because pest control operators had inquired about “mystery ants” which I positively identified as *B. patagonicus*, it was apparent that the actual invasive range for this species in South Carolina was unknown, but broader than Horry County.
Each county was sampled individually. Specific target sites were chosen within each county that were likely to contain invasive ant species, including dark rover ants, because they contained a high volume of traffic of people from many different areas. Examples of target sites chosen included hospitals, parks, schools and universities, government buildings, outlet malls, and tourist attractions.

Dark rover ants were found to be present in all 46 counties of South Carolina, and in most instances, were not difficult to locate. These findings indicate that dark rover ants have most likely been established in South Carolina for some time and have already colonized most of the state. A map was developed which showed that *B. patagonicus* inhabited all 46 counties of the state, and also included the first state record for this species. The remaining 45 counties also represent 45 new county records for this species in South Carolina. This information should prove useful to pest control operators to become more aware of the presence of this previously overlooked species in the state.

The second study in this research had three main objectives: 1) to determine if there is an increase in foraging activity by *B. patagonicus* after suppression of *S. invicta*, 2) to determine whether or not *B. patagonicus* will become the dominant forager on baits after the suppression of *S. invicta*, and 3) to establish a list of common co-occurring ant fauna with *B. patagonicus*, both native and invasive. There have been reports from pest control operators in the southeastern United States that dark rover ants become a secondary pest in accounts after the suppression of the red imported fire ant, *Solenopsis invicta* Buren.
Ten trees were selected on Clemson University Main Campus in two distinct areas (5 trees each). One area was selected to receive treatment to suppress *S. invicta* and the other was left untreated to serve as a control. Each tree was sampled once using pitfall traps, baits, and tree scans, and then was sampled three more times after *S. invicta* was suppressed in the treated area. The results from pitfall traps indicated that there was not a significant difference in the mean number dark rover ants found in pitfall traps before and after suppression of *S. invicta* in the treated area. There also was not a significant difference in the number of dark rover ants found in pitfall traps between the treated and untreated areas. Furthermore, there was no linear relationship between the amount of dark rover ants and red imported fire ants in pitfalls. These results contradict reports from pest control operators in the southeast.

Previously, I hypothesized that *B. patagonicus* could become the dominant forager after the suppression of *S. invicta*, which would account for reports from pest control operators. However, results from bait samples found no evidence that *B. patagonicus* would become the dominant forager after the suppression of *S. invicta*. *Brachymyrmex patagonicus* was not found to be more likely to respond to a bait when *S. invicta* had been suppressed, and there was no significant difference in the number of responding *B. patagonicus* workers to a bait when *S. invicta* was present than when they had been suppressed. There were however a few instances of dark rover ants recruiting to a bait at the same time as *S. invicta* with no apparent signs of aggression between the two, which may suggest that dark rover ants exploit a variety of food sources in part, instead of dominating a single food source. Results from tree scans found that the most
common species of co-occurring ants with *B. patagonicus* on trees are the red imported fire ant (*S. invicta*), the Argentine ant (*Linepithema humile* (Mayr)), the black carpenter ant (*Camponotus pennsylvanicus* (De Geer)), and Field ants (*Formica* sp.).

These results contradict reports from pest control operators in the southeast. However, this could be the result of an error in methodology. Although no increase in dark rover ant activity was detected by pitfalls, an increase was noticed personally after *S. invicta* suppression. However, their activity was heavier on root systems of trees, and their foraging behavior is uneven on the ground. This combined with the fact that dark rover ants are excellent climbers may mean that they are less likely to fall into a pitfall trap if they come into contact with one. Future research may choose to sample *B. patagonicus* in a different way to further investigate these claims. The results from bait sampling are consistent with personal observations of dark rover ants foraging on food sources elsewhere. Often, there are very few dark rover ants on a food source, which makes it seem doubtful that they would dominate a bait.

The third study in this research had two main objectives: 1) to investigate whether or not *B. patagonicus* will be met with the same lack of aggression from co-occurring ants that is observed in the field, and 2) to quantify the levels of aggression *B. patagonicus* receives from co-occurring ant species relative to other interactions between invasive ant species. It has been widely reported *B. patagonicus* will co-occur and co-forage with several ecologically dominant, aggressive, and invasive species (MacGown et al. 2007). However, *B. patagonicus* is not met with aggression by these usually aggressive ants in the field.
The dark rover ant and three other ecologically dominant or invasive species (Linepithema humile, Solenopsis invicta, and Dorymyrmex bureni (Trager)) were chosen to investigate in this study, specifically because they often co-occur with B. patagonicus. These species were paired against each other in an experimental arena and their interactions were observed and scored according to a previously established agonism scale (Suarez et al. 1999). Dark rover ants were paired against each of the other species, as well as against non-nestmate dark rover ants. Argentine ants and pyramid ants were also paired against red imported fire ants to serve as a positive control for aggression since these species are often observed fighting in the field.

I found that all combinations of dark rover ants versus another ecologically dominant species had a mean lower aggression score than those combinations which did not include dark rover ants, and that the difference between them was statistically significant. I also found that there was no significant difference between the mean aggression scores for the combinations that included dark rover ants versus other species, which indicates that dark rover ants do experience lower levels of aggression from ecologically dominant species, than other ants do, and that this interaction is the same in the field as it is in a laboratory setting. The results also showed that the combination including dark rover ants versus non-nestmate dark rover ants had a higher mean aggression score than any combination of ants including dark rover ants versus ecologically dominant species. This would seem to suggest that dark rover ants face higher levels of aggression from their own species than they do from other species. However, I believe this is inaccurate. The agonism scale used rates active avoidance as a
more aggressive interaction than both light interaction and complete ignorance of one another. This is not a fault in logic of the scale, but likely rather an indication that the scale is not best used between ants of the same species. It would be expected that ants of the same species would need to interact to identify one another as either nest mates or non-nestmates, while ants of different species may completely ignore one another if it is obvious to them that they are different.

There have been two main hypotheses as to why this interaction occurs. One is that size is a factor and the other is that it has to do with chemical protection (MacGown et al. 2007). While this research did not seek to identify the cause of this interaction, future research should focus on this because it could better help to understand how *B. patagonicus* is potentially using other species to expand its invasive range. This study should be repeated with the inclusion of another very small species of ant, as well as some other large species of ants to better understand how and if size plays a role in this interaction.

This research has helped to better understand the distribution and behavior of the dark rover ant in South Carolina. However, much of the dark rover ant’s behavior and biology still remains unknown. This species has only increased its invasive range since it has arrived, and the timeline of state records in South Carolina illustrate how this species can easily fly under the radar. There were nearly 8 years between the first record for this ant in the state and the remaining 45 counties. Better monitoring is needed to more quickly pick up on future invaders as they arrive, especially with invasive species which have a more destructive potential, such as *Nylanderia fulva* (Mayr). On multiple
occasions, I have had pest control operators tell me that they were not aware of this ant in
the state, but had seen it on accounts before and only came to realize what it was after
speaking with me. This research will provide much needed awareness for this species in
the state, as well as stress the importance of identifying pests before treatment.

References Cited


APPENDICES
Appendix A

SUPPORTING DATA FOR CHAPTER 2

Table A-1. Collection information for each county record of *Brachymyrmex patagonicus* Mayr in South Carolina, including location, GPS coordinates, date, microhabitat type, and co-occurring ant species.

<table>
<thead>
<tr>
<th>County</th>
<th>Location</th>
<th>GPS</th>
<th>Date</th>
<th>Microhabitat</th>
<th>Co-occurring Ant Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbeville</td>
<td>Calhoun Falls State Park</td>
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<td>Hardwood Tree</td>
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</tr>
<tr>
<td>Aiken</td>
<td>Aiken Co. Chamber of Commerce</td>
<td>33.3336N, -81.4308W</td>
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<td>Parking Lot Sidewalk</td>
<td><em>S. invicta</em></td>
</tr>
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<td>Allendale</td>
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<td>Crate Myrtle Tree</td>
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<td>Starlight Cinemas</td>
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<td><em>S. invicta</em></td>
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<td>Exxon Gas Station</td>
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<td>8.5.17</td>
<td>Hardwood Tree</td>
<td><em>S. invicta</em></td>
</tr>
<tr>
<td>Beaufort</td>
<td>Old Sheldon Church</td>
<td>32.3707N, -80.4650W</td>
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<td>Coordinates</td>
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<td>Type</td>
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<td>------------------------------</td>
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<td>Sandy Patch</td>
<td>D. bureni</td>
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Appendix B

SUPPORTING DATA FOR CHAPTER 3

Table B-1. The number of *B. patagonicus* Mayr and *S. invicta* Buren collected in each pitfall trap across four sampling dates.

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Figure B-1. Satellite image of Clemson University from Google Earth® showing both the treated and untreated areas and the distance between them.