BAIT ACCEPTANCE AND SEASONAL ACTIVITY OF THE ASIAN NEEDLE ANT, *BRACHYPONERA* (=PACHYCONDYLA) *CHINENSIS* (EMERY), AN EMERGING MEDICALLY IMPORTANT SPECIES, IN CENTRAL GEORGIA

by

KAREN ANN CORSETTI

(Under the Direction of Daniel R. Suiter)

ABSTRACT

The Asian needle ant (Hymenoptera: Formicidae) has become a well-established invasive species in natural habitats and suburban environments. The objectives of this thesis was to investigate bait acceptance, foraging activity, seasonal activity, and food preference in the field and laboratory. Five hundred ninety-five bait acceptance trials were conducted in the field from August 2020 through September 2022 by randomly placing one granular bait where a worker would eventually encounter the granule. This research indicates bait acceptance is greatest from July to September, and Advance® bait (abamectin), Advion® fire ant bait, and Advion® granular bait (indoxacarb) were preferred over Niban® bait (boric acid). In field efficacy trials, indoxacarb resulted in the greatest decrease in foraging activity, followed by abamectin. Seasonal activity trials using pitfall traps indicated increased foraging activity from June to August and maintained a high level through September, with July having the highest foraging activity. A one-year macronutrient choice assay field study evaluated food preferences for four protein choices: sardine, anchovy, egg yolk, and tuna/sucrose. Results indicated that sardine was preferred over the other choices.

INDEX WORDS: *Brachyponera chinensis* foraging activity, integrated pest management, seasonal activity, bait preference, food preference

BAIT ACCEPTANCE AND SEASONAL ACTIVITY OF THE ASIAN NEEDLE ANT, *BRACHYPONERA* (=PACHYCONDYLA) *CHINENSIS* (EMERY), AN EMERGING MEDICALLY IMPORTANT SPECIES, IN CENTRAL GEORGIA

by

KAREN ANN CORSETTI

M.S., Georgia Southern University 1992

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2024

BAIT ACCEPTANCE AND SEASONAL ACTIVITY OF THE ASIAN NEEDLE ANT,

BRACHYPONERA (=PACHYCONDYLA) *CHINENSIS* (EMERY), AN EMERGING

MEDICALLY IMPORTANT SPECIES, IN CENTRAL GEORGIA

by

KAREN ANN CORSETTI

Major Professor:	Daniel R. Suiter
Committee:	David G. Buntin
	Shimat V. Joseph

Electronic Version Approved:

Ron Walcott
Vice Provost for Graduate Education and Dean of the Graduate School
The University of Georgia
December 2024

DEDICATION

This is in loving memory of my parents, Louis and Marie Corsetti, and my godparents, Gloria and Patrick Iafrate.

ACKNOWLEDGEMENTS

Most people start graduate school to earn a degree; however, my intentions were quite different. I enrolled in graduate school to take a few courses that would enhance my military career, with the plan to leave afterward. Before I departed, I wanted to take a course in Taxonomy. This course significantly altered what I had expected to be a brief journey through graduate school. Therefore, my journey continued for the next three years.

There is an old African proverb that states, “It takes a village to raise a child”. This statement should include graduate students because I could not have achieved my goals without the assistance and support of many people. I thank my advisor, Dr. Dan Suiter, for his patience, mentorship, and countless hours of guidance throughout my research. I would also like to thank my committee members, Dr. David Buntin and Dr. Shimat Joseph, for their assistance, and thank you to Dr. Conor Fair for the statistical expertise he brought to my thesis. I also want to thank Dr. Brian Forschler for the opportunity to present my research work at pest control conferences. I also want to thank my fire department for listening to my presentations about ants, assisting me in collecting insects, and allowing me to set up my insect-collecting lab on our kitchen table. Special thanks to Anthony Rutledge, John Bruschetti, Josh Teal, Brian Glennie, Benjamin Hsieh, and John Nevil for their support and encouragement.

I want to express my sincere thanks to my dear friend Kathleen Schmidt for her help and suggestions regarding my research. I would also like to thank Tristan Davis for his dedication and assistance. It is not easy to find someone willing to spend hours in the field baiting Asian needle ants, deploying pitfall traps, and reviewing research references.

Finally, I express my deepest gratitude to my family for their support and understanding, especially to my mom, who would spend countless hours watching me work on my thesis during the few times I could travel home.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND REVIEW OF THE SCIENTIFIC LITERATURE OF THE ASIAN NEEDLE ANT, AN EMERGING MEDICALLY IMPORTANT SPECIES IN CENTRAL GEORGIA

Introduction

Understanding the target pest's life cycle, behavior, foraging, and seasonal activity is essential for developing an effective pest management program. Limited research is available on the Asian needle ant, *Brachyponera chinensis* (Emery), to develop an effective management program. This ant poses challenges that include cryptic nest sites; it is a protein feeder rather than a carbohydrate feeder, which limits insecticide bait products; and it is a stinging ant, making it a threat to people. This research presents the results for finding an acceptable bait, evaluation for the efficacy of four granular baits, documentation of seasonal foraging behavior, and food preference trials. This thesis is divided into a literature review of the history of invasive ants and the life history of *B. chinensis*, a review of management programs, and the remaining four research chapters.

Literature Review

History of invasive ants

Ants are one of the most dominant arthropods in most ecosystems. They reshape ecosystems by redirecting energy flow, turning and aerating soil, and dispersing seeds; they have a significant role in food webs (Plentovich et al. 2007; Vogt et al. 2022). They maintain ecosystem cleanliness by aiding in the destruction and decomposition of plant and animal matter (Hölldobler and Wilson 1990; Plentovich et al. 2007; Vogt et al. 2022). The destructive potential of invasive ants, capable of causing extensive and irreversible damage to naive environments, presents a pressing concern for ecological balance. Invasive ants prey upon native ant taxa and other native invertebrate fauna (Hölldobler and Wilson 1990; Holway et al. 2002; Plentovich et al. 2007; Rodriguez-Cabal et al. 2011; Vogt et al. 2022). The ecological impacts of invasive ants change ecosystem processes that affect the existence of native insects, fauna, and flora and may enhance the populations of plant pests such as aphids and scale insects (Siddiqui et al. 2021). Non-native species invasion may also disrupt members of other trophic levels (Rodriguez-Cabal et al. 2011; Merchlinsky et al. 2023), such as mammals, birds, and reptiles that feed on native insect species (Holway et al. 2002).

A classic example of a trophic level cascade occurred on Christmas Island in Australia. The yellow crazy ant, *Anoplolepis gracilipes* (F.Smith), known as the most destructive ant species in the world, formed supercolonies that covered over 30% of the 10,000 ha of rainforest (Abbott 2006; GISD 2021). The ants rapidly consumed a keystone species, the endemic red land crab, *Gecarcoidea natalis* (Pocock), as they ventured into supercolonies, which resulted in a trophic cascade within the island's ecosystem (O'Dowd et al. 1999; Abbott 2004; Thomas et al. 2010; Lee and Lang 2022).

Gecarcoidea natalis forage on leaf litter that covers the forest floor (O'Dowd et al. 1999; Thomas et al. 2010; Baumgartner and Ryan 2020). When the crabs migrate through ant-infested areas, the ants spray formic acid that overwhelms the crabs. The formic acid blinds the crabs; they will die of dehydration within 48 h of exposure (Lee and Lang 2022; Abbot 2004; Baumgartner and Ryan 2020). The ants take over the crab burrows as nesting sites (O'Dowd et al. 1999; Lee and Lang 2022). *Gecarcoidea natalis* is the primary seed, seedling, and litter consumer in the rainforest of Christmas Island (Lee and Lang 2022).

The crab's removal, or reduction in numbers, caused by *A. gracilipes* resulted in alterations in the rates of seedling recruitment and litter breakdown that can affect the nutrient availability of the ecosystem (O'Dowd et al. 1999; Abbot 2004).

The reduction in the population allowed the crab's prey, the invasive giant African land snail, *Achatina fulica*, to increase (Emery et al. 2020; Lee and Lang 2022; Abbott 2004). *Achatina fulica* is an intermediate host of rat lungworm, *Angiostrongylus cantonensis*, which causes eosinophilic meningitis in humans through handling and eating, so increased snail numbers could result in increased risk for humans (Cowie 2013; Lee and Lang 2022). When crab populations decline, increased plant growth increases forest floor litter accumulation (Baumgartner and Ryan 2020; Lee and Lang 2022). *Anoplolepis gracilipes* population increase is also responsible for the decline and nesting failure of three endemic bird species: the ground-foraging emerald dove, *Chalcophaps indica* (Linnaeus), the island thrush, *Turdus poliocephalus erythropleurus* (Sharpe); and the white tern, *Gygis alba* (Sparrman) (Feare 1999; Abbot 2006).

Anoplolepis gracilipes also devastated the population and habitat of three native lizards, resulting in the extinction of the Christmas Island forest skink, *Emoia nativitatis* (Boulenger), and the blue-tailed skink, *Cryptoblepharus egeriae* (Boulenger), which became extinct from the wild (Emery et al. 2020; Mitchell and Woinarski 2022). Parks Australia and Taronga Zoo started a captive breeding program that prevented the total extinction of the blue-tailed skink (Emery et al. 2022). This ant is also responsible for the severe decimation of the Christmas Island giant gecko, *Cyrtodactylus sadlieri* (Wells and Wellington) (Emery et al. 2020; Mitchell and Woinarski 2022).

Anoplolepis gracilipes also significantly reduced the biodiversity on Bird Island, Seychelles, off the coast of East Africa (Gerlach 2004). They spread into the island's colony of nesting sooty terns, *Sterna fuscata* (Linnaeus). Ants swarmed over the ground, covering and killing the chicks (Feare 1999; Gerlach 2004).

In the agricultural environment, the little fire ant, *Wasmannia auropunctata* (Roger), causes extensive damage to people, plants, and crops. They exhibit a mutualistic interaction with honeydew-producing Hemiptera, such as aphids, scales, and mealybugs, leading to decreased crop yields (Fasi et al. 2012). In the 1970s, *W. auropunctata* was introduced to the Solomon Islands as a biological control agent of the nut-fall bug, *Amblypelta* sp. in coconut and cocoa (Wetterer 2003; Fasi et al. 2012). However, this tactic became disastrous because of the population increase in sapsucking plant pests and the threat to human health as a stinging pest (Fasi et al. 2012).

The ecology of invasive and native ants. Native ants are very effective in the distribution of seeds in their habitat. Seed dispersal by ants, or myrmecochory, is a highly evolved mutualism between ants and plants (Holway et al. 2002). Ants in the genus *Aphaenogaster* are the keystone seed-dispersing ants in their habitat (Warren and McMillan 2015; Merchlinsky et al. 2023). They have a mutualistic relationship with myrmecochorous plants such as white and red trilliums, bloodroot, and bleeding hearts (Clark and King 2012). Worker ants disperse myrmecochorous plant seeds after they consume the elaiosomes, a white food structure attached to the seed rich in lipids, amino acids, and other nutrients (Zettler et al. 2001; Holway et al. 2002; Cumberland and Kirkman 2013). Native ants take seeds into their tunnel to eat the nutritious elaiosomes (Holway et al. 2002; Clark and King 2012). The workers may disperse and deposit the seeds into nutrient-rich trash piles either on the surface of or below the ground, where they germinate (Cumberland and Kirkman 2013).

Native ants are negatively affected by invasive ants. Invasive ants are highly aggressive toward native ant species; they dominate habitats and nesting sites, compete for food resources, disrupt foraging activity (Siddiqui et al. 2021; Merchlinsky et al. 2023), and use native and exotic species as food resources (Holway et al. 2002). When an invasive ant is established in a habitat, they are likely to cause economic and environmental threats to humans, suburban and undisturbed forested habitats, and other insect species and ecosystems (Gentili et al. 2021; Siddiqui et al. 2021; Merchlinsky et al. 2023). Newly established populations of invasive ants may remain undetected for years (MacGown 2009). Once an invasive species becomes established and its population reaches high densities over a broad range of territories, it becomes difficult to manage (Buczkowski 2016). With early detection and management strategies, growing populations can be detected before accelerating to uncontrollable numbers (Buczkowski 2016). The driving forces of increased trade and commerce have led to the accidental transport of ants worldwide (King and Tschinkel 2008; Malone et al. 2023). Invasive ants can enter a country on imported sea and air cargo, machinery, cargo containers, plants, soil, nursery stock, timber, and other imported commodities (Siddiqui et al. 2021).

Ants are among the most successful and widespread invasive species worldwide (Merchlinsky et al. 2023). The Global Invasive Species Database (GISD 2021) lists five invasive ant species in the following order:

6 – Yellow crazy ant *Anoplolepis gracilipes* (F. Smith)

48 - Argentine ant *Linepithema humile* (Mayr)

68 - Big-headed ant *Pheidole megacephala* (Fabricius)

86 - Red imported fire ant *Solenopsis invicta* (Buren)

100 - Little fire ant *Wasmannia auropunctata* (Roger), (GISD 2023).

History of the Asian needle ant, *Brachyponera chinensis*

The Asian needle ant, *Brachyponera chinensis (*formerly *Pachycondyla chinensis),* is an invasive ant species detected in the U.S. in the 1930s (Smith 1934; Smith 1947; Nelder et al. 2006). They are native to China, Japan, and Korea (Guénard and Silverman 2011) and have been found in other regions of the globe, such as New Zealand, Vietnam, Thailand, Sri Lanka, India, Guam, Papua New Guinea, the Philippines, and Nepal (Nelder et al. 2006; Mo 2013).

Brachyponera chinensis was initially described and named *Euponera solitaria* by F. Smith in 1874. It has undergone five taxonomic changes:

1. *Ponera solitaria* (Smith) 1874; original description of worker, Hiogo, Japan, (Brown 1958)

2. *Ponera nigrita chinensis* (Emery) 1895; description of worker, Shanghai, China; junior synonym (Brown 1958; Bolton 2016)

3. *Euponera* (*Brachyponera) chinensis* (Emery) 1909; generic combination (Bolton 2016)

4. *Brachyponera chinensis* (Brown) 1958; Junior synonym of *B. solitaria* Junior homonym of *B. solitaria* (Bolton 2016)

5. *Pachycondyla chinensis* (Bolton) 1995; genetic combination. (Smith 1934; Paysen 2007; Bolton 2016).

In 2014, Schmidt and Shattuck removed *B. chinensis* from *Pachycondyla*, placing it in the newly revived genus *Brachyponera* (Guénard et al. 2018).

Nelder et al. (2006) referenced Bolton (1995) that described ≈ 200 species in the genus *Pachycondyla* (Formicidae: Ponerinae), predominantly from tropical and subtropical regions of the world (Nelder et al. 2006**;** Wanandy et al. 2021**).** In North America, there are four documented species:

(1) *Pachycondyla stigma* (Fabricius 1804), Florida. *P. stigma* can sting; this species rarely encounters humans and is found in rotten logs. It seems unlikely that *P. stigma* will ever achieve pest status (Wetterer 2012).

(2) *Pachycondyla harpax* (Fabricius 1804), Louisiana, California, and Texas: is one of the few ant species capable of producing a sticky foam. This defense behavior repels attacks from other ants (Mackay and Mackay 2010).

(3) *Pachycondyla villosa* (Fabricius 1804) Texas and New York. This is a very aggressive ant with a painful sting. The sharp pain mostly subsides after thirty minutes but remains tender for a few days. It is the most common and widely distributed member of the genus in the New World (Mackay and Mackay 2010).

(4) *Brachyponera chinensis* (Emery 1895)*,* the Asian needle ant was moved from the genus *Pachycondyla* into the genus *Brachyponera.*

History in the United States (U.S.). Yashiro (2010) conducted a molecular analysis based on mitochondrial sequences of the cytochrome oxidase I (COI) region. COI is the standard marker for DNA barcoding. The analysis showed no difference between *B. chinensis* from the U.S. and those from temperate areas in Japan (Yashiro et al. 2010). Both documentation and phylogenetic analysis suggest that *B. chinensis* entered the U.S. from Japan (Yashiro et al. 2010).

Although the exact time of introduction into the U.S. is unclear, *B. chinensis* was introduced by accident into the southeastern U.S. from Japan sometime before 1932 (Smith 1934), where it remained unnoticed in suburban and forested habitats for several decades (MacGown 2009; Rodriguez-Cabal et al. 2011; Buczkowski 2016). Plant shipments from foreign countries contribute to the accidental introduction of *B.chinensis* into new territories. (Smith 1934). For example, Smith (1934), while reviewing the literature, noted that this species was intercepted during the early 20th century in Hamburg, Germany, in plant (*Prunus* spp.) shipments originating in Japan.

In 1932, H.T. Vanderford collected the first documented specimens from forests near Decatur, Dekalb County, Georgia (Smith 1934; Nelder et al. 2006; Guénard et al. 2018; Merchlinsky et al. 2023). According to Smith (1934), Mr. Vanderford also found *B. chinensis* in other locations: Wilmington, NC; Newbern, NC; Washington, NC; Elizabeth City, NC; Norfolk, VA; Petersburg, VA; and Richmond, VA (Smith 1934).

Since 1932, *B. chinensis* has broadened its range in the southeastern U.S. (Alabama, Georgia, North Carolina, South Carolina, and Virginia) (Guénard and Dunn 2010; Rice and Silverman 2012; Zungoli and Benson 2008). Because *B. chinensis* can tolerate cooler temperatures (Rice 2012), this may give them the ability to colonize northern states such as New York (Pecarevic et al. 2010), Connecticut, Washington, New Jersey, Tennessee, and Rhode Island (Waters et al. 2022), making its way into suburban and undisturbed hardwood habitats (MacGown et al. 2013).

Morphology. *Brachyponera chinensis* is a monomorphic black ant with a one-segmented waist and a large sting (MacGown 2009). Workers are 4-5 mm long and have 3-segmented antennae with nine flagellomeres and long antennal scapes, which visibly surpass the posterior border of the head (Smith 1934; Allen 2017). Worker ants have large compound elliptical eyes placed about their greatest diameter from the base of the mandibles and smooth and shining mesopleural region (Smith 1934; Allen 2017).

Brachyponera chinensis queens are up to 5-6 mm long and are dark brown to black (Allen 2017) with long, light reddish-brown legs (MacGown 2009). Their head is longer than wide and covered with dense, appressed setae, eyes ovoid with the posterior edge at the midline of the head; three ocelli present; antennae are 12-segmented (Allen 2017).

Male ants are yellow-brown to dark brown and 3.5 to 4.0 mm long. The head and mouthparts are reduced, and the antennae are 13-segmented (Allen 2017). The scape surpasses the posterior margin of the head, and all funicular segments are longer than wide (Yashiro et al. 2010).

Medical Importance. The painful sting of *B. chinensis* can be mistaken for the sting from another invasive ant, the red imported fire ant, *Solenopsis invicta*. Incorrect identification of *B. chinensis* can lead to improper medical treatment. The allergens in the ant's venom are responsible for various human symptoms (Allen 2017). Their sting can cause a mild allergic reaction to anaphylactic shock, especially in people who are hyper-allergic, e.g., small children and elderly (Cho et al. 2002; Nelder et al. 2006; Wanandy et al. 2021). Minor symptoms include localized redness, minor pain sensation at the time of the sting, and mild urticaria without swelling, with symptoms lasting <1 h (Nelder et al. 2006).

The venom, consisting of several different histolytic and neurotoxic peptides, is injected into the victim by a large ovipositor (Nelder et al. 2006; Allen 2017). Lee et al.

(2009) determined that the major allergens, 23 kDa and 25 kDa proteins belonging to the antigen 5 family, are the major reactive *B. chinensis* venom components.

Significant local reactions include <5 cm diameter swelling around the site, recurring pain, skin redness, mild-to-severe urticaria, and symptoms lasting 3-14 d (Kim et al. 2001; Nelder et al. 2006; Wanandy et al. 2021). Patients described the sting as itchy, intense pain that fades away and returns in several hours, and the pain may not be contained at the original site (Nelder et al. 2006). Anaphylactic symptoms can include painful legions, edematous, severe urticaria, respiratory distress, wheezing, and hypotension with or without loss of consciousness (Kim et al. 2001; Fukuzawa et al. 2001; Cho et al. 2002; Wanandy et al. 2021).

An example of an influx of *B. chinensis* and increased stings occurred in Greenville, SC. In 1997, zookeepers of the Greenville Zoo (Greenville, S.C.) first reported ant stings on their campus while constructing a new exhibit (Nelder et al. 2006). Stings occurred while zookeepers placed mulch around the zoo's indoor and outdoor exhibits (Nelder et al. 2006). In 2004, an investigation at the zoo concluded that *B. chinensis* entered the zoo via landscaping materials, off-site potted plants, or soil used in construction for the new exbibit (Nelder et al. 2006).

The zoo has not reported any ant stings within its animal population other than humans. Veterinary concerns exist at the zoo since *B. chinensis* was identified in animal bedding, and animal stings may have occurred without adverse effects on the animals (Nelder et al. 2006). In Japan, *B. chinensis* is an intermediate host for the chicken tapeworm *Raillietina kashiwarensis* (Fuhrmann) (Cestoidea: Davaineidae) (Sawada 1959;

Nelder et al. 2006). In the U.S., there is no documentation that *B. chinensis* is a mechanical vector of animal pathogens (Nelder et al. 2006).

Brachyponera chinensis is not aggressive (MacGown et al. 2013; Wanandy et al. 2021). Stings occur when people disrupt their nesting habitat with bare hands or feet, move logs in gardens, wooded areas, and lawns, or trap winged females between clothing and skin (MacGown 2009; MacGown et al. 2013).

Habitat. *Brachyponera chinensis* does not build recognizable nest sites. They select their nesting site in dark, damp areas in moist soil under outdoor furniture, patio bricks, or lawn equipment, in rotting logs or the soil beneath objects, under patio bricks, in mulch, leaf litter, dirt piles, railroad ties, within rotting and decayed logs, and under ornamental stones (Smith 1934; Smith 1947; Zungoli and Benson 2008; Guénard and Dunn 2010; Bednar and Silverman 2011; Bednar et al 2013: MacGown 2016; Wanandy et al. 2021). .They colonize natural habitats such as undisturbed, deciduous hardwood forests and suburban areas (Guénard and Dunn 2010). They are ground-dwelling ants rarely found on vegetation (Guénard and Silverman 2011).

Several key characteristics shared by invasive ants in introduced territories are polydomy, in which an ant colony occupies two or more spatially separated nests but is socially connected; polygyny, having at least two reproductive queens present in a colony (Robinson 2014); weak inter-nest aggression, and acceptance of non-nestmates (Murata et al. 2017). The five invasive ant species on the world's 100 worst invasive species are polydomous (GISD 2021). Debout et al. (2007) observed polydomy in 166 ant species belonging to 49 genera.

Depending on the habitat available, *B. chinensis* colonies are either monodomous or polydomous (Paysen 2007; Mo 2013). For example, Paysen (2007) found monodomous colonies occupying active and abundant galleries of subterranean termites, *Reticulitermes* spp. in decaying wood and oak tree roots. Polydomous colonies were found underneath rocks, boards, and concrete materials. Colonies can range from several hundred workers (MacGown 2016) to over 5,000 individuals (Paysen 2007; Zungoli and Benson 2008) by simultaneously occupying multiple nests (Zungoli and Benson 2008; Bednar et al. 2013; Robinson 2014; Merchlinsky et al. 2023), and if a large area of contiguous, suitable habitat is available, the colonies may expand to fill the void **(**Zungoli et al. 2014).

Polydomous colonies are associated with substantial advantages that may ensure their survival (Debout et al. 2007; Ellis et al. 2016). Benefits include an even distribution of risks among nests, increasing the size and population of colonies within their habitat, inter-nest movement extending communication and recruitment, and reducing the time to forage for food, thereby increasing foraging efficiency (Robinson 2014; Ellis et al. 2016). *Brachyponera chinensis* is polygynous, with colonies having multiple queens (Paysen 2007; Allen 2017; Murata et al. 2017).

Ecosystem impact on native and invasive ant taxa. *Brachyponera chinensis* are better adapted to colder temperatures than *L. humile* (Rice 2012). During March, when temperatures are low, *B. chinensis* becomes active and begins to expand its colonies, forage for food, and reproduce before *L. humile* foragers become active (Rice 2012). In early May, when *L. humble* workers slowly emerge, *B. chinensis* may consume *L. humile* as a primary food resource (Rice 2012).

When *B. chinensis* consumes *L. humile* as a food resource, it changes *B. chinensis'* cuticular lipid profile to resemble that of *L. humile* (Rice 2012). This change in the chemical profile leads to the acceptance of *B. chinensis* into *L. humil*e's colony, thereby avoiding negative encounters between the two ant species (Rice). Rice (2012) suggested that this "chemical mimicry" is a survival tactic to compensate for the lower colony numbers of *B. chinensis* compared to the higher colony numbers of *L. humile* (Rice 2012).

Foraging and food resources. *Brachyponera chinensis* are diurnal, solitary foragers and will forage until they find a food source. Smith (1934) recorded *B. chinensis* feeding on dead insects, fish scraps, and the juices of decayed fruits, dead terrestrial invertebrates, birds, and mammals (Zungoli et al. 2014). They also feed on small arthropods like springtails, pill bugs, and spiders (Andersen 2000). Their strategic technique for dominating live food resources in mature temperate forests and suburban habitats is crucial to their survival (Bednar and Silverman 2011). *Brachyponera chinensis* are specialist predators (Andersen 2000) that prefer protein-rich foods such as live termites (MacGown 2009; Bednar and Silverman 2013) and will occupy subterranean termite galleries, preying on *Reticulitermes virginicus* (Banks) (Paysen 2007; Bednar and Silverman 2011). They also prey on native ant species *Aphaenogaster rudis* (Enzmann) (Bednar and Silverman 2011; Rodriguez-Cabal et al. 2012; Merchlinsky et al. 2023) and invasive ant species *L. humile* (Rice 2012). In natural habitats, reducing insects, such as termites, that contribute to litter decomposition, turning and aerating soil, and carbon recycling may negatively impact the ecosystem and initiate cascading effects at other trophic levels (Bednar and Silverman 2011; Vogt et al. 2022).

Foraging strategies used by *Brachyponera chinensis.* Most invasive ants use mass recruitment via trail pheromones to collect food (Beckers et al.1989; Traniello 1989; Roulston and Silverman 2002; Guénard and Silverman 2011). *Brachyponera chinensis* does not employ trail pheromones when they forage (Guénard and Silverman 2011; Buczkowski 2016). The workers are solitary foragers. They forage randomly without organized cooperation or communication to locate, capture, or transport a food source (Beckers et al. 1989). When the worker encounters a food source too large to transport to the nest, it will return to the colony to recruit a nestmate for assistance. According to Guénard and Silverman (2011), they incorporate a form of a foraging strategy called tandem carrying, a simple recruitment strategy observed in Ponerinae species essential for colony survival. The scout will drum its antennae on potential recruits until a worker ant responds to their request. The chosen worker positions itself onto the scout, now called the carrier, for transportation to the food source, where the carrier releases it. Workers then dismantle the food source and return it to the nest.

Brachyponera chinensis workers cannot climb vertical surfaces, likely preventing them from foraging in trees or shrubs (Guénard and Dunn 2010). They have a reduced tarsal arolium structure, a pad-like structure that helps ants hold on to smooth surfaces and acts like a suction cup, a characteristic of most ground-dwelling *Brachyponera* species (Guénard and Dunn 2010; Allen 2017). Ants of the subfamily Ponerinae do not tend honeydew producers such as aphids and scale insects, unlike other ant species, because they do not possess a crop (Hölldobler and Wilson 1990; Bednar and Silverman 2011). The crop allows ants to share liquid resources among nest mates through trophallaxis (Paul and Roces 2003; Bednar and Silverman 2011). Ponerinae species accumulate and transport fluid as droplets between their mandibles held by capillary forces (Paul and Roces 2003). Bednar and Silverman (2011) found no evidence that *B. chinensis* workers tended honeydew-excreting hemipterans at or above the soil surface.

Management Strategies. Control measures such as baits are utilized against *B. chinensis* in the suburban environment (Rice and Silverman 2012; Buczkowski 2023). Treating undisturbed hardwood forest habitats where colonies have been established for several years is almost impossible due to the costs involved and the potential for negatively affecting native insect species.

An integrated pest management plan (IPMP) is an effective way of managing invasive ants. The success of an IPMP and the baits incorporated into it relies on knowledge of the pest species, proper identification, monitoring, foraging activities, and life history (Zungoli and Benson 2008).

Most IPMPs list insecticide usage as a last resort. This philosophy will be practical for nuisance insect pests such as sugar ants and other insect fauna. However, for stinging ants and other Hymenoptera, such as *B. chinensis*, the best practice should be a zero-tolerance policy goal; pesticides may need to play a more critical role against this public health pest.

Integrated pest management relies on a combination of common-sense practices and approaches to manage pest species. IPM is founded on in-depth inspections and species identification. It uses multiple management tactics to reduce risks to people, non-target organisms, and the environment and manages non-health-threatening pest populations. Management tactics begin with preventing an insect from becoming a pest by incorporating sanitation, habitat manipulation, modification of cultural practices, and applying pesticides.

Habitat manipulation aids in decreasing the presence of invasive ants in a suburban environment. Removing potential nest sites, such as rotting or dead logs, and piles of mulch and limb branches may help reduce the abundance and presence of *B. chinensis*. Outdoor garden and farm equipment should be contained in a shed, away from moist soil and litter. Indoor mulch storage, pine litter, straw, and other types of compost should be secured on pallets and checked periodically for activity. Hardscapes such as movable bricks and walkways should be inspected for potential ant colonies. Deep crevices in driveways and patios allow ants to forage and form colonies. Cracks should be repaired when ant activity is present.

Cultural practices may help to control the presence of *B. chinensis* and other ant taxa. When mulch is placed around a structure or in a garden, place it one foot away from the structure. Apply a layer of fabric landscape between the soil and the mulch to deter ants from nesting in the mulch.

Brachyponera chinensis workers are not known to follow a conventional trail when foraging (Guénard and Silverman 2011; Buczkowski 2016). However, workers scouting for food particles in the same area may belong to different colonies, which may help decrease the population of more than one colony. According to label instructions, baits may be applied to areas where worker ants are present. The ants should be monitored for a few minutes to observe them retrieving the bait and leaving the site before applying additional bait.

Baits are an essential control method for ants. When baits are used according to label instructions, they can be a cost-effective management program tool (Klotz et al.1997). Baits contain a food-based material (carrier) that is a preferred food source, usually a grain or animal protein or a granule infused with a pheromone, a toxicant, and other materials such as emulsifiers, preservatives, waterproofing, or antimicrobial agents (Stanley 2004; Jordan et al. 2013). The most effective baits for ants are those with delayed toxicity or slow-acting toxicants that exploit the ants' recruitment process (Klotz et al. 2003; Stanley 2004; Rust et al. 2004; Jordan et al. 2013). These slow-acting insecticides allow time for the foragers to exchange bait, ensuring the toxicants are distributed throughout the colony (Barbani 2003; Klotz et al. 2003; Stanley 2004; Jordan et al. 2013).

If a nesting site is discovered, especially in undisturbed hardwood areas, several bait granules may be placed near the nest's opening for workers to locate the bait and bring it back to their colonies. This method can reduce the amount of bait distributed and can reduce collateral damage to non-target fauna. Residual sprays may be a disadvantage for *B. chinensis*. The workers are solitary foragers, and their nest is often cryptic. When bait and spray applications are used simultaneously, the spray may affect the nest sites and bait granules by acting as a repellent and preventing the worker from entering the nest with the bait granules (Barbani 2003). Although effective toxicants and different baiting techniques are available, invasive ant management programs need further research development for cost-effective products and large-scale application techniques for the growing populations of stinging ants in the suburban environment and undisturbed hardwood forests (Buczkowski 2023).

The U.S. Department of Agriculture safeguards and monitors natural resources against the entry, establishment, and spread of economically and environmentally significant pests in the United States and facilitates the safe trade of agricultural products (U.S. Department of Agriculture 2023). A quarantine of soil and plant materials might successfully slow the spread of *B. chinensis* in the United States (Nelder et al. 2006). Ponerine ants are generally not pests; therefore, control strategies are not warranted (Nelder et al. 2006). For effective control measures to monitor the movement of *B. chinensis* by the U.S. Department of Agriculture, further research documenting the medical importance to humans and the destruction of the environment that *B. chinensis* inflicts on an ecosystem is paramount to developing an effective quarantine program.

Farmers, conservation land managers, and zookeeper employees worldwide struggle to deal with invasive ant species. *Brachyponera chinensis* is a stinging ant with venom that can induce severe allergic reactions. Site-specific, goal-oriented management programs for suburban and hardwood forest areas are essential where this is a problem. IPM programs should focus on managing the ants where many are discovered rather than eliminating native and beneficial insects from the ecosystem.

References Cited

Abbott, K.L. (2004). Alien ant invasion on Christmas Island, Indian Ocean: the role of ant-scale associations in the dynamics of supercolonies of the yellow crazy ant, *Anoplolepis gracilipes*. PhD Thesis, Biological Sciences. Monash University, Melbourne.

Abbott, K. L. (2006). Spatial dynamics of supercolonies of the invasive yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean. Diversity and Distributions, (Diversity Distrib.) (2006) 101–110.

Allen, H. R. (2017). Biology and Behavior of the Asian Needle Ant, Dissertation, Clemson University, Tiger Prints 1-111.

Andersen, A. N. (2000). Global ecology of rainforest ants. Functional groups in relation to environmental stress and disturbance. Washington (D.C.): Smithsonian Institute Press.

Barbani, L. E. (2003). Foraging activity and food preferences of the odorous house ant (*Tapinoma sessile* Say) (Hymenoptera: Formicidae). M. S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Baumgartner, N. R. II, and S. D. Ryan. (2020). Interaction of red crabs with yellow crazy ants during migration on Christmas Island Mathematical Biosciences 330 https://doi.org/10.1016/j.mbs.2020.108486.

Beckers, R., S. Goss, J. L. Deneubourg, and J. M. Pasteels. (1989). Colony size, communication and ant foraging strategy. Psyche 96:239-256.

Bednar, D. M., and J. Silverman, (2011). Use of termites, *Reticulitermes virginicus*, as a springboard in the invasive success of a predatory ant, *Pachycondyla*

(=*Brachyponera*) *chinensis*. Insect Soc. 58: 459-467.

Bednar, D. M., J. Z. Shik, and J. Silverman, (2013). Prey handling performance facilitates competitive dominance of an invasive over native keystone ant. Behav. Ecol. 24:1312-1319.

Bolton, B. (2016). A new general catalogue of the ants of the world. Updated access on January -5- 2016. http://gap.entclub.org/ Brown, W. L. 1958. A review of the ants of New Zealand. Acta Hymenopterologica. 1(1): 22.

Bolton, B. (1995). A new general catalogue of the ants of the World. Harvard University Press, Cambridge, MA.

Brown, W. L. (1958). A review of the ants of New Zealand. Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts. Acta Hymenopterologica 1:1-50.

Buczkowski, G. (2016). The Trojan horse approach for managing invasive ants: a study with Asian needle ants *Pachycondyla chinensis*. Biol Invasions 18: 507-515.

Buczkowski G. (2023). Termite cuticular extracts improve the acceptance of bait for controlling invasive Asian needle ants, such as *Brachyponera chinensis*. (wileyonlinelibrary.com) DOI 10.1002/ps.7601.

Cho, Y.-S., Y.-M. Lee, C.-K. Lee, B. Yoo, H.-S. Park, and H.-B. Moon. (2002). Prevalence of *Pachycondyla chinensis* venom allergy in an ant-infested area in Korea. J. Allergy Clin. Immunol. 110: 54-57.

Clark, R. E., and J. R. King. (2012). The ant, *Aphaenogaster picea,* benefits from plant elaiosomes when insect prey is scarce. Environ. Entomol. 41(6): 1405-408; DOI: http://dx.doi.org/10.1603/EN12131.

Cowie, R. H. (2013). Pathways for transmission of angiostrongyliasis and the risk of disease associated with them. Hawaii J. Med. Public Health 72:70–74.

Cumberland, M. S., and L. K. Kirkman. (2013). The effects of the red imported fire ant on seed fate in the longleaf pine ecosystem. Plant Ecol 214:717-724 DOI 10.1007/sl 1258-013-0201-2.

Debout, G., Schatz, B., M. Elias, and D. McKey. (2007). Polydomy in ants: What we know, what we think we know, and what remains to be done. Biol J Linn Soc. 90: 319-348.

Emery, P., N. J. Mitchell, H. Cogger, J. Agius, P. Andrew, S. Arnall, T. Detto, D. A. Driscoll, S. Flakus, P. Green, P. Harlow, M. McFadden, C. Pink, K. Retallick, K. Rose, M. Sleeth, B. Tiernan, L. E. Valentine, and J. Z. Woinarski. (2020). The lost lizards of Christmas Island: A retrospective assessment of factors driving the collapse of a native reptile community. Conservation Science and Practice. 2021;3:e358. Https://doi.org/10.1111/csp2.358.

Ellis, S., D. S. Procter, P. Buckham-Bonnett, and E. J. H. Robinson. (2016). Inferring polydomy: a review of functional, spatial and genetic methods for identifying colony boundaries. Insect. Soc. 64:19–37 DOI 10.1007/s00040-016-0534-7.

Fasi, J., G. Brodie, and C. Vanderoude. (2012). Increases in crop pests caused by *Wasmannia auropunctata* in Solomon Islands subsistence gardens. Journal of Applied Entomology 137: 580-588.

Feare C. J. (1999). Ants take over from rats on Bird Island, Seychelles. Bird Consev. Int. 9: 95–96.

Fukuzawa, M., F. Arakura, Y. Yamazaki, H. Uhara and T. Saida. (2001). Urticaria and Anaphylaxis Due to Sting by an Ant (*Brachyponera chinensis*). Acta Dermato-Venereologica, 2002, Vol 82, Issue 1, p59. DOI 10.1080/000155502753600939.

Gentili, R., U. Schaffner, A. Martinoli, and S. Citterio. (2021). Invasive alien species and biodiversity: impacts and management Biodiversity, Vol. 22, NOS. 1–2, 1–3 https://doi.org/10.1080/14888386.2021.1929484.

Gerlach, J. (2004). Impact of the invasive crazy ant *Anoplolepis gracilipes* on Bird Island, Seychelles. Journal of Insect Conservation 8: 15–25.

GISD (2021). Global Invasive Species Database (GISD) [WWW Document]. URL https://www.cabi.org/isc/abstract/20097200135 (accessed 4.27.21).

Guénard B., and R. R. Dunn. (2010). A new (old), invasive ant in the hardwood forests of eastern North America and its potentially widespread impacts. PLoSONE 5(7): e11614. doi:10.1371/journal.pone. 0011614.

Guénard, B., and J. Silverman. (2011). Tandem carrying, a new foraging strategy in ants: description, function, and adaptive significance relative to other described foraging strategies. Naturwissenschaften. 98: 651-659.

Guénard, B., J. K. Wetterer, and J. A. MacGown. (2018). Global and temporal spread of a taxonomically challenging invasive ant, *Brachyponera chinensis* (Hymenoptera: Formicidae). Florida Entomologist-Volume 101, No. 4.

Hölldobler, B., and E. O. Wilson. (1990). The Ants. Belknap Press of Harvard University Press, Cambridge p. 732.

Holway, D. A., L. Lach, A.V. Suarez, N. D. Tsutsui and T. J. Case. (2002). Causes and consequences of ant invasions. Annu Rev Ecol Evol Syst 33:181–233.

Jordan, B. W., B. E. Bayer, P. G. Koehler and R. M. Pereira. (2013). Bait evaluation methods for urban pest management, Pg. 447–471. In S. Trdan (ed.), Insecticides: Development of Safe and More Effective Technologies. InTech, Rijeka, Croatia.

King, J. R., and W. R. Tschinkel. (2008). "Experimental Evidence That Human Impacts Drive Fire Ant Invasions and Ecological Change." Proceedings of the National Academy of Sciences of the United States of America 105: 20339–43.

Kim, S. S., H. S. Park, H.Y. Kim, S. K. Lee, and D. O. Ham. (2001). Anaphylaxis caused by the new ant, *Pachycondyla chinensis*: demonstration of specific IgE and IgE binding components. Journal of Allergy and Clinical Immunology. 107: 1095–1099.

Klotz, J., D. F. Williams, B. Reid, K. M. Vail, and P. Koehler. (2003). Ant trails: A key to management with baits. University of Florida, Cooperative Extension service, Institute of Food and Agricultural Sciences.

Lee, Chow-Yang, and C.C.S. Yang. (2022). Biology, Ecology, and Management of the Invasive Longlegged Ant, *Anoplolepis gracilipes* Annu. Rev. Entomol. 67:43–63.

Lee, E-K., K-Y. Jeongw, D-P. Lyuz, Y-W. Lee, J-H. Sohn, K-J. Lim, C-S. Hong, and J.W. Park. (2009). Characterization of the major allergens of *Pachycondyla chinensis* in ant sting anaphylaxis patients. Clinical and Experimental Allergy. 39: 602-607.

MacGown, J.A. (2009). The Asian needle ant, *Pachycondyla chinensis* (Emery) (Hymenoptera: Formicidae), reported from Alabama. Midsouth Entomologist 2: 88–89.

MacGown, J. A., H. Richter, and R. L. Brown. (2013). Notes and new distributional records of invasive ants (Hymenoptera: Formicidae) in the southeastern United States. Midsouth Entomologist 6: 104–114.

Mackay, W. P., and E. E. Mackay. (2010). The systematics and biology of the New World ants of the genus *Pachycondyla* (Hymenoptera: Formicidae). Edwin Mellon Press, Lewiston.

Malone, M., K. Ivanov, S. V. Taylor, and R. Schürch. (2023). Fast range expansion of the red imported fire ant in Virginia and prediction of future spread in the United States Ecosphere, an unrestricted access journal, DOI: 10.1002/ecs2.4652.

Merchlinsky, A., P. T. Frankson, R. Gitzen, C. A. Lepczyk, J. E.Mohan and R. J. II. Warren. (2023). Warming promotes non-native invasive ants while inhibiting native ant communities. Ecological Entomology, 48(5), 588–596. Available from: https://doi.org/10.1111/een.13256.

Mitchell, N. and J. Woinaeski. (2022). Options beyond captivity for two Critically Endangered Christmas Island reptiles. Threatened Species Recovery Hub.

Mo, Y. (2013). Temporal food preference and effectiveness of selected bait products against *Pachycondyla chinensis* (Emery) (Hymenoptera: Formicidae). (Masters Thesis) Retrieved from http://tigerprints.clemson.edu/cgi/viewcontent.cgi? article=2650&context=all_thes es. Accessed 12 May 2016.

Murata, N., T. Kazuki, and T. Kikuchi. (2017). Social structure and nestmate discrimination in two species of *Brachyponera* ants distributed in Japan Entomol.

Sci. 20(1): 86-95.

Nelder, M. B., E. S. Paysen, P. A. Zungoli, and E. B. Benson. (2006). The emergence of the introduced ant *Pachycondyla chinensis (*Formicidae: Ponerinae) as a public health threat in the southeastern United States. J. Med. Entomol. 43: 1094-1098.

O'Dowd, D. J., P. T. Green, and P. S. Lake. (1999). Status, Impact, and Recommendations for Research and Management of Exotic Invasive Ants in Christmas Island National Park. Centre for the Analysis and Management of Biological Invasions Monash University.

Paul, J., and F. Roces. (2003). Fluid intake rates in ants correlate with their feeding habits. J. Insect Physiol. 49: 347–357.

Paysen, E. (2007). Diversity and abundance of ants at forest edges. (Doctoral dissertation). https://tigerprints.clemson.edu/ all_dissertations/174.

Pecarevic, M. Z., J. Danoff-Burg, and R. R. Dunn. (2010). Biodiversity on broadway – enigmatic diversity of the societies of ants (Formicidae) on the streets of New York City. Plos One 5(10): e13222. Accessed 23 August 2015.

Plentovich, S., A. Hebshi, and S. Conant. (2009). Detrimental effects of two widespread invasive ant species on weight and survival of colonial nesting seabirds in the Hawaiian Islands. Biol Invasions 11:289–298 DOI 10.1007/s10530-008-9233-2.

Rice, E. (2012). The species overlap of *Pachycondyla chinensis* and *Linepithema humile*: contributing mechanisms and consequences. Dissertation, North Carolina State University, Raleigh, NC.

Rice, E., J. Z., Shik and J. Silverman. (2012). Effect of scattered and discrete hydramethylnon bait placement on the Asian needle ant. J Econ Entomol 105:1751–1757.

Robinson, E. J. (2014). Polydomy: the organization and adaptive function of complex nest systems in ants. Curr Opin Insect Sci. 5: 37–43.

Rodriguez-Cabal, M. A., K. L. Stuble, B. Guénard, R. R. Dunn, and N. J. Sanders. (2011). Disruption of ant-seed dispersal mutualisms by the invasive Asian needle ant (*Pachycondyla chinensis*). Biological Invasions. 14: 557-565.

Roulston, T. H., and J. Silverman. (2002). The Effect of Food Size and Dispersion Pattern on Retrieval Rate by the Argentine Ant, *Linepithema humile* (Hymenoptera: Formicidae). Journal of Insect Behavior, Vol. 15, No. 5.

Rust, M. K., D. A. Reierson, and J. H. Klotz. (2004). Delayed Toxicity as a Critical Factor in the Efficacy of Aqueous Baits for Controlling Argentine Ants (Hymenoptera: Formicidae). J. Econ. Entomol. 97(3): 1017-1024.

Sawada, I. (1959). Studies on the life history of the chicken tapeworm *Raillietina* (*Paroniella*) *kashiwarensis* Sawada. J. Nara Gakugei Univ. 8: 31Ð63.

Schmidt, C.A., S. O. Shattuck. (2014). The higher classification of the ant subfamily Ponerinae (Hymenoptera: Formicidae), with a review of ponerine ecology and behavior. Zootaxa 3817: 1–242.

Siddiqui, J. A., B. S. Bamisile, M. M. Khan, W. Islam, M. Hafeez, I. Bodlah, and Y. Xu. (2021). Impact of invasive ant species on native fauna across similar habitats under global environmental changes Environmental Science and Pollution Research 28:54362–54382 https://doi.org/10.1007/s11356-021-15961-5.

Smith, M. R. (1934). Ponerine ants of the genus *Euponera* in the United States. Ann.

Entomol. Soc. Am. 27: 558-564.

Smith, M. R. (1947). A generic and subgeneric synopsis of the United States ants, based on the workers. Am Midl Nat 37: 521–647.

Stanley, M. C. (2004). Review of Efficacy of Baits Used for Ant Control and Eradication. Landcare Research Contract Report: LC0405/044.

Thomas, M. L., K. Becker, K. Abbott, and H. Feldhaar. (2010). Supercolony mosaics: two different invasions by the yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean Biol Invasions 12:677–687 DOI 10.1007/s10530-009-9473-9.

Traniello, J. R. (1989). Foraging strategies in ants. Ann. Rev. Entomol. 34: 191-210.

Vogt, J. T., J. A. MacGown, J. Lewis, S. Horn, and M. D. Ulyshen. (2022). Diversity and Seasonal Occurrence of Native and Nonnative Ants (Hymenoptera: Formicidae) in LongTerm Experimental Chinese Privet (Lamiales: Oleaceae) Plots in Georgia, USA. J. Entomol. Sci. 57(2): 297–309. https://doi.org/10.18474/JES21-58.

Wanandy, T., E. Mulcahy, W. Y.Lau, S. G. A. Brown, and M. D. Wiese. (2021). Global View on Ant Venom Allergy: from Allergenic Components to Clinical Management. Published online: 1 June 2021 © Crown 2021.

Waters, J. S., N. W. Keough, J. Burt, J. D. Eckel, T. Hutchinson, J. Ewanchuk, M. Rock, J. A. Markert, H. J. Axen, and D. Gregg. (2022). Survey of ants (Hymenoptera, Formicidae) in the city of Providence (Rhode Island, United States) and a new northernmost record for *Brachyponera chinensis* (Emery, 1895.). Check List 18 (6): 1347–1368. https://doi.org/10.15560/18.6.1347.

Warren, R.J. II, A. McMillan, J. R. King, L. Chick, and M. A. Bradford. **(2015).** Forest invader replaces predation but not dispersal services by a keystone species. Biol Invasions (2015) 17:3153–3162 DOI 10.1007/s10530-015-0942-z.

Wetterer, J. K. and S. D. Porter. (2003). The little fire ant, *Wasmannia auropunctata*: Distribution, impact, and control. Sociobiology Vol. 41, No. 3.

Wetterer, J. K. (2012). Worldwide spread of the stigma ant, *Pachycondyla stigma* (Hymenoptera: Formicidae) Myrmecol. News 16: 39-44 (online 12 August 2011) ISSN 1994-4136 (print), ISSN 1997-3500 (online).

Yashiro, T., K. Matsuura, B. Guénard, M. Terayama and R. R. Dunn. (2010). On the evolution of the species complex *Pachycondyla chinensis* (Hymenoptera: Formicidae: Ponerinae), including the origin of its invasive form and description of a new species. Zootaxa. 2685: 39-50.

Zettler, J. A., Spira, T. P., and C. R. Allen. (2001). "Ant–seed mutualisms: can red imported fire ants sour the relationship?" Nebraska Cooperative Fish & Wildlife Research Unit -- https://digitalcommons.unl.edu/ncfwrustaff/3.

Zungoli, P. A. and E. P. Benson. (2008). Seasonal occurrence of swarming activity and worker abundance of *Pachycondyla chinensis* (Hymenoptera: Formicidae). Proceedings of the Sixth International Conference on Urban Pests. pp. 51-57. Budapest, Hungary.

Zungoli, P. A., E. P. Benson, and B. Ellis. (2014). Challenges for successful management of *Pachycondyla chinensis* (Hymenoptera: Formicidae). Proceedings of the Eighth International Conference on Urban Pests. https://cabidigitallibrary.org by 70.33.100.30. pp. 259–263.

CHAPTER 2

COMMERICAL BAIT ACCEPTANCE BY THE ASIAN NEEDLE ANT, *BRACHYPONERA CHINENSIS* (EMERY) (HYMENOPTERA: FORMICIDAE)

Karen Corsetti To be submitted to a peer-reviewed journal.

Abstract The Asian needle ant, *Brachyponera chinensis*, is an invasive insect that has a negative impact on native ant habitats and can disrupt the balance of ant-seed dispersal mutualisms by displacing native ant species. They have a venomous sting that can cause severe allergic reactions in people sensitive to arthropod stings. Effective management is limited due to the lack of research for effective bait products and the life history of the Asian needle ant. Bait formulations commonly used to control ants include granular, liquid, and ant stations**.** Here, we assessed the bait acceptance of the Asian needle ant under field conditions from August 2020 to September 2022. This study evaluated the bait acceptance for four commercial granular baits, Advion granular bait and Advion fire ant bait (FAB) (AI indoxacarb); Advance 375A (active ingredient (AI) abamectin) and Niban (AI orthoboric acid). Bait granules were randomly placed in areas where *B. chinensis* colonies were active. For each trial, the behavior and activity of the worker with the bait granule were visually recorded. Advance® and Advion® fire ant bait (FAB) had the highest probability of acceptance. Advance® resulted in an 80% probability of a positive response, and Advion® FAB has a 75% probability of a positive response.

Key Words Asian needle ant, baits, *Brachyponera chinensis*, invasive species, granular baits

Introduction

The global impact of invasive ants affects continental and island ecosystems worldwide (Holway et al. 2002). According to ant-invasive databases, 23 species of invasive ants have been recorded worldwide (Siddiqui et al. 2021). The ecological impacts of invasive ants often reduce and outnumber native ant diversity, directly impacting native fauna and plant flora, causing habitat disturbance, and limiting the foraging activity of native species (Holway et al. 2002; Rodriguez-Cabal et al. 2011; Siddiqui et al. 2021). In the United States (U.S.), invasive ants have invaded and adapted to various suburban, agricultural, and undisturbed habitats (Lowe et al. 2000; Holway et al. 2002; Lach and Hooper-Bùi 2010; Buczkowski 2017).

The Asian needle ant, *Brachyponera chinensis*, was introduced into the U.S. by accident in the early 1930s (Smith 1934; Smith 1947; Nelder et al. 2006). In 1932, the first record of *B. chinensis* in the continental U.S. was collected by H.T. Vanderford in forests near Decatur, Dekalb County, Georgia (Smith 1934; Smith 1947; Nelder et al. 2006). Since 1932, *B. chinensis* has become one of the most widespread and vital invasive ant species in the U.S. (Guénard et al. 2018). They have established a strong presence in southern states and the east coast (Smith 1934; Nelder et al. 2006; Guénard et al. 2018), and their ability to tolerate cooler temperatures (Guénard and Dunn 2010) has allowed them to expand into territories such as Wisconsin, Virginia, Ohio, Kentucky, Mississippi, Arkansas, and Washington DC (Guénard et al. 2018), Rhode Island (Waters et al. 2022) and New York (Pecarevic et al. 2010).

Asian needle ants are predatory termite hunters (Matsuura 2002; MacGown 2009; Bednar and Silverman 2011; Buczkowski 2023). Their unique ability to invade undisturbed hardwood forest areas, which often contain abundant colonies of termites, has allowed them to remain isolated from human interference for decades (MacGown 2009; Bednaer and Silverman 2011; Buczkowski 2015). In Georgia, they can be found in the Oconee National Forest, in areas far removed from human activity (E. Poole, personal communication). They inhabit rotting logs and tree stumps in moist soil often associated with termite colonies (Smith 1934; Smith 1947; MacGown 2009; Mo 2013; Zungoli et al. 2014). *B.chinensis* may move into the active galleries of subterranean termites (*Reticulitermes* spp.) that reside in these logs and use them as a food source (Bender and Silverman 2011; Buczkowski 2015). *Brachyponera chinensis* does not build dome-shaped mounds; their nest entrances are hidden and cryptic (Mo 2013), making it challenging to locate nesting sites and colonies in undisturbed forests and human-populated areas. Early detection and rapid response are crucial in managing *B. chinensis*, as detection can prevent the spread of the ant and minimize its impact on the environment and human health.

Asian needle ants colonize natural habitats characterized by undisturbed, deciduous hardwood forests, where they live in logs underneath bark. In suburban areas, they live underneath stones, flowerpots, sidewalks, patio areas, or other human-made debris (Smith 1934; Smith 1947; Guénard and Dunn 2010; Zungoli et al. 2014).

In the suburban environment, *B. chinensis* can threaten humans and animals. Their venomous sting can cause severe allergic reactions resulting in anaphylactic shock (Kim et al. 2001; Leath et al. 2006; Lee et al. 2009; Rice 2012; Wanandy et al. 2021) in humans who may be hypersensitive to Hymenoptera venom (Nelder et al. 2006). Anaphylactic reactions have been documented in Korea (Kim et al. 2001; Fukuzawa et al. 2001;

Cho et al. 2002; Wanandy et al. 2021); however, according to Nelder (2006), there has been one case of anaphylactic shock reported in the U.S. by an individual who suffered a severe allergic reaction to multiple stings from a single ant (Nelder et al. 2006). A 2024 University of Georgia (UGA) account by a concerned homeowner indicated that she was stung by *B. chinensis* and suffered an anaphylactic reaction (S.Vigil, personal communication). In 2004, in another case at the Greenville Zoo in South Carolina, several zookeepers complained of painful and itchy stings from *B. chinensis* while working with mulch for an outdoor exhibit (Nelder et al. 2006). The zookeepers experienced mild symptoms, and hospitalization or advanced treatment was unnecessary (Nelder et al. 2006). The economic impact of *B. chinensis* infestations can be significant, with costs associated with specific economic impacts.

Recent studies conducted by Guénard and Dunn (2010) and Rice (2012) concluded that *B. chinensis* negatively impacts *Aphaenogaster rudis*, a keystone seed-dispersing ant, thereby reducing some local myrmecochorous plant abundance (Rodriguez-Cabal et al. 2011; Buczkowski 2017; Merchlinsky et al. 2023). The invasion of *B. chinensis* can lead to a decline in native ant populations, disrupting seed dispersal and other ecological processes (Guénard and Dunn 2010; Rodriguez-Cabal et al. 2011; Warren et al. 2015; Buczkowski 2017; Merchlinsky et al. 2023). These long-term effects highlight the need for conservation efforts to protect native ant species from the threat of *B. chinensis*.

Despite the advanced management methods to control ants, effective control measures have yet to be developed for *B. chinensis* (Mo 2013; Buczkowski 2023). This underscores the urgent need for innovative and effective strategies to manage this invasive species. No commercial baits are labeled explicitly for *B. chinensis* (Mo 2013). Baits can be a cost-effective tool for ant management (Klotz et al.1997). They contain an attractant (food substance) that encourages the worker to return the bait to the colony (Klotz et al. 1997; Mo 2013;). The most effective baits for ants are those with delayed toxicity that exploit the ants' recruitment process (Klotz et al. 1997; Stanley 2004; Rust et al. 2004). Slow-acting insecticides allow time for the foragers to exchange bait, ensuring the toxicants are distributed throughout the colony (Klotz et al.1997; Barbani 2003; Klotz et al. 2003). Using an active ingredient with delayed toxicity can effectively deliver mortality to different castes within the colony (Stringer et al.1964; Hooper-Bùi and Rust 2002; Jordan et al. 2013). Placing bait near active nest sites can reduce the amount of bait used and labor involved in applying it (Tripp et al. 2000).

The objective of my study was to evaluate the acceptance of four commercially available granular baits (Table 2.1; Figure 2.1) by *B. chinensis* under field conditions. My experiment was designed so the worker, while foraging, would encounter a bait granule by chance-i.e., an organic encounter. The response to the bait was recorded as positive or negative, and the time (seconds) from encounter to removal from a camera's field of view (FOV) was noted.

Materials and Methods

Granular Bait Acceptance

The objective of my study was to evaluate the acceptance of four commercially available granular baits (Table 2.1; Figure 2.1) by *B. chinensis* under field conditions. My experiment was designed so the worker, while foraging, would encounter a bait granule by chance-i.e., an organic encounter. The response to the bait was recorded as

positive or negative, and the time (seconds) from encounter to removal from a camera's field of view (FOV) was noted.

A residential property in Griffin, GA (33°13'52" N84°15'08" W) was chosen for field trials. The property was heavily populated with *B. chinensis* and other non-*Brachyponera* genera. The study site was a suburban neighborhood driveway and backyard porch with English ivy vines, *Hedera helix*, and mature hardwood trees (*Quercus* spp.) on the property and throughout neighboring properties. Experiments were conducted from August to September 2020, March to September 2021, and June to September 2022.

On the day of each trial, the head of a house cricket, *Acheta domesticus* (Linnaeus), was placed on the outdoor substrate in the middle of the camera's field of view (FOV) to confirm the ants' food-collecting behavior. This process was repeated before the start of each trial day to confirm positive foraging. If the cricket head was unacceptable to the worker ant, bait preference trials were not conducted.

In a random location on the porch or driveway, a tripod-mounted camera (Sony HD wide-angle 29.8mm HDR-XR260: New York, NY, U.S.A.) was positioned 28 cm above and perpendicular to the substrate (Figure 2.2). A single bait granule (Figure 2.2) was placed in the center of the camera's FOV (Figure 2.3). If the granule was not encouunted by *B.chinensis* after 15 minutes, the camera and bait were repositioned to a nearby area. The recording started when a worker ant entered the camera's FOV and ended when the worker exited the FOV.

This assay was designed so worker ants would eventually encounter a bait granule while foraging. *Brachyponera chinensis* does not establish trails while foraging; instead, they forage as individuals (Guénard and Silverman 2011; Rice 2012) (personal observation) in what appears to be a random manner.

Upon entering the camera's FOV, the worker's activity and behavior toward the bait granule were recorded. There were two types of behavioral responses: a positive response and a negative response. A positive response was characterized as cautionary acceptance or full acceptance. For cautionary acceptance, the worker manipulated the bait with her antennae for several seconds and then positioned herself to retrieve it; for full acceptance, the worker encountered the granule, immediately retrieved it, and exited the camera's FOV without hesitation.

A negative response was defined as no contact or investigation. For no contact, the worker showed no interest in the granule and ignored it. For investigative response, the worker investigated the bait with her antennae but did not retrieve it. Recordings were later reviewed, and the time (seconds) from granule discovery until granule removal (left the camera's FOV) was recorded for data analysis. The workers were collected and placed in a vial containing 75% ethanol along with a label for further identification. The used bait was discarded according to label instructions at a remote site.

Dry weights of ants and baits. Ten live, whole worker ants were immersed in 75% ethanol until dead. The dead ants were extracted from the ethanol, allowed to air dry at room temperature, and placed in an oven at 60°C for three days and weighed. One replicate of 10 ants from each of the four lab colonies (N=4 replicates, 40 ants total) was used. Five replicates of 10 bait granules were weighed to obtain bait granule weights. All weights were obtained with an analytical scale (Mettler Toledo, Switzerland).

Statistical analysis. Analyses and visualizations of data were completed using R 4.3.2 (R Core Team, 2023). I tested ordinal logistic regression models using the function “polr” in the MASS package (Venables and Ripley 2002) to determine if the workers preferred a specific type of bait and the behavioral responses toward the bait. The ordinal logistic regression model is appropriate given that the positive and negative behavioral responses are discrete categories in ascending order (Hosmer et al. 2013). The proportional odds assumption was tested and met using the Brant (1990) test. Model coefficients estimated by the ordered logistic regression model as log (odds ratios) were exponentiated to produce odds ratios for ease of interpretation. A linear model was fit to test the relationship between the interaction of bait and response type (cautionary acceptance vs. full acceptance) and the time it took for an ant to leave the field of view. A log transformation of the response variable was performed to meet model assumptions of normally distributed residuals. Post-hoc tests using emmeans package (Lenth 2024) with Tukey’s HSD adjustment for multiple comparisons were performed to determine significant differences among levels of categorical variables. Following the post-hoc test, results were back-transformed for data visualization and interpretation.

Results

Five hundred ninety-five video-recorded trials were acquired from August 2020 through September 2022 (Table 2.2). *Brachyponera chinensis* foraging activity varied by month. In March, ants were not active. Several attempts were made to locate foraging workers; however, no workers were found. Worker activity was scarce in April; however, two negative responses were recorded. Ant foraging increased in May N=20 trials. In June, foraging activity increased by N=91 trials, July to N=169, and in August to N=179. Trials remained steady until September (N=129) and declined in October to N=5 trials (Table 2.2). Trials were not conducted from November to March because of ant inactivity. The most significant foraging activity occurred in July and August (personal observation). From 0800 to 1200h, acceptance trials were recorded when *B. chinensis* and other ant taxa were actively foraging (personal observation; Rice 2012).

Worker ants retrieved the bait for both categories of positive response (Table 2.3). For a cautionary acceptance, the workers were "more cautious" in retrieving the granule; for full acceptance, the worker immediately retrieved the granule upon encounter. The predictive probability of workers exhibiting a positive response to bait selection resulted in Advance® and Advion® FAB having the highest acceptance rate. (Figure 2.4A). Advance® resulted in an 85.2% probability of a positive response, and Advion® FAB 76.1%. There was no significant difference in acceptance probability of Niban®, Advion FAB®, and Advion®, where positive responses ranged from 65.9% to 76.1%. Advance® had a greater acceptance rate than Advion® and Niban®.

Brachyponera chinensis was most active (personal observation) in June, July, and August. August had the most observations (N=179) compared to other months (Table 2.2). When August data (N=179) were isolated and analyzed (Figure 2.4 B), the predicted positive probability of acceptance for Advance® and Advion® FAB was nearly identical. In summary, the pattern of granular bait acceptance did not change from the total observations of all the trials (N=595; Figure 2.4A) or in isolated trials when ants were most active (August only, N=179; Figure 2.4B).

The time required to remove a bait granule from the camera's FOV (Figure 2.5) was analyzed for each recording. For cautionary acceptance, there was no significant difference in the time for the workers to remove the bait granules (Figure 2.5, red). For full acceptance, there was a significant time difference between Advion® FAB and Niban®.

The weight of Niban® was less than Advion® FAB: 0.86 ± 0.15 and 1.96 ± 0.99 mgs, respectively. The response for the average time to remove Advion® FAB was 20 seconds, and for Niban, it was 9.5 seconds.

Discussion

The objective of this study was to evaluate bait acceptance, behavioral responses to baits, and the time required to remove the bait granule. Based on the results of this study, the order of bait acceptance was Advance 375A®,> Advion® FAB, >Advion® IG, >and Niban® (Figure 2.4). The results from these trials are insightful to understanding the ant's reaction to the bait, a crucial element in formulating effective ant-bait suppression strategies. Among the four baits tested, Advance® 375A and Advion® FAB were the most preferred by *B. chinensis* (Figure 2.4). Advance® was easily retrieved by *B. chinensis* with minor manipulations while exiting the camera's FOV.

When *B. chinensis* workers encountered a granule, they probed the bait with their antennae and legs. When the bait was deemed an acceptable food source, the worker positioned the granule with their front legs and positioned it into their mandibles. For the heaviest Advion® granules, the worker turned their body to one side of the bait to maneuver their long legs over the bait, lifted their bodies, and positioned them on top of the bait with mandibles and legs. Once in this position, she would maneuver the bait to her second pair of legs. The worker carried the bait with its mandibles while positioning it underneath its body. Workers are medium-sized with long, slender legs. Manipulating a long, heavy bait may be difficult for smaller ant species. Worker ants were able to carry the bait away from the FOV. In the data from August (Figure 2.4B, N=179 trials), the most active foraging month, the order of bait preference did not change (Figure 2.4A, N=595 trials).

Bait weight was compared to the ant's weight (Table 2.4). Niban® is a small, lightweight granule compared to Advion®, Advion® FAB, and Advance®. Advance® bait granules are small, irregular in shape, with jagged edges, and perhaps less cumbersome than Advion®. Advion® weighed 5.8X more than the ant and was the heaviest bait. Advion is a cylindrical-shaped bait, usually longer than the ant. Compared to the other baits, the shape and weight of Advion® granules did not appear to influence the worker's ability to retrieve and position the bait for transport.

Due to the shape and weight of Niban®, it may have been easier for the ant to select a lighter bait that can be easily carried without having to stop and reposition the bait. This may suggest that a round lighter is easier to carry than a jagged-edge bait.

While the four commercial baits used in this assay varied in shape and size, evaluating the appropriate particle size for *B. chinensis* was outside the scope of this study. Particle size preference trials have not been established for *B. chinensis*, an essential characteristic unknown for this ant species. Future research should be conducted to determine the appropriate particle size for a bait granule and match it to *B. chinensis* to produce the maximum toxicant output to eliminate a colony.

Determining the proper particle size is crucial for the effectiveness of granular ant baits (Hooper-Bùi et al. 2002). Understanding particle size profiles among different ant species and matching these profiles to a specific target species can significantly enhance the efficacy of granular baits (Jordan et al. 2013). This approach can also help reduce the impact on native ant species (Ipser and Gardner 2019) and increase the amount of toxicant brought into the colony (Hooper-Bùi et al. 2002).

Hooper-Bùi et al. (2002) conducted a food particle size preference study among two large ant species and four small ant species using a toxicant-free anchovy-based bait offered to foraging workers. The larger species were the California harvester ant, *Pogonomyrmex californicus* (Buckley), Allegheny mound ant, *Formica* spp., and the red imported fire ant (RIFA), *Solenopsis invicta* (Buren); the smaller ants included the Argentine ant, *Linepithema humile* (Mayr), southern fire ant, *Solenopsis xyloni* McCook and the pharaoh ant, *Monomorium pharaonis* (Linnaeus). According to optimal foraging theory (OFT), ants will secure the largest food particles. The Allegheny mound ant, the RIFA, and the California harvester ant preferred the larger particle size >2,000 µm. However, the findings for the smaller ants concluded that when given a choice, they prefer smaller particle sizes of 840 to 1,000 µm to larger particle sizes, compared to the larger ant species. The authors concluded that if a granular bait can be altered to fit the profile of the target ant species, it may be conceivable to increase the efficacy of the bait to the target pest.

Ipser and Gardner (2019) conducted bait particle size preferences by four non-invasive and two invasive ant species. The author's results for the RIFA corroborate those of Hooper-Bùi et al. (2002); however, the results of Isper and Gardner (2019) for Georgia *L. humile* differed slightly. Isper and Gardner (2019) results show that the Georgia *L. humile* population from Georgia preferred larger particles of 2.0 mm, and the mean width of the head capsule was larger than California *L. humile* and Alabama *L. humile*. Their data also showed that the size of the bait particle foraged was positively correlated with the width of worker head capsules for the six ant species tested. Neff (2010) reported that ants with larger head capsule widths would remove larger bait particle sizes (Hooper-Bùi and Rust 1997; Hooper-Bùi et al. 2002). These studies validate the belief that bait particle size preference is related to head capsule width and, thus, worker size.

In the Optimal Foraging Theory (OFT), worker ants adopt a foraging strategy to secure the largest food particles that provide the most benefit to maximize net energy gained (Jordan et al. 2013). OFT is a process in which ants decide to maximize energy gained or minimize time spent to obtain a fixed amount of energy (Jordan et al. 2013). For social insects such as ants, the colony's survival depends upon the quantity of food obtained by workers to maintain the colony's growth and reproductive status (Nonacs and Dill 1990). The OFT has been tested for *S. invicta* (Roeder et al. 2020), the leafcutter ants, *Atta cephalotes* (Linnaeus) (Snowden 2011), and the desert harvester ant, *Veromessor pergandei* (Mayr) (Boewn et al. 2019). Future research should include *B. chinensis* since managing this ant is vital to human health due to its ability to cause severe allergic reactions.

Retrieving large granule-size baits is a significant aspect of ant foraging. It introduces active ingredients into the colony with fewer particles collected by the foragers (Jordan et al. 2013). This concept may disadvantage ant species not cooperating in transport when foragers encounter complex and challenging situations of navigating larger bait across rough terrain to their colonies (Nonacs and Dill 1990; Jordan et al. 2013). Because of the increased surface area-to-volume ratio, smaller particles may bring much more toxicants into the colony per gram of bait taken by ants (Hooper-Bùi et al. 2002). When workers break up and subdivide larger bait particles before returning to the colony, the resulting piece may have a small amount or no toxicant left to affect the colony (Hooper-Bùi et al. 2002; Jordan et al. 2013).

Oi et al. (2022) conducted laboratory and field trials to decrease the effects of precipitation on fire ant baits on large land acreage such as golf courses. Their study assessed the effect of irrigation on the efficacy of a water-resistant and standard fire ant bait formulation, Advion® FAB, by (0.045% indoxacarb). To improve water resistance, Advion® FAB was treated with zein, a plant protein isolated from corn that can provide a moisture barrier surrounding the bait granule to improve bait performance. In laboratory studies, turfgrass squares were treated with a water-resistant formulation of zein-coated and uncoated Advion® FAB. They were placed under an outdoor irrigation system; only the water-resistant zein+Advion® treatment exhibited significantly higher reductions in workers and brood, and none of the queens survived. In field studies, the fire ants fed on both water-soaked baits. Their results concluded that irrigation did not fully compromise the efficacy of either bait type against fire ant colonies.

My research on the acceptance of bait products has practical implications for effective ant pest management strategies. It offers insights into *B. chinensis's* behavior and the acceptance of bait products, underscoring its potential to develop and implement management strategies. For instance, the findings can guide the selection of the most effective bait products in attracting *B. chinensis*, leading to more targeted and efficient pest control measures.

While these trials have provided valuable behavioral information for designing an effective management program, they do not reveal efficacy. This crucial question remains to be answered whether the workers transport the bait to the colony for consumption or abandon it before reaching their colony. Therefore, efficacy trials must be conducted (Chapter 3) to fill in the unknown gaps of this ant species.

References Cited

Barbani, L. E. (2003). Foraging activity and food preferences of the odorous house ant (*Tapinoma sessile* (Say) (Hymenoptera: Formicidae). M. S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Bednar, D. M., and J. Silverman. (2011). Use of termites, *Reticulitermes virginicus,* as a springboard in the invasive success of a predatory ant, *Pachycondyla (=Brachyponera) chinensis*. Insect Soc. 58: 459-467.

Bowen, C.G., Eshander, B. Lai and M. Samy. (2019). Optimal foraging behavior in desert harvester ants (Veromessor pergandei). https://doi.org/10.21973/N3PW9W.

Buczkowski, G. (2015). The Trojan horse approach for managing invasive ants: a study with Asian needle ants *Pachycondyla chinensis*. Biol Invasions 18: 507-515.

Buczkowski, G. (2017). Prey-baiting as a conservation tool: selective control of invasive ants with minimal non-target effects. Insect Conservation and Diversity 10: 302–309.

Buczkowski, G. (2023). Termite cuticular extracts improve the acceptance of bait for controlling invasive Asian needle ants, such as *Brachyponera chinensis*. (wileyonlinelibrary.com) DOI 10.1002/ps.7601.

Cho, Y. S., Y. M. Lee, C. K. Lee, B. Yoo, H. S. Park, and H. B. Moon. (2002). Prevalence of *Pachycondyla chinensis* venom allergy in an ant-infested area in Korea. J. Allergy Clin. Immunol. 110: 54-57.

Fukuzawa, M., F. Arakura, Y. Yamazaki, H. Uhara and T. Saida. (2001). Urticaria and Anaphylaxis Due to Sting by an Ant (*Brachyponera chinensis*). Acta Dermato-Venereologica, 2002, Vol 82, Issue 1, p59. DOI 10.1080/000155502753600939.

Guénard, B., and R. R. Dunn. (2010). A new (old), invasive ant in the hardwood forests of eastern North America and its potentially widespread impacts. PLoSONE 5(7): e11614. doi:10.1371/journal.pone. 0011614.

Guénard, B., and J. Silverman. (2011). Tandem carrying, a new foraging strategy in ants: description, function, and adaptive significance relative to other described foraging strategies. Naturwissenschaften 98:651–659 DOI 10.1007/s00114-011-0814-z.

Guénard, B., J. K. Wetterer, and J. A. MacGown, (2018). Global and temporal spread of a taxonomically challenging invasive ant, *Brachyponera chinensis* (Hymenoptera: Formicidae). Florida Entomologist-Volume 101, No. 4.

Holway, D. A., L. Lach., L., A.V. Suarez, N. D. Tsutsui and T. J. Case. (2002). Causes and consequences of ant invasions. Annu Rev Ecol Syst 33:181–233.

Hooper, L. M., and M. K. Rust. (1997). Food Preference and Patterns of Foraging Activity of the Southern Fire Ant (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 90(2): 246-253.

Hooper-Bùi, L. M., Appel, A. G, and M. K. Rust. (2002). Preference of food particle size among several urban ant species. Journal of Economic Entomology 95: 1222–1228.

Hosmer, D.W., S. Lemeshow, and R. X. Sturdivant. (2013). Applied logistic regression. New York: John Wiley and Sons, p. 398.

Ipser, R. M. and W. A. Gardner. (2019). Particle Size Preference of Six Ant Species (Hymenoptera: Formicidae). J. Entomol. Sci. 54(4): 370–377.

Jordan, B. W., B. E. Bayer, P. G. Koehler, and R. M. Pereira. (2013). Bait evaluation methods for urban pest management, Pg. 447–471. In S. Trdan (ed.), Insecticides: Development of Safe and More Effective Technologies. InTech, Rijeka, Croatia.

Kim, S. S., H. S. Park, H. Y. Kim, S. K. Lee, and D. O. Ham. (2001). Anaphylaxis caused by the new ant, *Pachycondyla chinensis*: demonstration of specific IgE and IgE binding components. Journal of Allergy and Clinical Immunology. 107: 1095–1099.

Klotz, J., D. F. Williams, B. Reid, K. M. Vail, and P. Koehler. (1997). Ant trails: A key to management with baits. University of Florida, Cooperative Extension service, Institute of Food and Agricultural Sciences.

Klotz, J. H., M. K. Rust, D. Gonzalez, Les Greenberg, H. Costa, P. Phillips, C. Gispert, D. A. Reierson, and K. Kido (2003). Directed sprays and liquid baits to manage ants in vineyards and citrus groves. J. Agric UrbanEntomol 20:31–40.

Lach, L. and L. M. Hooper-Bui. (2010). Consequences of ant invasions. Ant Ecology (ed. by L. Lach, C.L. Parr and K.L. Abbott), pp. 261–286. Oxford University Press, Oxford, UK.

Leath, T., T. Grier, R. Jacobson, and M. Fontana-Penn. (2006). Anaphylaxis to *Pachycondyla Chinensis*. J. Allergy Clin. Immunol. 117:S129-S129.

Lenth, R. (2024). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.0. https://CRAN.R-project.org/package=emmeans.

Lee, E. K., K. Y. Jeongw, D. P. Lyuz, Y-W. Lee, J. H. Sohn, K. J. Lim, C-S Hong and J. W. Park. (2009). Characterization of the major allergens of *Pachycondyla chinensis* in ant sting anaphylaxis patients. Clinical & Experimental Allergy, 39, 602–607, doi: 10.1111/j.1365-2222.2008.03181.x.

Lowe, S., M. Browne, and S. Boudlejas. (2000). 100 of the world's worst invasive alien species. Alien, 12, 1–12.

MacGown, J.A. (2009). The Asian needle ant, *Pachycondyla chinensis* (Emery) (Hymenoptera: Formicidae), reported from Alabama. Midsouth Entomologist 2: 88–89.

Matsuura, K. (2002). Colony-level stabilization of soldier head width for head-plug defense in the termite *Reticulitermes speratus* (Isoptera: Rhinotermitidae). Behav. Ecol. Sociobiol. 51: 172–179.

Merchlinsky, A., P. T. Frankson, R. Gitzen, C. A. Lepczyk, J. E. Mohan, and R. J. Warren II. (2023). Warming promotes non-native invasive ants while inhibiting native ant communities. Ecological Entomology. 2023;48:588–596.

Mo, Y. (2013). Temporal food preference and effectiveness of selected bait products against *Pachycondyla chinensis* (Emery) (Hymenoptera: Formicidae). (Masters Thesis) Retrieved from http://tigerprints.clemson.edu/cgi/viewcontent.cgi?article=2650&context=all_theses. Accessed 12 May 2016.

Neff, R., R. Puckett and R. Gold. (2011). Particle size and bait preference of the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Sociobiology 58: 473–488.

Nelder, M. B., E. S. Paysen, P. A. Zungoli, and E. B. Benson. (2006). The introduced ant *Pachycondyla chinensis* (Formicidae: Ponerinae) emerged as a public health threat in the southeastern United States. J. Med. Entomol. 43: 1094-1098.

Nonacs, P., and L. M. Dill. (1990). Mortality Risk vs. food quality trade-offs in common currency: an patch preferences. Ecology. 71: 1886-1892.

Oi, D. H., R. A. Atchison, G. Chuzel, J. Chen, J. A. Henke, and R. D. Weeks (2022). Effect of Irrigation on the Control of Red Imported Fire Ants (Hymenoptera: Formicidae) by Water-Resistant and Standard Fire Ant Baits. Journal of Economic Entomology, Volume 115, Issue 1, February 2022, Pages 266–272, https://doi.org/10.1093/jee/toab242.

Pecarevic, M. Z., J. D. Burg, and R. R. Dunn. (2010). Biodiversity on broadway - enigmatic diversity of the societies of ants (Formicidae) on the streets of New York City. Plos One 5(10): e13222. Accessed 23 August 2015.

R Core Team. (2023). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R- project.org.

Rice, E. (2012). The species overlap of *Pachycondyla chinensis* and *Linepithema humile*: contributing mechanisms and consequences. Dissertation, North Carolina State University, Raleigh, NC.

Rodriguez-Cabal, M.A, K. L. Stuble, R. R. Guénard, and N. Sanders. (2011). Disruption of ant-seed dispersal mutualisms by the invasive Asian needle ant (*Pachycondyla chinensis*). Biol Invasions 14:557–565.

Roeder, K.A., R.M. Prather, A.W. Paraskevopoulos, and D.V. Roeder. (2020). The Economics of Optimal Foraging by the Red Imported Fire Ant. Environmental Entomology, 49(2), 2020, 304–311 doi: 10.1093/ee/nvaa016.

Rust, M. K., D. A. Reierson, and J. H. Klotz. (2004). Delayed Toxicity as a Critical

Factor in the Efficacy of Aqueous Baits for Controlling Argentine Ants (Hymenoptera: Formicidae). J. Econ. Entomol. 97(3): 1017-1024.

Siddiqui, J. A., B. S. Bamisile, M. M. Khan, W. Islam, M. Hafeez, I. Bodlah, and Y. Xu. (2021). Impact of invasive ant species on native fauna across similar habitats under global environmental changes Environmental Science and Pollution Research 28:54362–54382 https://doi.org/10.1007/s11356-021-15961-5.

Smith, M. R. (1934). Ponerine ants of the genus Euponera in the United States. Ann. Entomol. Soc. Am. 27: 558-564.

Smith, M. R. (1947). A generic and subgeneric synopsis of the United States ants, based

Snowden, R. O. (2011). Optimal foraging in leafcutter ants (*Atta cephalotes*) selection of leaves based on proximity to the nest. Tropical Ecology and Conservation [Monteverde Institute]. 338. https://digitalcommons.usf.edu/tropical_ecology/338.

on the workers. Am Midl Nat 37: 521–647.

Stanley, M. C. (2004). Review of Efficacy of Baits Used for Ant Control and Eradication. Landcare Research Contract Report: LC0405/044.

Stringer, Jr C. E., C. S. Logren, and F. J. Bartlett. (1964). Imported fire ant toxic bait studies: evaluation of toxicants. Journal of Economic Entomology 57: 941- 945.

Tripp, J. M., D. R. Suiter, G. W. Bennett, J. H. Klotz, and B. L. Reid. (2000). Evaluation of Control Measures for Black Carpenter Ant (Hymenoptera: Formicidae). J. Econ. Entomol. 93(5): 1493—1497.

Venables, W. N., and B. D. Ripley. (2002). Modern applied statistics with S. Fourth Edition. Spring, New York. ISBN 0-387-95457-0.

Wanandy, T., E. Mulcahy, W. Y. Lau, S. G. A. Brown, and M. D. Wiese. (2021) Global View on Ant Venom Allergy: from Allergenic Components to Clinical Management Clinical Reviews in Allergy And Immunology. 62:123–44 https://doi.org/10.1007/s12016-021- 08858-1.

Waters, J. S., N. W. Keough, J. Burt, J. D. Eckel, T. Hutchinson, J. Ewanchuk, M. Rock, J. A. Markert, H. J. Axen, and D. Gregg. (2022). Survey of ants (Hymenoptera, Formicidae) in the city of Providence (Rhode Island, United States) and a new northernmost record for Br*achyponera chinensis* (Emery, 1895). Check List 18 (6): 1347–1368. https://doi.org/10.15560/18.6.1347.

Zungoli, P. A., E. P. Benson, and B. Ellis. (2014). Challenges for successful management of *Pachycondyla chinensis* (Hymenoptera: Formicidae). Proceedings of the Eighth International Conference on Urban Pests. https://cabidigitallibrary.org by 70.33.100.30. pp. 259–263.

Table 2.1. Granular bait products used to evaluate acceptance by *Brachyponera chinensis*

Product	Active Ingredient (%)	Manufacturer
Advance® 375 A bait	Abamectin (0.011%)	BASF Corporation, Triangle Park, NC USA
Advion® IG Granular bait	Indoxacarb (0.22%)	Syngenta Crop Protection, LLC Greensboro, NC, USA
Advion® Fire Ant bait	Indoxacarb (0.045%)	Syngenta Crop Protection, LLC Greensboro, NC, USA
Niban® bait	Orthoboric Acid (5%)	Nisus Corporation, Rockford, TN, USA

Table 2.2. Monthly bait granule acceptance data, August 2020-August-September 2022

Month	Advance	Advion	Advion Fire ant	Niban	Total
March	X	X	X	X	**0**
April	X	2	X	X	**2**
May	X	20	X	X	**20**
June	17	40	X	34	**91**
July	54	60	19	36	**169**
August	59	71	10	39	**179**
September	28	47	19	35	**129**
October	3	1	X	1	**5**
Total	**161**	**241**	**48**	**145**	**595**

A total number of trials (positive and negative) recorded for each month.

The X represents trials that were not conducted due to a lack of foraging activity during November through February.

Table 2.3. The outcome of bait granules encounters by *B. chinensis* workers.

Bait	Negative Response		Positive Response		Total
	No Contact	Investigate	Cautionary	Full	
Advance®	9	14	26	112	161
Advion®	21	57	56	107	241
Advion® FAB	1	10	11	26	48
Niban®	25	25	27	68	145
Total	56	106	120	313	595

Trials were recorded from August 2020 to September 2022 (N=595 trials).

Table 2.4 Weight of the four granular baits and *B. chinensis* workers.

Product	Granular Weight (mg)	Ratio (granular weight/ant)
Advance® 375A Bait	2.91±0.92	4.04
Advion® Granule Bait	4.15±1.30	5.86
Advion® Fire Ant Bait	1.99±0.99	2.86
Niban® Bait	0.86±1.50	1.20
Brachyponera chinensis	0.72±2.46	------

Figure 2.1.Granular baits used in the acceptance trials: Advion® and Advion® fire ant bait (Syngenta Crop Protection, LLC Greensboro); Advance 375®A (BASF corporation, Triangle Park, NC USA); Niban® Bait (Nisus Corporation, Rockford, TN, USA).

Figure. 2.2. The behavioral activity of *B. chinensis* toward four granular baits was recorded using a Sony HD wide-angle 29.8 mm HDR-XR260 video camera (New York, NY, U.S.A.). A single bait granule (blue arrow), e.g., Advion®, was placed in the middle of the camera's FOV, and the worker ant's response was recorded. All trials were conducted outdoors in Griffin, GA.

Figure. 2.3. The bait granule is placed in the middle of the camera's FOV. Recording started when the ant entered the field of view (FOV) (blue square). The FOV refers to the area that the camera lens can capture, and this area was kept constant for all trials. When a worker entered the camera's FOV, the recording began. When the worker exited the FOV, the recording stopped.

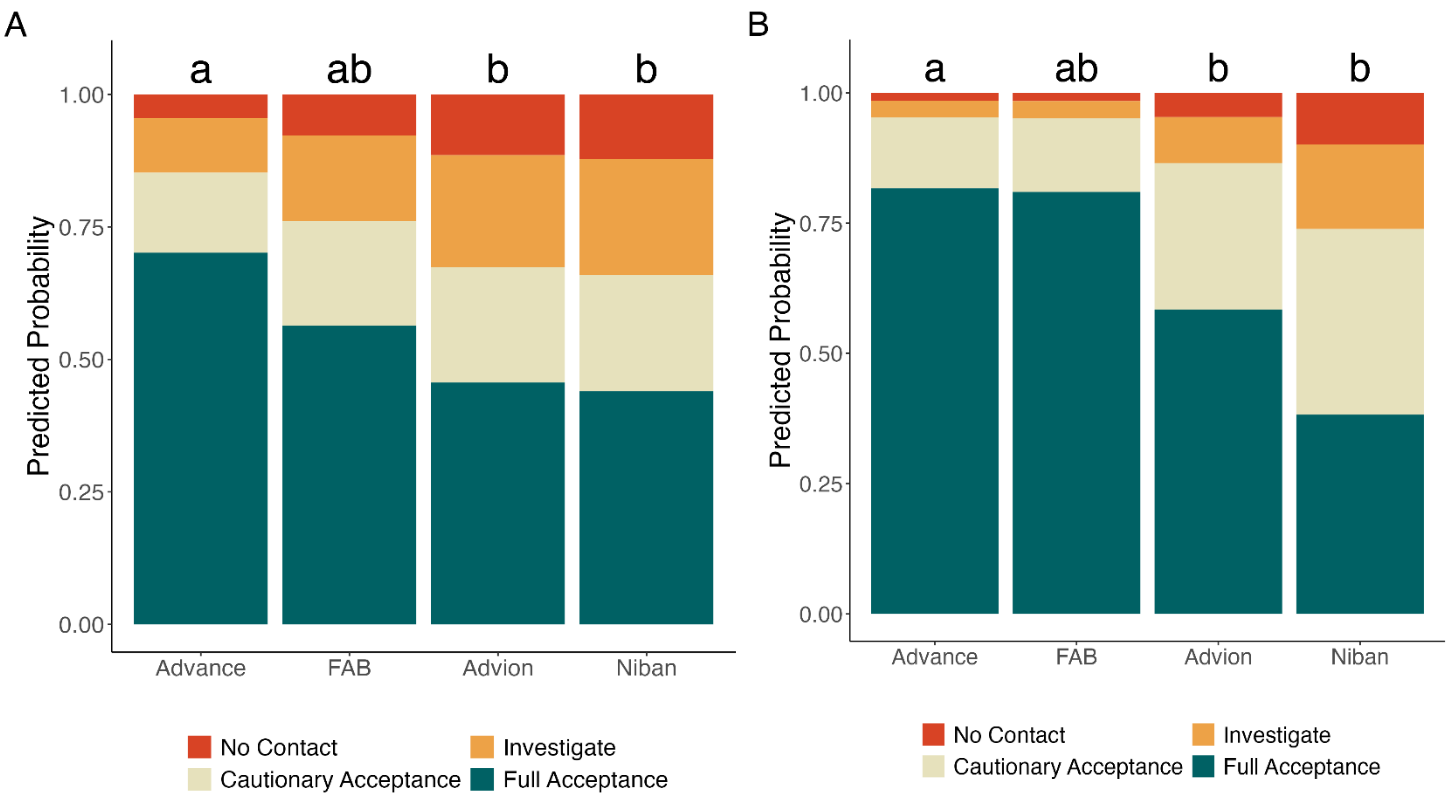


Figure. 2.4. Relative probably of *B. chinensis* exhibiting one of the four types of bait acceptance in field trials from August 2020 to September 2022. (A) All trials (N=595; August 2020-September 2022); (B) August trials only (N=179). Four behaviors were documented: negative response: (1) *no contact*, ant ignored the bait; (2) *investigate*, the ant probed the bait and did not accept it; positive response: (3) *cautionary acceptance*, the ant accepted the bait after the ant probed it; (4) *full acceptance*, the ant retrieved the bait without probing it. Advion® and Advion® fire ant bait (Syngenta Crop Protection, LLC Greensboro); Advance® 375A (BASF corporation, Triangle Park, NC USA); Niban® Bait (Nisus Corporation, Rockford, TN, USA). For both (A) and (B), means followed by the same letter are not significantly different.

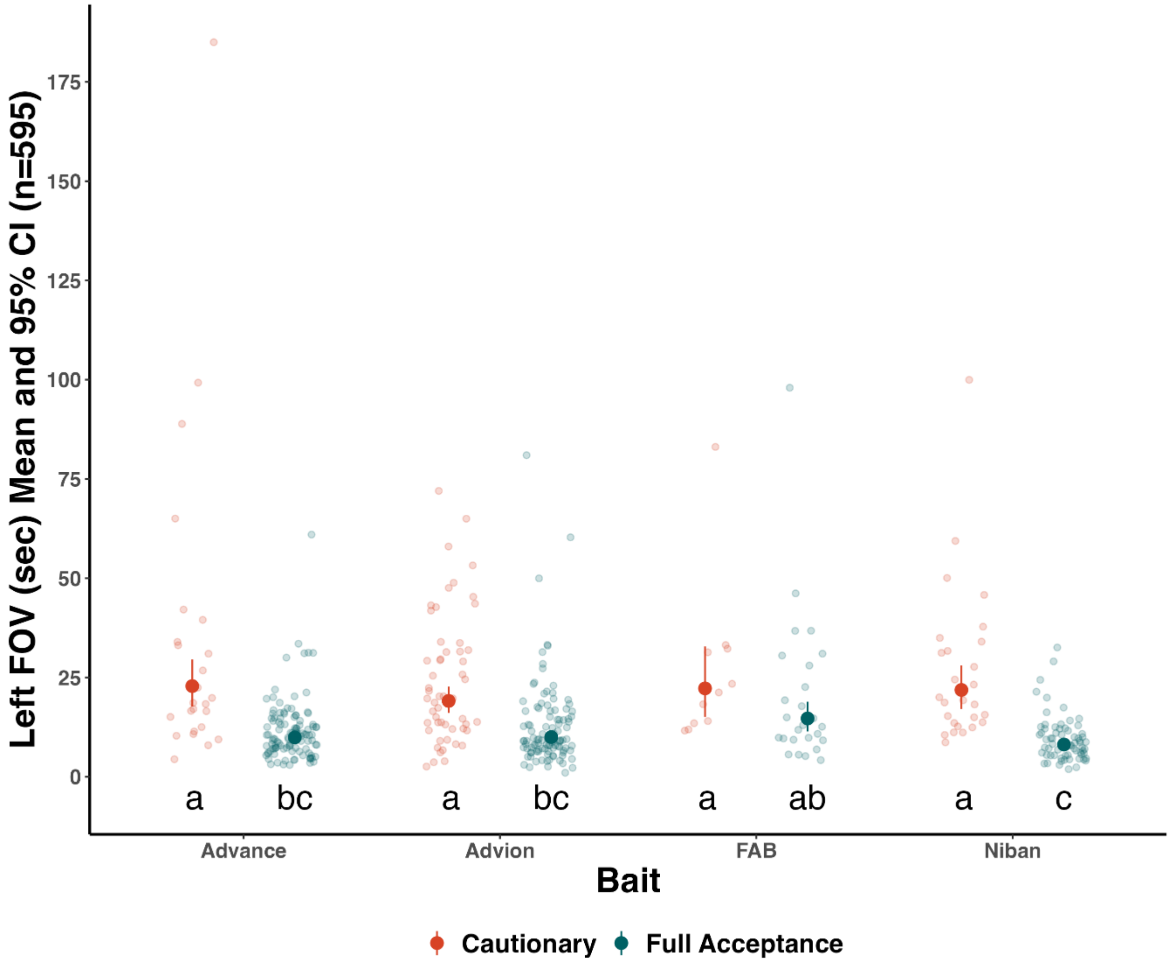


Figure. 2.5. Mean time in seconds required for *B. chinensis* walking to remove bait granules from the camera's field of view for four ant baits. For each acceptance type (cautionary or full), means followed by the same letter are not sufficiently different. Raw data points were incorporated into the box plots to help interpret the data. Advion® and Advion® fire ant bait (FAB) (Syngenta Crop Protection, LLC Greensboro); Advance® 375A (BASF corporation, Triangle Park, NC USA); Niban® Bait (Nisus Corporation, Rockford, TN, USA).

CHAPTER 3

EFFICACY OF SELECTED BAIT PRODUCTS AGAINST AN INVASIVE ANT, *BRACHYPONERA CHINENSIS* (EMERY) (HYMENOPTERA: FORMICIDAE)

Karen Corsetti To be submitted to a peer-reviewed journal.

Abstract. This research focuses on testing baits, an essential aspect of managing invasive ants. *Brachyponera chinensis* (Emery), the Asian needle ant, is a well-established threat in suburban and woodland areas in Georgia. Its venomous sting can trigger severe allergic reactions, posing a significant health risk to humans and animals. Moreover, it is an environmental concern, threatening our native seed-dispersing ant species. The importance of this research lies in its potential to provide practical solutions to the pressing issue of invasive ant management.

This study evaluated four bait products against *B. chinensis* in field and laboratory settings. In the field, we used the transect sampling method to measure the foraging activity of *B. chinensis*. The evaluation of Advion® granular bait in large-scale plots revealed a significant difference between the control and the ants observed in the Advion® plots. The foraging activity steadily decreased, resulting in a 100% reduction in the field population over seven weeks. This promising result underscores the potential of effective bait products in managing invasive ant populations.

Small plot trials were conducted to test Advion® fire ant bait (FAB), Advion®, Advance®, and Niban® granular bait. Advion® produced a 100% reduction at the end of the six-week trial. There was no significant difference between Advion® and Advance®, and there was no significant difference between Advion® FAB and Advance®. Niban® was the least effective bait, and there was no significant difference between the control and Niban®. In the laboratory, a choice/no choice study was conducted. Advion® fire ant bait (FAB), Advance®, and Advion® achieved a significantly lower survival rate than Niban® at the end of the ten-day trial for choice and no-choice trials.

Key Words Granular bait, *Brachyponera chinensis,* Asian needle ant, foragers

Introduction

Ants (Hymenoptera: Formicidae) are successful and destructive invaders that profoundly impact our planet. Invasive ants have been transported around the globe and successfully invaded new regions, severely impacting animal and human health and the agricultural industry (Nelder et al. 2006; Mo 2013; Buczkowski et al. 2017; Vogt et al. 2022). They also reduce the biodiversity of natural and human habitats (Holway et al. 2002; Zungoli and Benson 2008; Guénard and Dunn 2010; Buczkowski et al. 2017; Merchlinsky et al. 2023). Invasive ants such as the red imported fire ant (RIFA), *Solenopsis invicta* (Buren 1972), and the Asian needle ant, *Brachyponera chinensis* (Emery 1895), compete with our native keystone ant species for resources, habitat, and nesting sites and will use native ants as a food resource (Holway et al. 2002; Warren and McMillan 2015; Merchlinsky et al. 2023). The yellow crazy ant, *Anoplolepis gracilipes* (F. Smith 1857), has caused severe damage to native biodiversity and has contributed to a drastic change in the structure of the Christmas Island rainforest (O'Dowd et al. 2009; Thomas et al. 2010) and Bird Island, Seychelles (Gerlach 2004). Therefore, the importance of managing invasive ant populations cannot be overstated for ecosystems and biodiversity health.

The success of baits for the management of ants relies on knowledge of the pest species, including identification, monitoring, foraging activities, and life history (Zungoli and Benson 2008). Additional factors contributing to the success of bait are dietary requirements, appropriate particle size, and the physical state of bait, which can determine the worker's ability to retrieve it (Hooper-Bùi and Rust 2000; Stanley 2004; Nyamukondiwa and Addison 2014). A palatable food-base material acceptable to the ant may include a grain, animal protein, carbohydrates or fats, or a granule infused with a pheromone (Klotz et al. 1997; Stanley 2004; Mo 2013). All baits must have a toxicant; baits without a toxin are just a food source. Baits are often infused with emulsifiers, preservatives, waterproofing, or antimicrobial agents (Klotz et al. 1997). However, commercial bait components are trade secrets (Mo 2013). Residual chemical sprays should not be used in habitats where granular baits are applied, as they may contaminate the bait, making it unpalatable (Barbani 2003). When sprays are used near the colony's entrance, it may prevent the workers from returning to the colony and distributing the bait to nestmates (Barbani 2003).

Hydrogels are super absorbent water-storing crystals (polyacrylamide spheres) that can absorb ≈ 300 times their weight in water (Buczkowski et al. 2014). Water-soluble insecticides are mixed with the hydrogels and applied as slow-release liquid baits (Buczkowski et al. 2014). The highly absorbent hydrogel acts as a controlled-release formulation, ensuring the liquid bait remains available and appetizing to target pests (Tay et al. 2020).

The most effective baits for ants are those with delayed toxicity that exploit the ants' recruitment process (Klotz et al.1997; Stanley 2004; Rust et al. 2004). Slow-acting insecticides allow time for the foragers to exchange bait, ensuring the toxicants are distributed throughout the colony (Klotz et al. 1997; Barbani 2003; Klotz et al. 2003). Stringer et al. (1964) introduced the concept of delayed toxicity, which was applied to formulations of bait toxicants for the RIFA (Williams et al. 2001) and the Argentine ant, *Linepithema humile* (Mayr 1868) (Rust et al. 2004). The critical attributes of ant bait toxicants are that they are palatable, exhibit a wide range of effective dosages of 100-fold or greater, and be readily transferred from one ant to another (Hooper-Bùi and Rust 2000). Some of the most effective ant baits, such as those formulated by Williams et al. (2001), consisted of corn grit and soybean oil as an inert bait carrier with a toxicant for the RIFA, resulting in practical implications for pest control (Lofgren et al. 1975). Bait development for *L. humile* consisted of sucrose water as a food attractant (Rust et al. 2004), and it also has significant and practical applications in managing the population growth of this ant (Rust et al. 2004; Tay et al. 2017).

Delayed toxicity is a crucial component of an effective bait to control *L. humile* and *S. invicta*. Toxicants are diluted during trophallaxis; therefore, the workers must survive long enough to make multiple return trips to their colonies with toxic bait (Lofgren and Williams 1982; Stanley 2004; Rust et al. 2004). Using an active ingredient with delayed toxicity can effectively deliver mortality to different castes within the colony (Jordan et al. 2013). An additional advantage of delayed-action toxicants is that they maximize feeding, trail following, and mass recruitment (Traniello 1989; Beckers et al. 1989; Roulston and Silverman 2002; Rust et al. 2004). Fast-acting toxicants eliminate foraging ants before distributing the bait among nestmates (Barbani 2003; Klotz et al. 2003).

Oi and Oi (2006) conducted field and lab studies to test the efficacy and speed of action of fire ant baits against the RIFA. The authors used Ortho® Fire Ant Killer Bait Granules (0.015% Spinosad), Advion® Fire ant bait (FAB) (0.045% indoxacarb), Siege® Pro (0.73% hydramethylnon), and a control (once-refined soybean oil, 30% by weight). More than 85% of laboratory colonies receiving indoxacarb died within 3 d, and all colonies died in 6 d. Standard bait containing hydramethylnon killed 60% of the colonies in 9 d. Bait containing spinosad did not cause colony death. Under field conditions, the areas treated with the indoxacarb bait had no active fire ant nests within 3 d, whereas 11 d was needed to reach the same level of control with the hydramethylnon bait. Based on laboratory and field studies, baits containing indoxacarb or hydramethylnon baits were most effective. The delayed toxicity characteristics of the fast-acting indoxacarb bait may be helpful in the development of other fast-acting ant baits, as it allows for the effective distribution of the toxicant throughout the colony, leading to colony death.

Rust et al. (2004) conducted laboratory trials against *L. humile* to determine the solubility for the following three toxicants in a 25% aqueous sucrose solution with delayed toxicity. The authors focused on the speed of toxic action, defined as the time required to produce 50% mortality (LT50). Baits that provided an LT50 between days 1 and 4 were considered to have delayed toxic effects. Their studies showed that 9.2×10^{-3} to 7.1×10^{-4} percent imidacloprid and 3×10^{-4} to 2×10^{-5} percent thiamethoxam displayed a range of toxicities >10-fold, producing an LT50 within 1-4 d. The concentration of boric acid needed to produce an LT50 on day 1 was 3.63 percent, but it was only 0.55 percent on day 4, a nearly seven-fold lower rate.

In field studies, Forschler and Evans (1994A, 1994B) conducted a seven-week trial to test the efficacy of two commercial containerized baits against *L.humile*. The baits chosen were Pro-Control (0.5% sulfluramid in a peanut butter carrier) and Maxforce® bait container (0.9% hydramethylnon in a macerated silkworm pupae/fish meal carrier). Maxforce® was accepted more often, resulting in reduced foraging activity.

The hydramethylnon treatments reduced foraging ant numbers one week before the sulfluramid treatments. Two colonies treated with sulfluramid treatment that did not consume the equivalent of one bait station were not eradicated; however, reduced foraging activity was observed. The authors concluded that the amount of toxic bait contained in one bait station from either of the two commercially available ant baits was sufficient to reduce the foraging activity of *L.humile*.

Hooper-Bùi and Rust (2000) conducted laboratory baiting studies against *L. humile.* Serial dilutions of abamectin, boric acid, fipronil, and hydramethylnon prepared in a 25% sucrose solution were provided to groups of workers and queens for 24 h or 14 d. Baits containing$1x10^{-5}$ percent fipronil and 0.1% hydramethylnon provided complete mortality of *L. humile* workers within 24 h; however, hydramethylnon provided 40-60% kill of queens. Baits containing $1x10^{-4}$ and $1x10^{-5\%}$ fipronil provided 100% kill of queens. The queens and workers provided baits containing $1x10^{-5\%}$ fipronil and 0.5% boric acid; all died within 14 d. Sucrose preparations containing 0.001-0.1% abamectin provided 80% kill of queens at the highest dose (0.10%) and significantly higher worker kill rates than the three lowest concentrations tested within 24 h.

Avermectin B_1a has shown potential in managing RIFA populations. Avermectin B_1a is a natural compound derived from the soil microorganism *Streptomyces avermililis* (Lofgren and Williams 1982). It causes irreversible cell and tissue damage to the ovaries of queens (Glancey et al. 1982). The colony will die if the queen is killed or her reproductive ability is lost (Glancey et al. 1982). At high concentrations, Avermectin B_1a can also kill worker ants (Williams et al. 2001). Since the primary effect of Avermectin B_1a targets the reproductive capacity of the queen rather than acute toxicity for workers, the death rate of treated colonies at lower application rates was low (Lofgren and Williams 1982). Other insecticides such as methoprene, boric acid, teflubenzuron, spinosyn, and fipronil are successful in RIFA baits (Williams et al. 2001). Methoprene prevents insects from reproducing; boric acid is a stomach poison; teflubenzuron inhibits chitin synthesis and molting, and spinosyn enhances the action of nicotinic acetylcholine, resulting in paralysis of the insect (Williams et al. 2001; BASF 2013). Fipronil blocks chloride channels in the nervous system that are gated by gamma-aminobutyric acid (GABA) or glutamate system in insects (Williams et al. 2001; BASF 2013). The muscle is kept excited and cannot relax, eventually leading to death.

Brachyponera chinensis is native to East Asia and was accidentally introduced into the United States in the 1930s (Smith 1934; Creighton 1950; Nelder et al. 2006). They were first documented in Georgia when H.T. Vanderford collected specimens from forests near Decatur, DeKalb County, Georgia (Smith 1934; Nelder et al. 2006; Guénard et al. 2018; Merchlinsky et al. 2023). Since 1932, *B. chinensis* has broadened its range in the Southeastern United States: Alabama, Georgia, North Carolina, South Carolina, Louisiana, Tennessee, Mississippi, Florida, and Virginia (MacGown 2009; Guénard and Dunn 2010; Rice and Silverman 2013; MacGown et al. 2013; Zungoli and Benson 2014). Because *B. chinensis* can tolerate cooler temperatures; they have also been established in New York (Pecarevic et al. 2010), Wisconsin, Kentucky, Arkansas, Connecticut, Washington DC, New Mexico (MacGown et al. 2013), and Rhode Island (Waters et al. 2022).

Brachyponera chinensis invasion of various habitats can be attributed to their adaptability to a wide range of temperatures, and foraging behavior allows them to outcompete native ant species. It has a well-defined sting capable of injecting prey or potential predators with neurotoxic venom, causing anaphylactic reactions in humans (Kim et al. 2001; Fukuzawa et al. 2001; Cho et al. 2002; Nelder 2006; Wanandy et al. 2021). The recognition of *B. chinensis* as a health threat to humans and natural ecosystems has increased the need for more effective management approaches.

In addition to their medical importance, the invasive nature of *B. chinensis* makes it a significant threat to natural habitats. Worker ants can potentially negatively affect suburban and natural deciduous hardwood forests and devastate environments by excluding and displacing native ant species (Guénard et al. 2010; Buczkowski et al. 2017).

Current control methods for managing *B. chinensis* are limited compared to the RIFA and the Argentine ant. Liquid and hydrogel baits, commonly effective against other ant species, are ineffective against *B. chinensis*. This is due to the unique feeding behavior of ants of the subfamily Ponerinae, who do not tend honeydew producers because they do not possess a crop to store liquids (Hölldobler and Wilson 1990; Bednar and Silverman 2011). The crop found in other ant species allows ants to share liquid resources among nest mates through trophallaxis, a behavior not used by *B. chinensis* to transfer food among adults (Paul and Roces 2003; Bednar and Silverman 2011). Ponerine species accumulate and transport the fluid as a droplet between their mandibles held by capillary forces (Paul and Roces 2003).

Buczkowski (2016) introduced a novel management tool, the Trojan horse approach, for managing *B. chinensis* in natural habitats. This approach is based on live, poisoned prey: Eastern subterranean termites (*Reticulitermes flavipes* (Kollar). *Brachyponera chinensis* prefers protein-rich foods such as live termites (Matsuura 2002; Nelder et al. 2006; MacGown 2009). In contrast, very few native ants actively hunt termites. Termites are a food source for *B. chinensis* and are critical for their successful colonization in new habitats (Bednar and Silverman 2011). Buczkowski (2016), termites were exposed to fipronil and then presented to *B. chinensis*. In laboratory assays, workers were offered fipronil-treated termites within experimental arenas. A single termite exposed to 25 ppm fipronil for 1h killed 100 *B. chinensis* workers in 9 h. Field studies were conducted in forested areas invaded by *B. chinensis* to evaluate the population effects of fipronil-treated termites. Termites were exposed to 25 ppm fipronil-treated construction sand of (Termidor; SC (9.1 % fipronil) was dissolved in 100 ml water and mixed with 500 g sand in a sealed Ziploc bag. The mixture was placed in a Petri dish, and ≈ 250 termites were added to 150 g of the fipronil mixture. Fipronil-exposed termites were scattered on the forest floor, providing rapid control of *B. chinensis,* and ant densities throughout the treated plots declined by 98 ± 5 % within 28 days. The trojan horse concept holds the potential to limit the use of commercial bait formulations. This approach offers a less toxic alternative to pesticides and granular bait treatments and benefits the environment.

The objective of this study was to evaluate the relative effectiveness of four commercial bait products for managing *B. chinensis*. By evaluating selected bait products and combining knowledge of *B. chinensis* biology and foraging activity, more practical recommendations for managing *B. chinensis* can be made.

Materials and Methods

Bait Efficacy Trials: Small and Large Field Plots. Four commercial granular bait products with three active ingredients were chosen to evaluate efficacy against *B. chinensis* in small-scale field plots (Table 3.1). Two small-scale efficacy trials were conducted: one at Conyers International Horse Park, Conyers, GA (33°39’33”83°56’05”W) and one in Griffin, GA (33°13’52” N84°15’16”W). The small-scale trials were conducted from September to October 2022. I also constructed a large plot efficacy trial in Conyers, GA, from August to October 2023, when *B. chinensis* was active. The sites chosen were undisturbed hardwood forest areas and habitats suitable for *B. chinensis*. For Conyers small-scale trials, the transect contained 20 plots:16 randomly baited plots and four control plots (non-baited plots). There were four plots per bait and four controls. In the Griffin small-scale trials, the transect contained 10 plots: eight randomly baited plots and two control plots. Each small plot measured 3 m by 3 m, and each plot was separated by 12 m. The large transect in Conyers contained six plots: three baited plots and three control plots measuring 20 m by 20 m, and each plot was separated by 30 m. All three plots were baited with Advion® IG. The transects were in wooded areas near *B. chinensis* nesting sites with low human traffic.

To assess ant activity pre- and post-treatment, ≈ 6 gms of hard-boiled chicken egg yolk mixed with water was placed on a flat platform (half a weigh boat (10.2 cm X 7.6 cm Fisher Brand, Pittsburg, PA). For the small-scale trials, a single boat was placed in the center of the marked plot. For the large-scale trial, three boats were placed in the center of the plot in a triangle pattern, and boats were separated by ≈ 1 m. The number of needle ants feeding on the egg was counted after one hour (Figure 3.1).

Treatment and Application Method. The granular bait was scattered by hand in each plot for the small-scale trials. The total number of post-treatment needle ants feeding on egg yolk was made on days 3, 7, 14, 29, 37, and 46. For the large-scale field trial, each baited plot received the maximum labeled rate of Advion® IG (1.15 lbs./1,000 square feet) or 2,272 grams. The bait was scattered within the three plots with an electric spreader (Scotts electronic hand-held fertilizer spreader, The Whizz). Post-treatment assessments were made on days 3, 7, 14, 21, 28, and 37.

Bait Choice/No Choice Study – Laboratory

Colony collection. Ants in this study were collected from a residence in Decatur, GA (33°53'30"N 84°14'41"W). Dead bark was removed from several cut tree stumps, and exposed workers, pupae, and larvae were lightly brushed into a plastic container (58 cm x 38 cm x 15 cm). Individual nests from each stump sample were placed into separate containers and considered separate nests. Sample colonies were returned to the laboratory and provided with hard-boiled egg yolk, house crickets, water, and harborages *ad libitum*. Five colonies were maintained in their original plastic containers and under ambient laboratory conditions.

Harborage. A glass test tube (20 mm in diameter, 150 mm in length) was filled with 30 ml water and plugged with a cotton ball at the water's level. Forty grams of Castone® White (Ransom & Randolph, Maumee, OH 43537 USA) was mixed with 12 ml water and the wet slurry dripped onto the inside of the test tube to cover the cotton plug entirely with a thin trail that reached the lip of the test tube (Figure 3.2). The tube was then allowed to sit for 24 h. A red cellophane wrap was placed around the test tube, covering the Castone® (Maumeee, OH 43537) trail to the lip of the tube. A small cork plug (19 mm top diameter, 15 mm bottom diameter) was inserted into the test tube. A hole $\approx$ 2.5 mm diameter was drilled through the cork plug to allow ants to enter and exit the test tube freely (Figure 3.2). Worker ants moved into the test tube within three days to one week. Once the ants were in the test tube, I removed the bark and other debris from the tray and added additional test tube harborages to the trays. Colonies were maintained at 25 ± 3ºC and 65% relative humidity and fed a constant diet of crickets, hard-boiled chicken egg yolk, canned tuna in water, and occasionally worker termites.

Experimental Design. The laboratory efficacy of four granular ant baits (Table. 3.1) and untreated control (water and crickets only) were evaluated in a choice and no-choice situation in a 5 X 2 factorial design. A trial consisted of two replicates of each bait and control in a choice and no-choice situation, and five trials were conducted (10 replicates for every factor level combination). Each trial utilized ants from a single colony, and five colonies were used.

I collected ten workers using an aspirator from laboratory-maintained colonies and transferred them into a petri dish. I observed the worker ants under a microscope to check for and remove brood before placing them into a test tube harborage (described above) using a small glass funnel to guide them into the test tubes. The test tube was then placed in a plastic tray (58 cm x 38 cm x 15 cm). Ants were allowed to habituate for 24 hours with water only before bait was provided. After 24 h starvation, ants were provided bait only (without competitive food) or bait and a house cricket, *Acheta domesticus* (Linnaeus).

Control workers were fed *A. domesticus,* and all colonies were provided free water. Food and water were refreshed every day for ten days. After the 24-hour starvation period, bait products were provided as a one-time treatment (Table 3.1). The number of live ants was recorded every other day for ten days.

Statistical analysis. Analyses and visualizations of data were completed using R 4.3.2 (R Core Team, 2023). The zero-inflated generalized linear mixed effects models was tested using the function "glmmTMB" in the *glmmTMB* package (Brooks et al. 2017) to determine if the change in the abundance of ANA observed over time was different between the treatment groups tested. The zero-inflated generalized linear mixed effects model is appropriate, given that excessive zeros are within the count data (Lambert 1992).

The fixed effects included in the model were the interaction between days post-treatment and the different treatments and sites. The random effect included in the model was a random intercept for sampling location to account for the repeated measures nature of the data. The initial model was fit using the Poisson distribution, but when overdispersion was found, the negative binomial (nbinom2) distribution was used. The terms of the zero-inflated formula were tested, and the optimal formula ~ Site+Treatment*DPT was determined by the lowest AIC value. Post-hoc tests using *emmeans* package (Lenth 2024) with Tukey's HSD adjustment for multiple comparisons were performed to determine significant differences between the interaction of days post-treatment and baits. Cox proportional hazard models (1972) were tested using the function "coxph" in the *survival* package (Therneau 2023) to determine the mortality in lab choice and no-choice trials of *B. chinensis*. Ants alive at the end of the 10-day observation period were censored at ten days. I assessed the linearity and proportional hazard assumptions and checked for separation, collinarity, outliers, and influential observations. When the proportional hazard assumption was not met, the model was refitted to include a time interaction term which the categorical variables to model the changing baseline hazard function over time. Post-hoc tests using *emmeans* package (Lenth 2024) with Tukey's HSD adjustment for multiple comparisons were performed to determine significant differences among levels of categorical variables.

Results

1. Field Studies. After one week, there were significantly fewer ants in plots treated with Advion® compared to plots treated with the other three baits, and there were no significant differences among these three treatments (Figure 3.3A). *Brachyponera chinensis* foraging activity fluctuated during the next four weeks at every treatment and control plot except for Advion®. The foraging activity changes in the remaining two weeks decreased in the treatment and control plots. After the second week, there was no significant difference among the baits and the control (Figure 3.3B).

In the large plot test, the Advion® plots showed a decrease in foraging activity compared to the control (Figure 3.4A, B), but the difference was not significant. The foraging trend remained unchanged throughout the seven-week trial (Figure 3.4B).

2. Choice/No Choice Lab Study

Laboratory. In both choice (Figure 3.5A) and no-choice (Figure 3.5B) trials, the survival probability of Niban® and control were not significantly different. The control was 62 to 80%, while Niban® ranged from 60 to 64%. (Figure 3.5A, B). Likewise, there was no significant difference in ant survival after 10 d in ants provided Advion®, Advion® FAB, or Advance® in both choice and no-choice trials. For both trials, the survival rate of FAB was lower than that of the other baits. After ten days, the survival rate in the control group was approximately 80% in the choice trial and 60% in the no-choice test. In the choice test (Figure 3.5A), the survival rate of *B. chinensis* exposed to Advion® FAB, Advance®, and Advion® was 26%-36 %, significantly less than that of workers exposed to Niban®. In the no-choice trial (Figure 3.5B), survival of ants provided Advion® FAB, Advance®, and Advion® were not significantly different, with a 25-33% survival rate after

10 days, and Niban® (60%) was significantly greater. Each bait treatment yielded a lower survival rate than the control. Based on observations during the 10-day test period, *B. chinensis* retrieved a significant amount of Niban® granules, as observed in the test tube; however, they likely did not consume the bait as compared with the other baits. Niban® granules may not contain an attractive or active food ingredient for this ant species.

Discussion

The small-scale trial began in late August when *B. chinensis* foraging activity was greatest (Chapter 2, Bait Acceptance; Chapter 4, Pitfall Trap Data). The number of ants responding to the egg yolk was highly variable for each bait and control. The decrease in forging activity fluctuated weekly except for Advion®. After bait application, Advion® plots exhibited a 100% decrease in foraging activity. Advion® FAB foraging activity steadily decreased for three weeks, then spiked in the fourth week. Foraging activity for the Advance® plots varied weekly throughout the eight weeks. There was a significant difference between Niban® and Advion® bait. Niban® was not as effective in reducing foraging activity as Advion®, Advion ®FAB, or Advance®. Other ant fauna were found in the bait and control plots but not in the same plots as *B. chinensis*.

The control plot activity should not have fluctuated with the baits but should have remained stationary. The exact foraging distance for these ants has yet to be discovered. I predicted the plots may have been too small or that there needed to be more distance between each plot. The workers visiting the control plots may have been colony members who consumed the bait.

Due to logistical constraints, the sampling window for the Brookwood and Conyers locations was offset by ~ 15 days. Some seasonal variations could impact the change in abundance, which would not be separated from the days post-treatment (time).

For the large-scale trial, Advion® IG was used based on results from the small plot trials. Ants provided Advion® showed a decrease in foraging activity compared to the control group. The bait products were only applied once according to label directions. Both laboratory and field studies demonstrated variable efficacy and concluded that the three baits may be an effective control management tool for *B. chinensis*.

Results from the laboratory study showed that Advion®, Advion® FAB, and Advance® were effective, suggesting that these three baits were palatable to *B.chinensis*. in the choice and no-choice assay. In both choice and no-choice tests, the control groups provided with food (adult crickets) and water had a survival rate of 62-80%. Competitive food was also provided during the choice tests. After 10 days, Advion®, Advion® FAB, and Advance® were not significantly different in the survival rate in both choice and no-choice tests. Moreover, Advion® FAB exhibited the lowest survival rate among these three baits. Niban® survival was not significantly different from the control in either choice or no-choice tests.

Based on observations during the ten-day test period, *B. chinensis* retrieved a significant amount of Niban® granules, as observed in the test tube; however, they did not consume the bait compared with the other baits (personal observation). Niban® granules may not contain an attractive food ingredient for this ant species, which results in a higher survival rate. Alternatively, Advion®, Advion® FAB, and Advance® granules were not readily found in test tubes, suggesting that the ants consumed the bait.

The studies discussed here offer insight into granular bait products that may reduce *B. chinensis's* foraging activity in suburban areas. Their nests are often cryptic and may be challenging to locate. Disrupting their nesting area may cause them to relocate (personal observation). These ants are solitary foragers and do not use a trail pheromone to locate food sources. Without recruitment by trailing, not all colonies may be able to locate the bait if the bait is not placed in the vicinity of their nesting site and foraging range (Zungoli et al. 2014).

Depending on environmental factors such as heavy or constant rainfall, high humidity, temperature, or short life span under field conditions, baits may lose effectiveness or become unpalatable to the worker ant (Zungoli et al. 2014; Buczkowski 2016). According to label instructions, proper baiting procedures are paramount when managing an invasive species that poses a human health threat due to a venomous sting (Nelder et al. 2006). Although effective toxicants and different baiting techniques are available, the management of invasive ants gives further research on cost-effective products and large-scale application techniques for the growing populations of stinging ants in the suburban environment and undisturbed hardwood forests (Buczkowski 2023).

References Cited

Baden Aniline and Soda Factory (BASF), (2013). Insecticide Mode of Action, Technical Training Manual.

Barbani, L. E. (2003). Foraging activity and food preferences of the odorous house ant (*Tapinoma sessile* (Say) (Hymenoptera: Formicidae). M. S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Beckers, R., S. Goss, J. L. Deneubourg, and J. M. Pasteels. (1989). Colony size, communication, and ant foraging strategy. Psyche 96:239-256.

Bednar, D. M., and J. Silverman. (2011). Use of termites, *Reticulitermes virginicus*, as a springboard in the invasive success of a predatory ant, *Pachycondyla* (=*Brachyponera*) *chinensis*. Insect Soc. 58: 459-467.

Brooks ME, K. Kristensen, K. J. Van Benthem, A. Magnusson, C. W. Berg, A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. (2017). GglmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal. 9: 378-400. Doi: 10.32614/RJ-2017-066.

Buczkowski, G. (2016). The Trojan horse approach for managing invasive ants: a study with Asian needle ants, *Pachycondyla chinensis*. Biological Invasions, 18, 507–515.

Buczkowski, G. (2017). Prey-baiting as a conservation tool: selective control of invasive ants with minimal non-target effects. Insect Conservation and Diversity 10, 302–309.

Buczkowski, Grzegorz, E. Roper, and D. Chin. (2014). Polyacrylamide Hydrogels: An Effective Tool for Delivering Liquid Baits to Pest Ants (Hymenoptera: Formicidae). J. Econ. Entomol. 107: 748Ð757 (2014); DOI: http://dx.doi.org/10.1603/EC13508.

Buczkowski G. (2023). Termite cuticular extracts improve the acceptance of bait for controlling invasive Asian needle ants, such as *Brachyponera chinensis*. (wileyonlinelibrary.com) DOI 10.1002/ps.7601.

Cho, Y. S., Y. M. Lee, C. K. Lee, B. Yoo, H. S. Park, and H. B. Moon. (2002). Prevalence of *Pachycondyla chinensis* venom allergy in an ant-infested area in Korea. J. Allergy Clin. Immunol. 110: 54-57.

Creighton, W. S. (1950). The Ants of North America. Bull. Mus. Comp. Zool. Harvard Univ. The Cosmos Press Inc. Cambridge, Massachusetts. USA. 104: 568.

Cox, D. R. (1972). Regression models and life-tables. Journal of the Royal Statistical Society Series B (Methodo- logical). 34(2):187–220.

Forschler, B. T., and G. M. Evans. (1994a). Argentine ant (Hymenoptera: Formicidae) foraging activity response to selected containerized baits. J. Entomol. Sci. 29: 209Ð 214.

Forschler, B. T., and G. M. Evans. (1994b). Perimeter treatment strategy using containerized baits to manage Argentine ants, *Linepithema humile* (Mayr)(Hymenoptera: Formicidae). J. Entomol. Sci. 29: 264D267.

Fukuzawa, M., F. Arakura, Y. Yamazaki, H. Uhara and T. Saida. (2001). Urticaria and Anaphylaxis Due to Sting by an Ant (*Brachyponera chinensis*). Acta Dermato-Venereologica, 2002, Vol 82, Issue 1, p59. DOI 10.1080/000155502753600939.

Gerlach, J. (2004). Impact of the invasive crazy ant *Anoplolepis gracilipes* on Bird Island, Seychelles. Journal of Insect Conservation 8: 15–25.

Glancey, M. B., C. S. Lofgren, and D. F. Williams. (1982). Avermectin B^: Effects On The Ovaries Of Red Imported Fire Ant Queens (Hymenoptera: Formicidae). J. Med. Entomol. Vol. 19, no. 6: 743-747.

Guénard B., and R. R. Dunn. (2010). A new (old), invasive ant in the hardwood forests of eastern North America and its potentially widespread impacts. PLoSONE 5(7): e11614. doi:10.1371/journal.pone. 0011614.

Guénard, B., J. K. Wetterer, J. A. MacGown. (2018). Global and temporal spread of a taxonomically challenging invasive ant, *Brachyponera chinensis* (Hymenoptera: Formicidae). Florida Entomologist-Volume 101, No. 4:649-656.

Hölldobler, B., and E. O. Wilson. (1990). The Ants. Belknap Press of Harvard University Press, Cambridge p. 732.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui, and T. J. Case. (2002). The causes and consequences of ant invasions. Annual Review of Ecology and Systematics, 33, 181–233.

Hooper- Bùi, L. M, M. K. Rust. (2000). Oral toxicity of Abamectin, boric acid, Fipronil and hydramethylnon to groups of workers and queens of the Argentine ant *Linepithema humile* (Mayr). Journal of Economic Entomology 93: 858–864.

Kim, S. S., H. S. Park, H. Y. Kim, S. K. Lee, and D. O. Ham. (2001). Anaphylaxis caused by the new ant, *Pachycondyla chinensis*: demonstration of specific IgE and IgE binding components. Journal of Allergy and Clinical Immunology. 107: 1095 –1099.

Klotz, J., D. F. Williams, B. Reid, K. M. Vail, and P. Koehler. (1997). Ant trails: A key to management with baits. University of Florida, Cooperative Extension service, Institute of Food and Agricultural Sciences.

Klotz J. H., M. K. Rust, D. Gonzalez, L. Greenberg, Heather Costa, P. Phillips, G. Carmen , D. A. Reierson, and K. Kido (2003). Directed sprays and liquid baits to manage ants in vineyards and citrus groves. J. Agric UrbanEntomol 20:31– 40.

Jordan, B. W., B. E. Bayer, P. G. Koehler and R. M. Pereira. (2013). Bait evaluation methods for urban pest management, Pg. 447–471. In S. Trdan (ed.), Insecticides: Development of Safe and More Effective Technologies. InTech, Rijeka, Croatia.

Lambert, D. 1992. Zero-inflated poisson regression, with an application to defects in manufacturing. Technometrics. 34: 1-14

Lenth, R. (2024). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.0. https://CRAN.R-project.org/package=emmeans.

Lofgren, C. S., W. A. Banks, and B. M. Glancey. (1975). Biology and control of imported fire ants. Annual Review of Entomology. 20: 1-30.

Lofgren, C. S., and D. F. Williams. (1982). Avermectin B1a: Highly Potent Inhibitor of Reproduction by Queens of the Red Imported Fire Ant (Hymenoptera: Formicidae). J. &00. Entomol. 75: 798-803.

MacGown, J.A. (2009). The Asian needle ant, *Pachycondyla chinensis* (Emery) (Hymenoptera: Formicidae), reported from Alabama. Midsouth Entomologist 2: 88–89.

MacGown, J. A., H. Richter, and R. L. Brown. (2013). Notes and new distributional records of invasive ants (Hymenoptera: Formicidae) in the southeastern United

States. Midsouth Entomologist 6: 104–114.

Matsuura, K. (2002). Colony-level stabilization of soldier head width for head-plug defense in the termite Reticulitermes speratus (Isoptera: Rhinotermitidae). Behav. Ecol. Sociobiol. 51: 172–179.

Merchlinsky, A., P. T. Frankson, R. Gitzen, C. A. Lepczyk, J. E. Mohan, and R. J. Warren II. (2023). Warming promotes non-native invasive ants while inhibiting native ant communities. Ecological Entomology. 48:588–596. DOI: 10.1111/een.13256.

Mo, Y. (2013). Temporal food preference and effectiveness of selected bait products against *Pachycondyla chinensis* (Emery) (Hymenoptera: Formicidae). (Masters Thesis) Retrieved from http://tigerprints.clemson.edu/cgi/viewcontent.cgi?article=2650&context=all_thes es. Accessed 12 May 2016

Nelder, M. B., E. S. Paysen, P. A. Zungoli, and E. B. Benson. (2006). Emergence of the introduced ant *Pachycondyla chinensis (*Formicidae: Ponerinae) as a public health threat in the southeastern United States. J. Med. Entomol. 43: 1094-1098.

Nyamukondiwa, C., and P. Addison.(2014). Food preference and foraging activity of ants: Recommendations for field applications of low-toxicity baits. Journal of Insect Science: Vol. 14 | Article 48.

O'Dowd, D. J., P. T. Green, and P. S. Lake. (2009). Status, Impact, and Recommendations for Research and Management of Exotic Invasive Ants in Christmas Island National Park. Centre for the Analysis and Management of Biological Invasions Monash University.

Oi, D. H., and F. M. Oi. (2006). Speed of Efficacy and Delayed Toxicity

Characteristics of Fast-Acting Fire Ant (Hymenoptera: Formicidae) Baits. J. Econ. Entomol. 99(5): 1739-1748.

Paul J., and F. Roces. (2003). Fluid intake rates in ants correlate with their feeding habits. J. Insect Physiol. 49: 347–357

Pecarevic, M. Z., J. Danoff-Burg and R. R. Dunn. (2010). Biodiversity on broadway – enigmatic diversity of the societies of ants (Formicidae) on the streets of New York City. Plos One 5(10): e13222. Accessed 23 August 2015.

R Core Team. (2023). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org.

Rice E. S., J. Silverman. (2013). Propagule Pressure and Climate Contribute to the Displacement of *Linepithema humile* by *Pachycondyla chinensis*. PLoS ONE 8(2): e56281.doi:10.1371/journal.pone.0056281.

Roulston, T'ai H., and J. Silverman. (2002). The Effect of Food Size and Dispersion Pattern on Retrieval Rate by the Argentine Ant, *Linepithema humile* (Hymenoptera: Formicidae). Journal of Insect Behavior, Vol. 15, No. 5.

Rust, M. K., D. A. Reierson, and J. H. Klotz. (2004). Delayed Toxicity as a Critical Factor in the Efficacy of Aqueous Baits for Controlling Argentine Ants (Hymenoptera: Formicidae). J. Econ. Entomol. 97(3): 1017-1024.

Smith, M. R. (1934). Ponerine ants of the genus *Euponera* in the United States. Ann. Entomol. Soc. Am. 27: 558-564.

Stanley, M. C. (2004). Review of Efficacy of Baits Used for Ant Control and Eradication. Landcare Research Contract Report: LC0405/044.

Stringer, C. E. Jr., C. S. Lofgren, and F. J. Bartlett. (1964). Imported fire ant toxic bait studies: evaluation of toxicants. J. Econ. Entomol. 57: 941-945.

Tay, J.W., D.H. Choe, A. Mulchandani, and M. K. Rust. (2020). Hydrogels: From Controlled Release to a New Bait Delivery for Insect Pest Management. Journal of Economic Entomology, 113(5), 2020, 2061–2068 doi: 10.1093/jee/toaa183.

Tay, J.W., M. S. Hoddle, A. Mulchandani, and D. H. Choe. (2017). Development of an alginate hydrogel to deliver aqueous bait for pest ant management. Pest Manag. Sci. 73, 2028–2038.

Therneau, T. M. (2023). A package for survival analysis in R: R package version 3.5–7. https://CRAN.R-project. org/package=survival. (7).

Thoms, M. L., K. Becker, K. Abbott, and H. Feldhaar. (2010). Supercolony mosaics: two different invasions by the yellow crazy ant, *Anoplolepis gracilipes*, on Christmas Island, Indian Ocean Biol Invasions 12:677–687 DOI 0.1007/s10530-009-9473-9.

Traniello, J. R. (1989). Foraging strategies in ants. Ann. Rev. Entomol. 34: 191-210.

Wanandy, T., E. Mulcahy, W. Y. Lau, S. G. A. Brown, and M. D. Wiese. (2021.) Global View on Ant Venom Allergy: from Allergenic Components to Clinical Management Clinical Reviews in Allergy & Immunology 62:123– 144 https://doi.org/10.1007/s12016-021-08858-1.

Warren, R. J., A. McMillan, R. J. King, L. Chick, and M. A. Bradford. (2015). Forest invader replaces predation but not dispersal services by a keystone species. Biological Invasions. 17: 3153-3162. https://doi.org/10.1007/s10530-015-0942-z.

Waters, J. S., N. W. Keough, J. Burt, J. D. Eckel, T. Hutchinson, J. Ewanchuk,

M. Rock, J. A. Markert, H. J. Axen, and D. Gregg. (2022). Survey of ants (Hymenoptera, in the city of Providence (Rhode Island, United States) and a new northernmost record for Br*achyponera chinensis* (Emery, 1895.). Check List 18 (6): 1347–1368. https://doi.org/10.15560/18.6.1347.

Williams, D. F., H, L. Collins, and D. H. Oi. (2001). The Red Important Fire Ant, (Hymenoptera: Formicidae): An Historical Perspective of Treatment Programs and the Development of Chemical Baits for Control. American Entomologist: Volume 47 Number 3: 146–179.

Vogt, J. T., J. A. MacGown, J. Lewis, S. Horn, and M. Ulyshen, (2022). Diversity and Seasonal Occurrence of Native and Nonnative Ants (Hymenoptera: Formicidae) in Long-Term Experimental Chinese Privet (Lamiales: Oleaceae) Plots in Georgia, USA. J.Entomol. Sci. 57(2): 297–309. https://doi.org/10.18474/JES21-58.

Zungoli P. A., and E. P. Benson. (2008). Seasonal occurrence of swarming activity and worker abundance of *Pachycondyla chinensis* (Hymenoptera: Formicidae). In: Robinson WH, Bajomi D (eds) Proceedings of the Sixth International Conference on Urban Pests. OOK-Press Kft, Veszprem.

Zungoli, P. A., E. P. Benson, and B. Ellis. (2014). Challenges for successful management of *Pachycondyla chinensis* (Hymenoptera: Formicidae). Proceedings of the Eighth International Conference on Urban Pests. https://cabidigitallibrary.org by 70.33.100.30. pp. 259–263.

Table 3.1 Granular bait products used to evaluate efficacy against *Brachyponera chinensis* (Emery) in outdoor small-scale plots and indoor lab trials.

Bait product	Active Ingredient (AI)	Label rate	Quantity (gms) Field trial [a]	Quantity (gms) Lab trial
Advance® 375 A bait[1]	Abamectin (0.011%)	1 lb. per Acre	1.0	0.023
Advion® Granule bait[2]	Indoxacarb (0.22%)	1.15 lbs./1,000 SF	52.2	1.15
Advion® Fire Ant bait[2]	Indoxacarb (0.045%)	1.5 lbs. per Acre	1.6	0.034
Niban® Granular bait[3]	Orthoboric Acid (5%)	2 lbs./1,000 SF	91.0	2.00

Abbreviations of product full name: [1]BASF Corporation, Triangle Park, NC USA; [2]Syngenta Crop Protection, LLC Greensboro, NC, USA; [3]Niban® Bait Nisus Corporation, Rockford, TN, USA. [a] small scale field trials.

Figure 3.1. *Brachyponera chinensis* foraging on egg yolk.

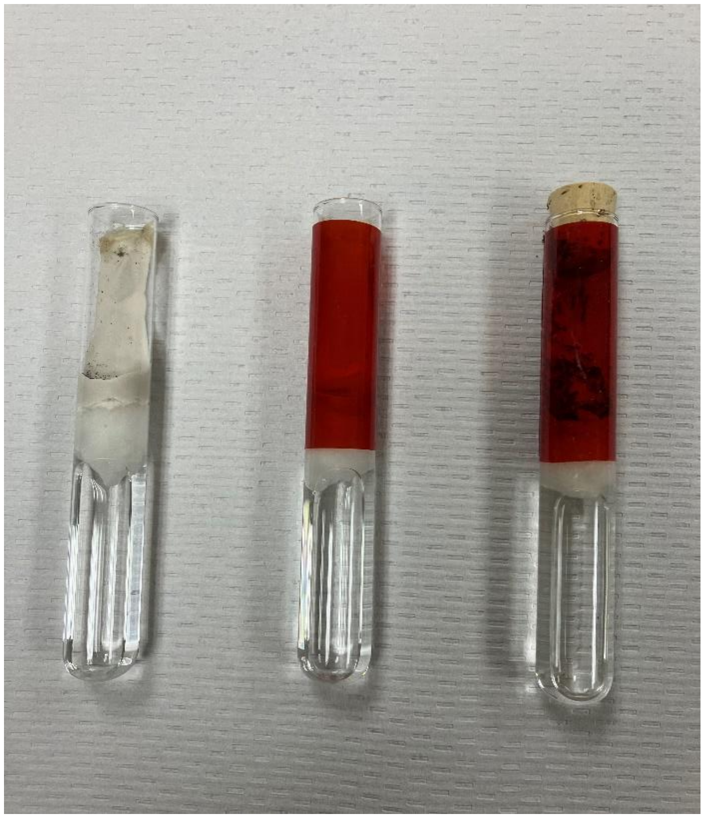


Figure 3.2. From left to right: ≈30 ml water, followed by a cotton plug and enough Castone® to cover the cotton plug with a trail leading to the edge of the lip, the middle tube wrapped in red cellophane wrap, the right tube a cork with a small hole inserted into the opening of the test tube for ant ants to enter and exit.

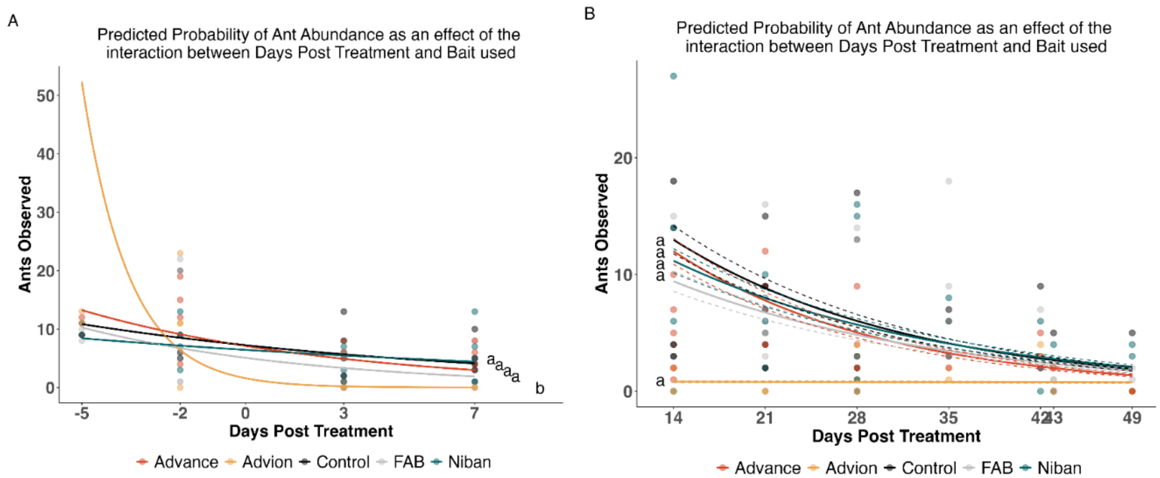


Figure. 3.3. Number of observed between pre-treatment and post-treatment. Four baits were tested in a small-scale field trial from September through October 2022. (A) There is a significant difference in the decrease of ants in the Advion® plots compared to the other baits. (B) After three weeks, there was no significant difference among the baits and the control. The ants observed in Advion® remained steady during the trial. Means followed by the same letter are not significantly different.

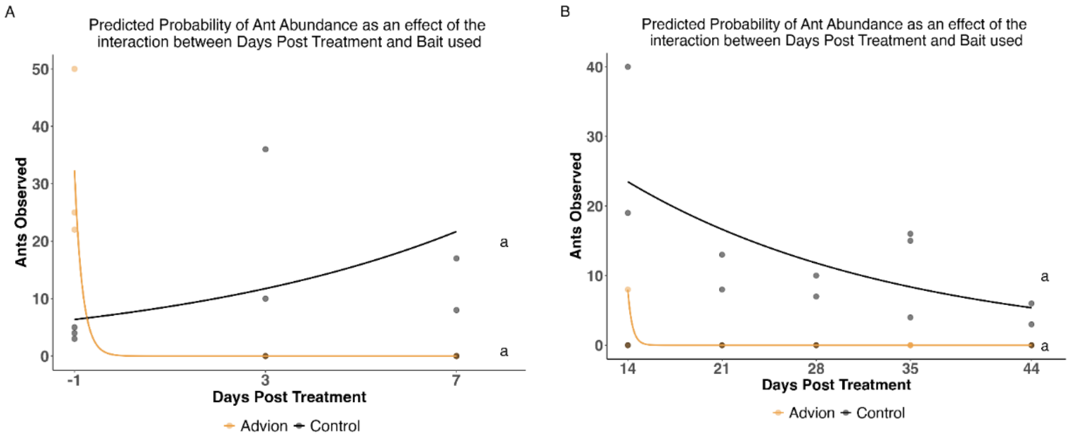


Figure 3.4. Predicted probability of ant abundance as an effect of the interaction between days post-treatment and bait tested in a large-scale field trial from August to September 2023. (A) There was no significant difference between Advion® and the control during the first week. (B) After the second week, the foraging activity decreased in the control group; however, there was no significant difference between Advion® and the control. Ants observed followed by the same letter are not significantly different.

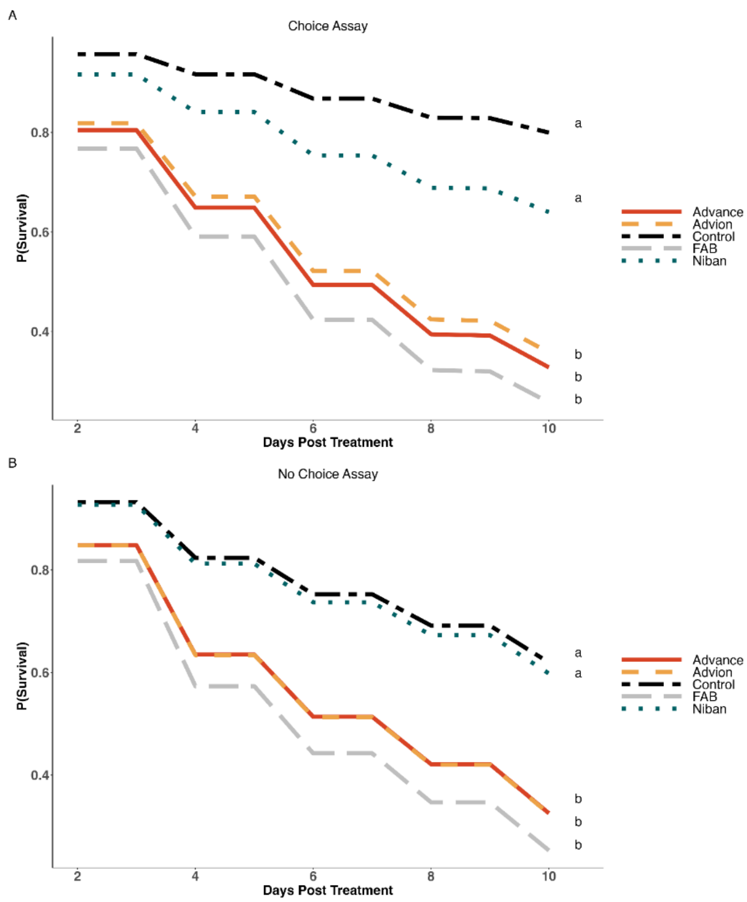


Figure 3.5. Mean probability of survival of *B. chinensis* exposed to selected commercial bait products. (A) a choice trial for ten days, and (B) a no-choice trial for ten days. Five colonies were used for each treatment (N=5). For (A) and (B), survival probabilities followed by the same letter are not significantly different.

CHAPTER 4

SEASONAL ACTIVITY OF THE ASIAN NEEDLE ANT, *BRACHYPONERA* (=*PACHYCONDYLA) CHINENSIS* (EMERY) IN CENTRAL GEORGIA

Karen Corsetti To be submitted to a peer-reviewed journal.

Abstract In this study, pitfall traps were deployed to evaluate the seasonal abundance of *B. chinensis* and other ant fauna. This method has been widely used in entomological research and has proven effective in providing insights into the ecology and behavior of ground-dwelling insects. My objective was to identify months when *B. chinensis* was more abundant than other ant species. Pitfall traps were randomly placed in hardwood forests and an English ivy (*Hedera helix)* area at a residence in Griffin, GA, and an undistributed hardwood forest in Conyers International Horse Park, Conyers, GA. The abundance of ant activity was recorded from August 2022 through August 2023. *Brachyponera chinensis* was most active from June through September. In October, more than half the population of *B. chinensis* declined as the other ant taxa increased. From November through March, documentation was inconsistent until April to August, when *B. chinensis* numbers increased.

Key Words pitfall traps, *Brachyponera chinensis,* Asian needle ant, ant abundance

Introduction

The Asian needle ant, *Brachyponera chinensis* (Emery) (Hymenoptera: Formicidae), is an invasive ant that entered the United States from Japan in the 1930s (Smith 1934). In 1932, H.T. Vanderford collected the first specimens from forests near Decatur, Dekalb County, Georgia (Smith 1934; Nelder et al. 2006; Guénard et al. 2018). Since its introduction, *B. chinensis* has broadened its range in the Southeast. Because they can tolerate cooler temperatures, it has also established in suburban and undisturbed hardwood habitats (MacGown et al. 2013) in northern states such as New York (Pecarevic et al. 2010), Connecticut, Virginia, Wisconsin, Washington DC, New Jersey, Tennessee, Arkansas (Guénard et al. 2018), and Rhode Island (Waters et al. 2022). Although it has expanded its range, the literature on this Ponerine ant's life history and seasonal abundance is deficient (Mo 2013).

Brachyponera chinensis is a significant health threat to humans and animals (Cho et al. 2002; Nelder et al. 2006; Guénard and Dunn 2010). It can inflict a painful, venomous sting that may result in a mild to moderate allergic reaction or anaphylactic shock (Cho et al. 2002; Nelder et al. 2006; Wanandy et al. 2021). Incorrect identification of *B.chinensis* can lead to improper medical attention; patients with anaphylactic reactions may not benefit from treatments designed for other stinging ant species. It also disrupts ecosystem biodiversity by outcompeting native seed-dispersing ants, such as *Aphaenogaster rudis*, and uses native ants as a food source (Rodriguez-Cabal et al. 2011; Warren et al. 2015; Merchlinsky et al. 2023).

The success of *B. chinensis* is credited to its ability to invade forested environments in suburban neighborhoods. They colonize natural habitats characterized by undisturbed deciduous hardwood forests in areas where humans live (Guénard and Dunn 2010; Rice and Silverman 2013; Warren et al. 2015). In recent studies, Grodsky et al. (2018) documented that *B. chinensis* also invades recently harvested pine forests, especially in areas with cut-retained coarse woody debris stored in field rows. Further, *B. chinensis* has been found nesting around buildings, residential areas, city sidewalks, and backyards (Smith 1934; Smith 1947; Guénard and Dunn 2010). They will nest around buildings and landscaping in urbanized areas (Paysen 2007; Rice and Silverman 2013), and they have been reported to inhabit mulch in exhibits at a zoo in Greenville, SC (Nelder et al. 2006). Their nests are commonly found in rotting logs, stumps, and the bark of dying or dead trees (Smith 1934; Creighton 1950; MacGown 2009; Zungoli and Benson 2014). They are ground-dwelling ants that rarely venture onto live foliage (Guénard and Silverman 2011). Their colonies tend to be small and discrete; however, colonies can be several thousand (MacGown 2009) to over 5,000 individuals (Paysen 2007; Zungoli and Benson 2008), and they achieve this by simultaneously occupying multiple nests (polydomy) (Zungoli and Benson 2008; Bednar et al. 2013).

Coexisting native and invasive ant species in the same habitat is a significant challenge to initiating an effective invasive ant management program. Native ants such as *Aphaenogaster rudis*, a seed-dispersing ant (Rodriguez-Cabal et al. 2011; Bednar and Silverman 2011; Buczkowski 2017; Merchlinsky et al. 2023), may be affected by toxic baits or residual sprays used to manage invasive ants (Barbani 2003). This complexity underscores the need for further research and the development of more effective management practices and tactics for invasive ants.

Appropriate sampling methods are essential for monitoring and assessing arthropod diversity and abundance under variable forest management and agricultural land schemes (Guénard and Dunn 2010; Rodriquez-Cabal et al. 2011; Grodsky et al. 2018). Pitfall trapping is a well-established procedure for confirming the presence of ground-dwelling invertebrates and measuring their ecological impact on the environment (Grodsky et al. 2018). Pitfall trapping has been used extensively for ant studies to determine the abundance of invasive and non-invasive ant species, species seasonality, regional distribution, and the impact invasive ants can inflict on native ant diversity (Guénard and Dunn, 2010; Rodriquez-Cabal et al. 2011; Grodsky et al. 2018). In this study, I investigated the seasonal abundance of *B. chinensis* in suburban habitats dominated by undisturbed deciduous hardwood forests in Central GA (U.S.A.). I used pitfall trapping to compare the seasonal activity of *B. chinensis* and other ant taxa.

Materials and Methods

Pitfall trap monitoring was conducted monthly from August 2022 through August 2023. Thirty traps were placed among three sites, each characterized by undisturbed areas dominated by undisturbed deciduous hardwood trees, to monitor the seasonal activity of *B. chinensis* and other ant fauna. Fifteen pitfall traps were placed at Conyers, GA International Horse Park (33°39’33” N83°56’05”W), ten at a residence in Griffin, GA (33°13’52” N84°15’16”W), and five in a wooded area on Brookwood Terrace, Griffin, GA (33°13’50” N84°15’16”W).

Trap placement was determined based on the surrounding habitats where *B. chinensis* colonies were active. Worker ants forage and live in rotting stumps, dead logs, and near hardwood trees, in mulched areas surrounding flower gardens, and under rocks and stones. The pitfall traps were 9-dram plastic vials half-filled with propylene glycol (Camco Mfg., Greensboro, NC, USA) (Figure 4.1A). Vials were deployed and then retrieved after 48 h. A 3.81 cm diameter hole was drilled in the soil (DeWalt D21008, Baltimore, MD), and the vial was inserted into the hole; the vial lip was placed flush with the soil surface (Figure 4.1B). The vial was covered with a red plastic plate suspended 5-7 cm above the vial to prevent litter and rain from entering the vial and to protect the vial from mammals (Figure 4.1C). After 48 h, the vials were collected and returned to the lab, and the ants were counted and separated into two groups: *B. chinensis* and non-*Brachyponera* genera. Counted ants were placed in plastic vials (Sarstedt, Nümbrecht (Germany), North Rhine-Westphalia) containing 5.0 ml of 75% ethanol.

Statistical analysis. Analyses and visualizations of data were completed using R 4.3.2 (R Core Team, 2023). I tested zero-inflated generalized linear mixed effects models using the function "glmmTMB" in the glmmTMB package (Brooks et al. 2017) to determine if the number of *B. chinensis* was more abundant than other ant species within sampling months. The zero-inflated generalized linear mixed effects model is appropriate given that there is an excessive number of zeros within the count data (Lambert 1992). The fixed effects included in the model were the interaction between ant species and the sampling period and site. The random effect included in the model was a random intercept for sampling location to account for the repeated measures nature of the data. The initial model was fit using the Poisson distribution, but when overdispersion was found, the negative binomial (nbinom2) distribution was used. There was a separation issue with the December and January sampling points where *B. chinensis* was not collected. These sampling periods were removed from the analysis to resolve the separation issue. The terms of the zero-inflated formula were tested, and the optimal formula ~ Site+Ant_sp+SamplingPeriod was determined by the lowest AIC value. Post-hoc tests using the emmeans package (Lenth 2024) with Tukey's HSD adjustment for multiple comparisons were performed to determine significant differences between ant species within sampling months.

Results

Three hundred ninety pitfall traps were installed from August 2022 through August 2023. *Brachyponera chinensis* workers captured in pitfall traps totaled 2,503, and other ant fauna totaled 1,410. Several *B. chinensis* workers were captured in November, and no workers were captured from December to January. From February through March, there was scattered foraging activity of *B.chinensis* and other ant fauna, with 18 *B. chinensis* workers captured in pitfall traps during the two months combined.

From April through September, *B.chinensis* was more abundant than non-*Brachyponera* species; in October, their numbers began to decrease. From November through March, very few ants were captured. Compared to other ant fauna, *B. chinensis* trap catch population was greatest in June, July, and August (Figure 4.2). From September to May, *B.chinensis* trap catch was not significantly greater than non-*Brachyponera* trap catches. In April, the foraging activity of *B. chinensis* increased as the population of other ant fauna decreased. The primary invertebrates collected with B. chinensis were sowbugs (Isopoda), millipedes (Diplopoda), and springtails (Collembola).

Discussion

The months that satisfy the objectives for these trials are June, July, and August, during which the abundance of *B. chinensis* exceeds that of other ant species (Figure 4.2). These three months would play a significant role in an effective management program.

Brachyponera chinensis workers and several other ant species were caught in pitfall traps. The dominant non-needle ant species identified were the winter ant, *Prenolepis imparis* (Say), and carpenter ants, *Camponotus* spp. *Prenolepis imparis* prefers cool temperatures between 7.2°C and 15°C (Talbot 1943; Tschinkel 1987). Their above-ground activity begins in November and ends in March or early April when most other ant species are inactive (Tschinkel 1987). *Brachyponera chinensis* has a dominant presence from late April to early October, with numbers almost non-existent from November to March. *Camponotus* spp. share a seasonal overlap with *B. chinensis* from early June until early October (Tripp et al. 2000; Sanders 2012).

Prenolepis imparis and *Camponotus* spp. are omnivores and share similar food resources. Both species collect honeydew and extrafloral nectar from sap-sucking insects such as aphids or scale insects during different times of the season. (Wheeler 1930; Sorrells et al. 201l; Rossi and Feldhaar 2019). Liquid food is stored in the crop, a thin-walled, enlarged part of the gut that stores food before digestion and allows ants to share liquid resources among nest mates through trophallaxis (Paul and Roces 2003; Bednar and Silverman 2011). Based on baiting trials, *P. imparis* also apparently prefers protein-fat food (Lynch et al. 1980), consumes rotting fruit, and exploits protein-rich sources such as dead annelids (Talbot 1943).

Carpenter ants (*Camponotus* spp.) live arboreal lifestyles and forage in tree canopies (Rossi and Feldhaar 2019). They are nocturnal; however, during early spring and summer, they will also forage during the day (Rossi and Feldhaar 2019). *Camponotus* ants also have large crops that allow the transport and storage of large quantities of liquid food (Rossi and Feldhaar 2019). Ants of the subfamily Ponerinae do not take honeydew because they do not possess a crop (Hölldobler and Wilson 1990; Bednar and Silverman 2011). *Bracyhyponera chinensis* has a reduced tarsal arolium, characteristic of ground-dwelling *Brachyponera* species, that prevents them from foraging in trees or shrubs (Guénard and Dunn 2010). Workers are active during the daytime and were observed foraging from 8:00 a.m. to 12:00 p.m. (K.A.C. personal observation; Rice 2012).

Zungoli and Benson (2008) conducted pitfall trap trials near Clemson, South Carolina, to collect *B. chinensis* workers from January 2007 through March 2008. Worker activity was noticed in late March; however, the trap catches spiked in April and gradually increased in May and June. July and August catches significantly increased and lasted through mid-September. The catches decreased in October, and workers were no longer present in pitfall traps by November.

Vogt et al. (2022) conducted pitfall trap trials in northern Georgia within the Oconee River watershed. *Brachyponera chinensis* was captured at Georgia State Botanical Gardens, Sandy Creek Nature Center, University of Georgia Warnell School of Forest Resources Watson Springs Forest, and Scull Shoals Experimental Forest. Fifty native ant species were collected and identified from the pitfall traps. *Brachyponera chinensis* was the most abundant ant captured in the pitfall traps and was 4X more abundant than the next-most collected species, *Lasius americanus* (Emery), a native North American species. Workers were most abundant in the Georgia State Botanical Gardens south of Athens, GA, where they were captured from March through October (excluding September) (N=2,665 needle ants captured). Counting ceased when the number of ants exceeded 1,000 in a sample. August and October samples totaled over 1,000 ants.

Grodsky et al. (2018) incorporated pitfall traps in Georgia pine plantations. Pitfall traps were placed in recently cut harvest residue piles to determine whether timber harvests may facilitate fostering interactions between non-native and native ants. *Brachyponera chinensis* was found to invade coarse woody debris retention in stand-scale treatments and at microsite locations. Several native ant species also favor these areas.

Applying granular baits when *B. chinensis* workers are foraging may reduce the population with only minor non-target ant mortality. *Brachyponera chinensis* is expanding its territory to other states, posing a health threat to humans and possibly animals due to its painful, venomous sting. Understanding an invasive pest's natural history is paramount to designing an integrated management program.

References Cited

Barbani, L. E. (2003). Foraging activity and food preferences of the odorous house ant (*Tapinoma sessile* (Say) (Hymenoptera: Formicidae). M. S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Bednar, D. M., J. Z. Shik, and J. Silverman. (2013). Prey handling performance facilitates competitive dominance of an invasive over native keystone ant. Behav. Ecol. 24:1312-1319.

Bednar, D. M., J. Silverman. (2011). Use of termites, *Reticulitermes virginicus,* as a springboard in the invasive success of a predatory ant, *Pachycondyla* (=*Brachyponera*) chinensis. Insect Soc. 58: 459-467.

Brooks M. E, K. Kristensen, K. J. Van Benthem, A. Magnusson, C. W. Berg A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. (2017). GglmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal. 9: 378-400. Doi: 10.32614/RJ-2017-066.

Buczkowski, G. (2017). Prey-baiting as a conservation tool: selective control of invasive ants with minimal non-target effects. Insect Conservation and Diversity 10, 302– 309.

Creighton, W. S. (1950). The Ants of North America. Bull. Mus. Comp. Zool. 104: 1-585.

Cho, Y.-S., Y.-M. Lee, C.-K. Lee, B. Yoo, H.-S. Park, and H.-B. Moon. (2002). Prevalence of P*achycondyla chinensis* venom allergy in an ant-infested area in Korea. J. Allergy Clin. Immunol. 110: 54-57.

Grodsky, S, M., J. W. Campbell, S. R. Fritts, T. B. Wigley, and C. E. Moorman. (2018). Variable responses of non-native and native ants to coarse woody debris removal following forest bioenergy harvests. Forest Ecology and Management 427 (2018) 414–422.

Guénard, B, and R. R. Dunn. (2010). A new (old) invasive ant in the hardwood forests of eastern North America and its potentially widespread impacts. PLoSONE 5(7): e11614. doi:10.1371/journal.pone. 0011614.

Guénard, B., and J. Silverman. (2011). Tandem carrying, a new foraging strategy in ants: description, function, and adaptive significance relative to other described foraging strategies. Naturwissenschaften. 98: 651–659.

Guénard, B., J. K. Wetterer, and J. A. MacGown. (2018). Global and temporal spread of a taxonomically challenging invasive ant, *Brachyponera chinensis* (Hymenoptera: Formicidae). Florida Entomologist-Volume 101, No. 4:649-656.

Hölldobler, B., and E.O. Wilson. (1990). The Ants. Belknap Press of Harvard University Press, Cambridge p. 732.

Lambert, D. (1992). Zero-inflated poisson regression, with an application to defects in manufacturing. Technometrics. 34: 1-14

Lenth, R (2024). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.0. https://CRAN.R-project.org/package=emmeans.

Lynch, J.F., E. C. Balinsky, and S. G. Vail. (1980). Foraging patterns in three sympatric forest ant species, *Prenolepis imparis, Paratrechina melanderi*, and *Aphaenogaster rudis* (Hymenoptera: Formicidae). Ecological Entomology 5: 353-371.

MacGown, J. A. (2009). The Asian needle ant, *Pachycondyla chinensis* (Emery)

(Hymenoptera: Formicidae), reported from Alabama. Midsouth Entomologist 2: 88–89.

MacGown, J.A., H. Richter, and R. L. Brown. (2013). Notes and new distributional records of invasive ants (Hymenoptera: Formicidae) in the southeastern United States. Midsouth Entomologist 6: 104–114.

Merchlinsky, A., P. T. Frankson, R. Gitzen, C. A. Lepczyk, J. E. Mohan, and R. J. Warren II. (2023). Warming promotes non-invasive ants while inhibiting native ant communities. Ecological Entomology.2023;48:588–596.

Mo, Y. (2013). Temporal food preference and effectiveness of selected bait products against *Pachycondyla chinensis* (Emery) (Hymenoptera: Formicidae). (Masters Thesis) Retrieved from http://tigerprints.clemson.edu/cgi/viewcontent.cgi?article=2650&context=all_theses. Accessed 12 May 2016.

Nelder, M. B., E. S. Paysen, P. A. Zungoli and E. B. Benson. (2006). The introduced ant *Pachycondyla chinensis* (Formicidae: Ponerinae) emerged as a public health threat in the southeastern United States. J. Med. Entomol. 43: 1094–1098.

Paul, J., and F. Roces. (2003). Fluid intake rates in ants correlate with their feeding habits. J. Insect Physiol. 49: 347–357.

Paysen, E. (2007). Diversity and abundance of ants at forest edges. (Doctoral dissertation). https://tigerprints.clemson.edu/ all dissertations/174.

Pecarevic, M. Z., J. Danoff-Burg, R. R. Dunn. (2010). Biodiversity on broadway – enigmatic diversity of the societies of ants (Formicidae) on the streets of New York

City. Plos One 5(10): e13222. Accessed 23 August 2015.

R Core Team. 2023. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org.

Rice, E. S., J. Silverman. (2013). Propagule Pressure and Climate Contribute to the Displacement of *Linepithema humile* by *Pachycondyla chinensis*. PLoS ONE 8(2): e56281.doi:10.1371/journal.pone.0056281.

Rodriguez-Cabal, M. A., K. L. Stuble, B. Guénard, R. R. Dunn, and N. J. Sanders. (2011). Disruption of ant-seed dispersal mutualisms by the invasive Asian needle ant (*Pachycondyla chinensis*). Biological Invasions. 14: 557–565.

Rossi, N. and H. Feldhaar. (2019). Carpenter Ants. Encyclopedia of Social Insects, https://doi.org/10.1007/978-3-319-90306-4_177-1.

Sanders, J. C. (2012). Seasonal And Daily Activity Patterns of Carpenter Ants (*Camponotus* Spp.) In Northwestern Ontario (Hymenoptera: Formicidae). The Canadian Entomologist, Volume 104, Issue 11, pp. 1681 – 1687. DOI: https://doi.org/10.4039/Ent1041681-11.

Smith, M. R. (1934). Ponerine ants of the genus *Euponera* in the United States. Ann. Entomol. Soc. Am. 27: 558–564.

Smith, M. R. (1947). A generic and subgeneric synopsis of the United States ants, based on the workers. Am Midl Nat 37: 521–647.

Sorrells, T. R., L. Y. Kuritzky P. G Kauhanen, K. Fitzgerald, S. J. Sturgis, J. Chen, C. A. Dijamco, K. N. Basurto, and D. M. Gordon. (2011). Chemical defense by the native winter ant (*Prenolepis imparis*) against the invasive Argentine ant (*Linepithema humile*). PLoS ONE 6: e18717.

Talbot, M. (1943). Population studies of the ant *Prenolepis imparis* (Say) Ecology 24: 31-34.

Tripp, Jason M., D. R. Suiter, G. W. Bennett, J. H. Klotz, and B. L. Reid. (2000). Evaluation of Control Measures for Black Carpenter Ant (Hymenoptera: Formicidae). J. Econ. Entomol. 93(5): 1493—1497.

Tschinkel, W. R. (1987). Seasonal life history and nest architecture of a winter-active ant, *Prenolepis imparis*. Insectes Sociaux 34: 143–164.

Vogt, J. T., J. A. MacGown, J. Lewis, S. Horn, and M. D. Ulyshen. (2022). Diversity and Seasonal Occurrence of Native and Nonnative Ants (Hymenoptera: Formicidae) in Long-Term Experimental Chinese Privet (Lamiales: Oleaceae) Plots in Georgia, USA.

Wanandy, T., E. Mulcahy, W. Y. Lau, S. G. A. Brown, M. D. Weise. (2021). Global View on Ant Venom Allergy: from Allergenic components to Clinical Management Clinical Reviews in Allergy & Immunology 62:123– 44 https://doi.org/10.1007/s12016-021-08858-1.

Warren, R. J. II, A. McMillan, J. R. King, L. Chick, and M. A. Bradford. (2015). Forest invader replaces predation but not dispersal services by a keystone species. Biol Invasions (2015) 17:3153–3162 DOI 10.1007/s10530-015-0942-z.

Waters, J. S., N. W. Keough, J. Burt, J. D. Eckel, T. Hutchinson, J. Ewanchuk, M. Rock, J. A. Markert, H. J. Axen, and D. Gregg. (2022). Survey of ants (Hymenoptera, Formicidae) in the city of Providence (Rhode Island, United States) and a new northernmost record for Br*achyponera chinensis* (Emery, 1895.). Check List 18 (6): 1347–1368. https://doi.org/10.15560/18.6.1347.

Wheeler, W. M. (1930). The ant *Prenolepis imparis* (Say). Annals of the Entomological Society of America, 23, 1-26.

Zungoli, P. A. and E. P. Benson. (2008). Seasonal occurrence of swarming activity and worker abundance of *Pachycondyla chinensis* (Hymenoptera: Formicidae). Proceedings of the Sixth International Conference on Urban Pests. pp. 51–57. Budapest, Hungary.

Zungoli, P. A., E. P. Benson,Y. Mo, and B. Ellis (2014). Challenges for successful management of *Pachycondyla chinensis* (Hymenoptera: Formicidae). Proceedings of the Eighth International Conference on Urban Pests. https://cabidigitallibrary.org by 70.33.100.30. pp. 259–263.

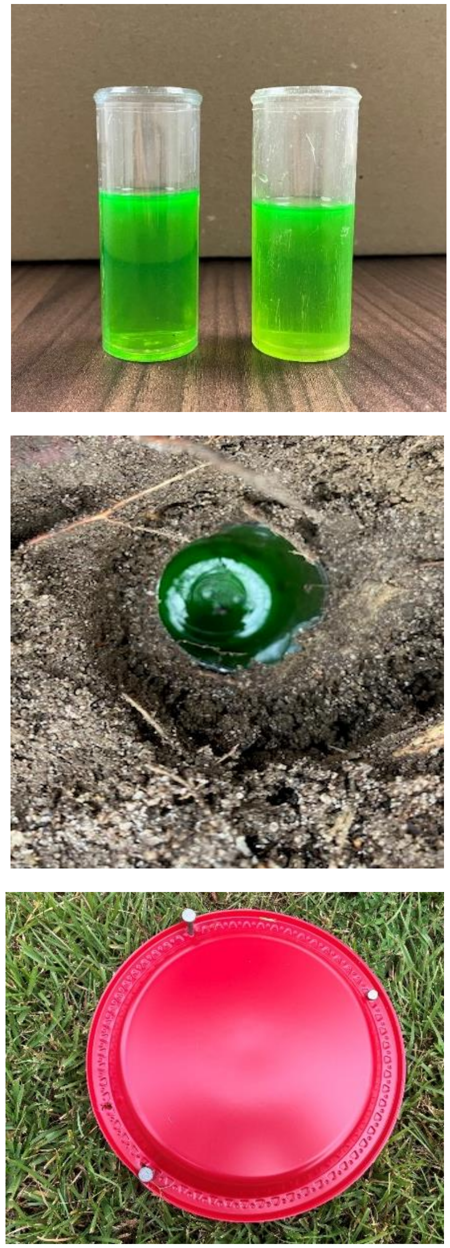


Figure 4.1. Pitfall trap set-up. Top: 9-dram vial half-filled with propylene glycol; Middle: vial drilled into the ground; Bottom: plate placed over the vial and secured with three nails to protect against excessive rain.

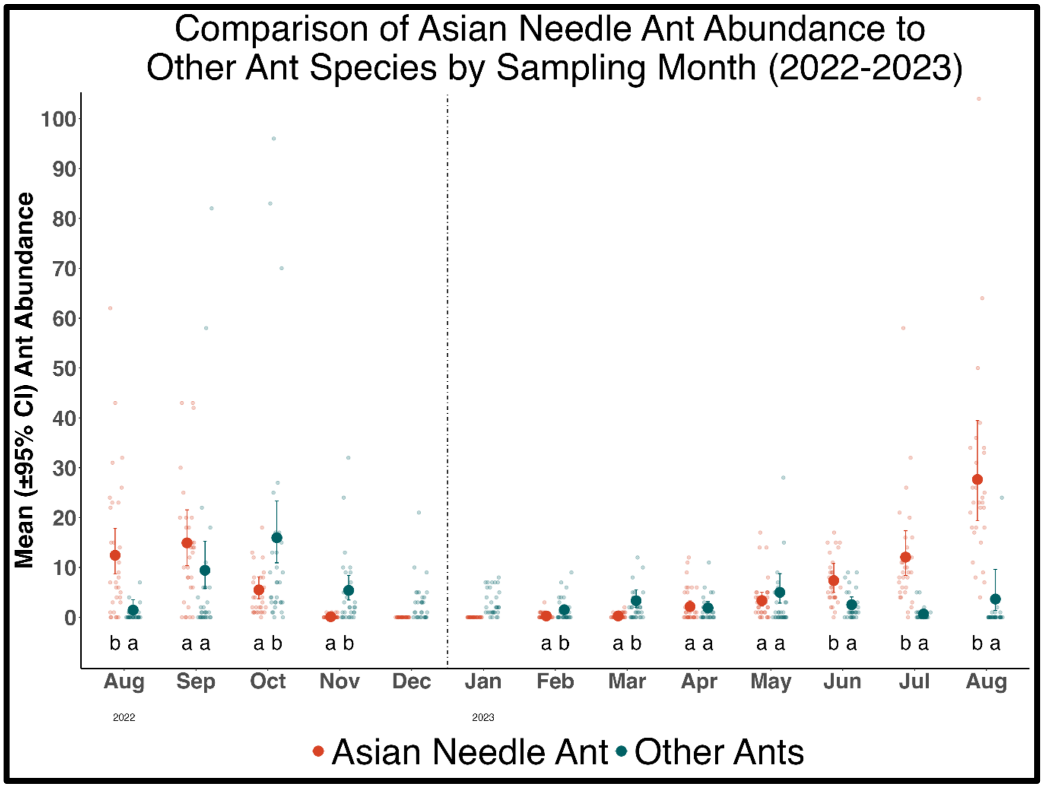


Figure 4.2. Abundance of *B. Chinensis* and other ant species in pitfall traps (N=30) from August 2022 through August 2023. Pairwise monthly comparisons indicate their abundance is significantly greater than other ant fauna. For each month, means followed by the same letter are not significantly different (P<0.05).

CHAPTER 5

TEMPORAL FOOD PREFERENCE IN *BRACHYPONERA CHINENSIS* (EMERY 1895) (HYMENOPTERA: FORMICIDAE)

Karen Corsetti To be submitted to a peer-reviewed journal.

Abstract Bait distribution can only be effective with knowledge of foraging activity patterns, biology, colony life cycle patterns, feeding preferences, and the attractiveness of bait carriers among foraging ant species. Food preference trials determine an attractive and palatable food source for foragers that consume and transport the bait to their colony. Here, we assessed the foraging activity of the Asian needle ant, *Brachyponera chinensis*, and food preference under field conditions from November 2022 through November 2023 by attracting them to ceramic tiles containing four food choices: egg yolk, sardine, anchovy, and tuna/sucrose. The tiles were randomly placed on the forest floor, and the number of ants in each food quadrant was recorded after one hour**.** My trials evaluated four food choices containing protein, carbohydrates, and lipids. Food preference trial results indicated that sardine, egg yolk, and tuna/sucrose were preferred over anchovy.
Key Words Asian needle ant, *Brachyponera chinensis*, bait, tandem carrying, food preference

Introduction

The Asian needle ant, *Brachyponera chinensis*, was introduced into the United States (U.S.) by accident in the early 1930s (Smith 1934; Nelder et al. 2006). In 1932 H.T. Vanderford collected the first record of *B. chinensis* in the continental United States in forests near Decatur, Dekalb County, Georgia (Smith 1934; Nelder et al. 2006; Guénard et al. 2011). Asian needle ants are native to China, Japan, Korea (Guénard and Silverman 2011), New Zealand, Vietnam, Thailand, Sri Lanka, India, Guam, Papua New Guinea, the Philippines, and Nepal (Nelder et al. 2006; Mo 2013).

Since 1932, *B. chinensis* has broadened its range in the southeastern U.S. (Alabama, Georgia, North Carolina, South Carolina, and Virginia). Because it can tolerate cooler temperatures (Rice and Silverman 2013), this affords them the ability to colonize northern states such as New York State (Pecarevic et al. 2010), Connecticut, Washington State, New Jersey, Tennessee, and Rhode Island (Waters et al. 2022), making its way into suburban and natural habitats (Smith 1934; Nelder et al. 2006; Guénard and Dunn 2010; Rodriguez-Cabal 2011; MacGown et al. 2013).

In mature temperate forests, *B. chinensis* is a potential threat to ecological systems that can cause a decline in native ant diversity and abundance (Guénard and Dunn 2010; Buczkowski 2017). They may disrupt ant-seed dispersal mutualisms by dislodging a native keystone ant species, *Aphaenogaster rudis* (Enzmann). *Brachyponera chinensis* may take over their nest sites, causing a failure in seed dispersal, and will also use *A. rudis* and other native ants as a food source (Guénard and Dunn 2010; Rodriguez-Cabal et al. 2011; Warren et al. 2015; Buczkowski 2017; Merchlinsky et al. 2023).

The biology of *B. chinensis* foraging behavior is unique among invasive ants (Buczkowski 2016), making it challenging to design and conduct an effective management program. Most invasive ants utilize carbohydrate-rich food consisting of floral nectar and hemipteran honeydew (Lanza et al.1993; Holway et al. 2002; Jordan et al. 2013; Buczkowski 2016). However, Bednar and Silverman (2011) found no evidence that *B. chinensis* workers tend to honeydew-excreting hemipterans. *Brachyponera chinensis* prefer live, protein-rich foods such as termites (MacGown 2009; Bednar and Silverman 2011; Mo 2013), native ant species, *A. rudis* (Bednar and Silverman 2011), and invasive ant species such as Argentine ants (*Linepithema humile* Mayr) (Rice 2012). They will also feed on small arthropods like springtails, pill bugs, dead insects, fish scraps, and spiders (Smith 1934; Smith 1947; MacGown 2009).

Ants may recruit nestmates via trail pheromones to retrieve food resources when the resource's size, density, or quality is too large and bulky for one worker (Breed et al. 1987; Traniello et al. 1989). Worker ants search for food as individuals or as groups. When workers locate a food item, they bring it directly to the nest, or the worker ant will return to their colony and recruit nestmates (Carroll and Janzen 1973). Ant species that are solitary foragers occur in the Ponerinae and Myrmeciinae subfamilies (Traniello 1989). Most invasive ant species use mass recruitment via trail pheromones to collect food (Beckers et al. 1989; Traniello 1989; Roulston and Silverman 2002; Guénard and Silverman 2011). *Brachyponera chinensis* (Ponerinae) does not employ trail pheromones when they forage (Mo 2013; Buczkowski 2016). They forage in a manner that appears random and without organized cooperation or communication to locate, capture, or transport a food source (Beckers et al. 1989). According to Guénard and Silverman (2011), when workers encounter a food source they incorporate a foraging strategy called tandem carrying, a simple recruitment strategy observed in Ponerine ant species. The scout will drum its antennae on potential recruits until the recruited ant responds to the request (Guénard and Silverman 2011). The recruit chosen worker positions itself onto the scout, now called the carrier, for transportation to the food source, where the carrier releases it (Guénard and Silverman 2011). The scout and recruit then deconstruct the food source and return it to the nest.

Brachyponera chinensis can colonize natural habitats, such as deciduous hardwood forests, and suburban habitats such as residential backyards characterized by hardwood trees (Smith 1934; Smith 1947; Guénard and Dunn 2010). These ants are ground-dwelling and do not build traditional flat or dome-shaped mounds (Mo 2013). This creates a challenge for homeowners when locating active colonies near their property. Colonies are found in damp soil within rotting logs or stumps, underneath the bark of dying hardwood trees (Smith 1934; Creighton 1950; MacGown 2009; Zungoli and Benson 2014), and in cracks and crevices in driveways and cement patios (personal experience).

The critical ingredient in designing a bait matrix is a preferred food source acceptable to the worker ants (Klotz et al. 1997; Mo 2013). Laboratory and field studies on food preference have used protein, lipids, and carbohydrates, which might be incorporated into a bait mixture (Barbani 2003; Mo 2013). Food preference trials focus on determining an attractive and palatable food source that might be used in bait (Klotz et al. 1997; Mo 2013).

Food preference trials for invasive ants. The foods used in previous trials for invasive ants ranged from protein products such as tuna, anchovies, sardines, dog food, fish meal, and eggs to various commercial oils such as peanut and vegetable oil and sugars such as honey and sucrose (Baker et al. 1985; Hooper and Rust 1997; Mashaly et al. 2013; Nyamukondiwa and Addison 2014). Understanding the specific food preferences of *B. chinensis* is crucial for developing an effective bait.

Buczkowski (2016, 2017) evaluated a novel method for managing *B. chinensis* using Eastern subterranean termites, *Reticulitermes flavipes* (Kollar) as live prey exposed to fipronil. These trials concluded that termites exposed to fipronil can cause complete mortality in *B. chinensis* field and lab colonies, underscoring the importance of understanding *B. chinensis's* food preferences.

Baker et al. (1985) performed bait-preference tests on the Argentine ant. In the field, the preferred foods were 25% honey or sucrose water over granulated brown sugar or other solid foods with high protein content, such as corn and tuna meal. In laboratory studies, egg white was the only protein significantly elevated feeding.

Hooper and Rust (1997) investigated the foraging activity and food preferences of the southern fire ant, *Solenopis xyloni* (McCook). The authors used a combination of readily available foods incorporated into a bait mixture and conducted choice tests to determine *S. xyloni's* most preferred diet. The Keller diet (K-diet) (Keller et al. 1989) was modified to substitute tuna, anchovy, sardine, or mealworms for beef hash as a protein source. Various K-diets were prepared from the chosen protein sources mixed with cornmeal. The authors speculated that *S. xyloni* may be attracted to fish (their personal observations), where *S. xyloni* was seen carrying and feeding on dead fish and bird scat. The diet was then baked or freeze-dried to formulate varying particle-sized granules. The authors concluded that freeze-dried diets containing anchovy were preferred over the other diet mixtures. Future studies are needed to determine the destination and use of each particle size within the colony.

In South Africa, Nyamukondiwa and Addison (2014) assessed ground and vine foraging activity and food preferences for three ant species: the Argentine ant, Cocktail ant, *Crematogaster peringueyi* (Emery), and the common pugnacious ant, *Anoplolepis custodiens* (F. Smith). The authors constructed a bait matrix using the following food attractants: 25% sugar solution, agar (in 25% sugar solution), tuna, honey, dog food, dry fish meal, dry sorghum grit, and 25% peanut butter (in distilled water). Food preference trials indicated that the ants preferred wet bait attractants over dry ones, making liquids the ideal carriers for baiting these ants.

Mashaly et al. (2013) investigated the foraging activity and food preferences of the samsum ant, *Pachycondyla sennaarensis* (Mayr). Trials were conducted at King Saud University, Kingdom of Saudi Arabia. Field and laboratory tests utilized a variety of proteins, carbohydrates, and lipids. The carbohydrate items were mixed fruit jam, strawberry jam, raspberry jam, marmalade, pineapple jam, fruit juice, and aqueous solutions of 20% maltose, sucrose, fructose, and glucose. The proteinaceous food items were dried fish, chicken sausage, tuna fish, cockroaches (animal protein), minced meat, wheat, beans, groats, rice, sesame, and lupine (vegetal proteins). Sunflower, fish, olive, and sesame oil were evaluated as lipid sources. *Pachycondyla sennaarensis* consistently preferred protein and carbohydrate to lipids. However, the results indicated that minced meat was consumed significantly more than other proteinaceous foods. Lipids were generally ignored by *P. sennaarensis*.

The objective of my study was to better understand the foraging activity and food preferences of *B. chinensis.* Asian needle ants are generally diurnal, solitary foragers who will forage until they find a food source. When an acceptable food source is encountered, the worker ant will retrieve the food and bring it to the colony or return to the colony to recruit a nestmate. Accurate information about the life history of the Asian needle ant will assist in executing better monitoring programs.

Materials and Methods

Food Preference Study in the Field. Food preference trials were conducted monthly from August 2022 to August 2023. Three protein foods and one protein/sucrose matrix were assessed for their palatability to *B. chinensis*. These included: (1) water-packed tuna (5-ounce can) (StarKist Co. Pittsburgh, PA) mixed with 5 ml Karo corn syrup (water was drained prior to adding the syrup) (ACH Food Companies, Inc. Chicago, IL), (2) King Oscar skinless & boneless sardines in olive oil (Tri-Union Seafoods, LLC EL Segundo, CA), (3) flat anchovy in vegetable oil (Kroger Company, Cincinnati, OH), (4) 5 gms boiled chicken egg yolk mixed with 15 ml of water to make the yolk stick together to form a ball. The protein foods were homogenized with a mortar and pestle to pulverize the food ingredients into a paste. All food was prepared in the laboratory.

Thirty food tiles were randomly placed among three sites, each characterized by undisturbed areas dominated by mature hardwood trees. Fifteen tiles were placed at the International Horse Park, Conyers, GA (33°39’33” N83°56’05”W),10 at a residence in Griffin, GA (33°13’52” N84°15’16” W), and five at Brookwood Terrace, Griffin, GA (33°13’50” N84°15’16” W). Tiles were placed in areas where *B. chinensis* colonies were active and foraging (Figure 5.1).

White ceramic tiles (15.24 cm x 15.24 cm) were divided into four equal-sized quadrants, and a ~ 4 gm dollop of food was placed in the center of each quadrant (Figure 5.1). The tiles were randomly placed and leveled with the soil to ensure foraging ants could walk on them. The number of workers in each food quadrant was recorded one hour after the tiles were deployed. A foraging ant was defined as moving onto a tile quadrant regardless of whether the ant was seen feeding on the food.

Statistical analysis. Analyses and visualizations of data were completed using R 4.3.2 (R Core Team, 2023). I tested zero-inflated generalized linear mixed effects models using the function "glmmTMB" in the glmmTMB package (Brooks et al. 2017) to determine if the number of *B. chinensis* workers was actively foraging compared to other ant species. The zero-inflated generalized linear mixed effects model is appropriate given that there is an excessive number of zeros within the count data (Lambert 1992). The fixed effects included in the model were the interaction between ant species and the sampling period and site. The random effect included in the model was a random intercept for sampling location to account for the repeated measures nature of the data. The initial model was fit using the Poisson distribution, but when overdispersion was found, the negative binomial (nbinom2) distribution was used. There was a separation issue with the early November to late July sampling points where *B. chinensis* was not foraging. These sampling periods were removed from the analysis to resolve the separation issue. The terms of the zero-inflated formula were tested, and the optimal formula ~ Site+Ant_sp+SamplingPeriod was determined by the lowest AIC value. Post-hoc tests using emmeans package (Lenth 2024) with Tukey's HSD adjustment for multiple comparisons were performed to determine significant differences between ant species within sampling months and perhaps bait development.

Results

The number of ants on the tiles varied throughout the study. From early November to early July, *B. chinensis* workers were rarely found on tiles. However, other ant fauna were found on the tiles during this time (Figure 5.1A). *Brachyponera chinensis* foraged on the tiles from late July to early September, when the presence of other ant fauna, especially the winter ant, *Prenolepis impairs,* decreased (Figure 5.1B).

During their active period, the mean number of worker ants visiting sardines (7.1) was significantly greater than the number of worker ants visiting anchovies (3.3) (Figure 5.2). Moreover, there was no significant difference in the number of *B.chinensis* ants visiting egg (3.6), sardine, or tuna/corn syrup (6.2). The mean number of workers visiting anchovy was not significantly different from the number visiting egg and tuna/sucrose. Food preference trials help determine a food choice that is an essential ingredient for a bait granule. If the bait is not palatable to the worker ant, it will reject it. *Brachyponera chinensis* are primarily protein feeders and will feed on all types of arthropods and fish scraps (Smith 1934). *Brachyponera chinensis* showed a preference for sardine, tuna/sucrose, egg yolk, and sardine, respectively.

Discussion

In this study, late July and early August were the height of *B. chinensis's* response to food baits. The last recording of *B. chinensis* was in early October when the ants visited the four food choice tiles. Food transport by visiting Asian needle ants was not recorded. However, during the hour trial, I observed worker ants visiting more than one quadrant and leaving with food pieces in their mandibles. I never observed tiles that contained *B. chinensis* and any other ant species simultaneously.

In conjunction with food preference trials, pitfall traps were also deployed to assess *B. chinensis* activity (Chapter 3). Worker foraging activity was first detected in pitfall traps in April, increased from June to August, and remained high through September, with July having the highest foraging activity (Figure 3.2). A noticeable decline in *B. chinensis* activity was noted from November to March, with no foraging activity detected during December and January, similar to the food preference trial months. From February to March, there was scattered foraging activity by *B.chinensis* (Chapter 3); however, during these months, no foraging activity was recorded in the food preference trials.

Food preferences are not solely determined by the type of food available. The size of the colony, environmental factors, foraging strategies, and the physical state of the foragers all play a significant role. The colony's size, the type of food exploited, and changes in nutritional demands associated with egg laying and reproduction may shift a colony's foraging activity among different food types (Beckers et al. 1989; Traniello 1989).

For a colony to flourish and survive, adequate nutrition is paramount for brood production and worker survival (Feldhaar 2014; Csata and Dussutour 2019; Renyard et al. 2024). When larvae are present in the colony, ants will collect more food overall, and a higher proportion of that food will be protein (Dussutour and Simpson 2012; Feldhaar 2014). Ants assess food quality through the presence and concentration of certain macronutrients (proteins, carbohydrates, lipids) and micronutrients (e.g., salts, vitamins) (Renyard et al. 2024; Csata and Dussutour 2019). However, ant nutritional needs change in response to varying environmental conditions and demands for growth, health, and reproduction (Csata and Dussutour 2019; Renyard et al. 2024).

Ant colonies require carbohydrates as an energy source to support worker ant longevity and activity (Shik and Silverman 2013; Renyard et al. 2024). Protein sources, in combination with carbohydrates, are essential for egg production by queens and brood development (Shik and Silverman 2013; Renyard et al. 2024), but in high amounts, protein shortens the lifespan in solitary insects (Dussutour and Simpson 2012; Renyard et al. 2024). Lipids are essential for egg production, larval growth, and ovary development (Blüthgen and Feldhaar 2009; Csata and Dussutour 2019) but do not support metabolism in adults nor for sperm production, which requires only carbohydrates (Blüthgen and Feldhaar 2009).

Tripp et al. (2000) conducted seasonal food preference assays of the black carpenter ant, *Camponotus pennsylvanicus.* During the evenings in June and August, foraging ants were provided a choice of diced mealworms for protein and clover honey for carbohydrates. The results indicated a strong dependency between food type and time of year. The ants preferred proteins in early June, then shifted to carbohydrates in late August.

To evaluate the ecological significance of changes in nectar among plants' effectiveness of ant attraction, different extrafloral nectaries were compared with nectar mimics that varied in either amino acid or sugar content. Extrafloral nectar is produced by plants on stems and leaves to attract predatory insects, such as ants, to protect plants from herbivores. Lanza et al. (1993) compared the feeding preferences of the imported fire ant, *Solenopsis invicta*, and the native fire ant, *S. geminate* (Fabricius), when offered amino acids and sugar components. Workers of both ant species were fed on artificial nectaries containing mimics of pre- and post-defoliation nectars of *Impatiens sultani* (Hook), which differed in amino acid content. They were also fed from artificial nectaries containing mimics of *Passiflora quadrangularis*, *Passiflora ambigua* (Hemsl), *and Passiflora talamancensis*, which varied in sugar composition. Results from the amino acid trials indicated that *S. geminata* preferred postdefoliation mimics of *I. sultani* extrafloral nectars, which are rich in amino acid content, over predefoliation mimic with a relatively lower amino acid content. *Solenopsis invicta* did not distinguish between pre-and postdefoliation nectars. For the sugar preference tests, *S. geminata* demonstrated a preference for the sugars of the extrafloral nectar of *P. ambigua* over those of the extrafloral nectar of *P. quadrangularis; S. geminata* did not discriminate between the sugars of *P. ambigua* and *P. talamancensis* nectars. The authors compared their results to the optimal foraging theory (OFT), which explains how ants forage to maximize their fitness (Kay 2002; Jordan et al. 2013). Sugar, for instance, is an energy substance. However, both ant species fed favorably from the nectar of *P. ambigua*, which was lowest in sugar concentration and energy content. Variables (viscosity, individual sugar concentrations, total sugar concentration, and sugar balance) presented by the authors may represent possible explanations contrasting with the optimal foraging theory.

References Cited

Baker, T. C., S. E. Van Vorhis Key , and L. K. Gaston. (1985). Bait-preference tests for the Argentine ant (Hymenoptera: Formicidae). Journal of Economic Entomology 78: 1083-1088.

Barbani, L. E. (2003). Foraging activity and food preferences of the odorous house ant (*Tapinoma sessile* Say) (Hymenoptera: Formicidae). M. S. Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Beckers, R., S. Goss, J. L. Deneubourg, and J. M. Pasteels. (1989). Colony size, communication, and ant foraging strategy. Psyche 96:239-256.

Blüthgen, N., and H. Feldhaar. (2009). Food and Shelter: How Resources Influence Ant Ecology. DOI: 10.1093/acprof:oso/9780199544639.003.0007.

Breed, M. D., J. H. Fewell, A. J. Moore and K. R. Williams. (1987). Graded recruitment in a Ponerine ant. Behavioral Ecology and Sociobiology. 20: 407-411.

Brooks, M. E, K. Kristensen, K. J. Van Benthem, A. Magnusson, C.W. B. A. Nielsen, H. J. Skaug, M. Maechler, and B. M. Bolker. (2017). GglmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal. 9: 378-400. Doi: 10.32614/RJ-2017-066.

Bednar, D. M., J. Silverman. (2011). Use of termites, *Reticulitermes virginicus*, as a springboard in the invasive success of a predatory ant, *Pachycondyla* (=*Brachyponera) chinensis*. Insect Soc. 58: 459-467.

Buczkowski, G. (2016). The Trojan horse approach for managing invasive ants: a study with Asian needle ants *Pachycondyla chinensis*. Biol Invasions 18: 507-515.

Buczkowski, G. (2017). Prey-baiting as a conservation tool: selective control of invasive ants with minimal non-target effects. Insect Conservation and Diversity 10, 302 –309.

Carroll, C. R., and D. H. Janzen. (1973). Ecology of foraging by ants. Ann. Rev. Ecol. Syst. 4:231-57.

Csata, E. and A. Dussutour. (2019). Nutrient regulation in ants (Hymenoptera: Formicidae): A review. *Myrmecol. News* 29, 111–124.

Creighton, W. S. (1950). The Ants of North America. Bull. Mus. Comp. Zool. 104: 1–585.

Dussutour, A., and S. J. Simpson. (2012). Ant workers die young and colonies collapse when fed a high-protein diet. R. Soc. B 279, 2402–2408 doi:10.1098/rspb.2012.0051.

Feldhaar, H. (2014). Ant nutritional ecology: Linking the nutritional niche plasticity on individual and colony-level to community ecology. *Curr. Opin. Insect Sci.* **5**, 25–30 (2014).

Guénard, B., R. R. Dunn (2010). A new (old) invasive ant in the hardwood forests of eastern North America and its potentially widespread impacts. PLoSONE 5(7): e11614. doi:10.1371/journal.pone. 0011614.

Guénard, B., and J. Silverman. (2011). Tandem carrying, a new foraging strategy in ants: description, function, and adaptive significance relative to other described foraging strategies. Naturwissenschaften. 98: 651-659.

Holway, D. A., L. Lach, A. V. Suarez, N. D.Tsutsui, and T. J. Case. (2002). Causes and consequences of ant invasions. Annu Rev Ecol Evol Syst 33:181–233.

Hooper, L. M., and M. K. Rust. (1997). Food preference and patterns of foraging activity of the southern fire ant (Hymenoptera: Formicidae). Annals of the Entomological Society of America 90(2): 246–253.

Jordan, B W., B. E. Bayer, P. G. Koehler and R. M. Pereira. (2013). Bait Evaluation Methods for Urban Pest Management. http://dx.doi.org/10.5772/53421.

Kay, A. (2002). Optimal Foraging Theory to Assess Nutrient Availability Ratios for Ants. Ecology, 83(7), pp. 1935–1944 q 2002.

Keller, L., D. Cherix, and P. Uoa -Chacon. (1989). Description of a new artificial diet for rearing ant colonies such as *Iridomyrmex humilis, Monomorium pharaonis,* and *Wasmannia auropunctata* (Hymenoptera: Formicidae). Insectes Soc. 36: 348-352.

Klotz, J., D. F. Williams, B. Reid, K. M. Vail, and P. Koehler. (1997). Ant trails: A key to management with baits. University of Florida, Cooperative Extension service, Institute of Food and Agricultural Sciences.

Lambert, D. (1992). Zero-inflated poisson regression, with an application to defects in manufacturing. Technometrics. 34: 1–14.

Lanza, J., E. L. Vargo, S. Pulim and Y. Z. Chang. (1993). Preferences of the fire ants *Solenopsis invicta* and *S. geminate* (Hymenoptera: Formicidae) for amino acid and sugar components of extrafloral nectars. Journal of Environmental Entomology 22(2): 411–417.

Lenth, R. (2024). emmeans: Estimated Marginal Means, aka Least-Squares Means. R package version 1.10.0. https://CRAN.R-project.org/package=emmeans.

Mashaly, A. A. M., F. A. Al-Mekhlafi1, and A. M. Al-Qahtani. (2013). Foraging activity and food preferences of the samsum ant, *Pachycondyla sennaarensis*. Bulletin of Insectology 66 (2): 187-193, ISSN 1721-8861.

MacGown, J.A. (2009). The Asian needle ant *Pachycondyla chinensis* (Emery) (Hymenoptera: Formicidae) was reported from Alabama. Midsouth Entomologist 2: 88–89.

MacGown, J.A., H. Richter, and R. L. Brown. (2013). Notes and new distributional records of invasive ants (Hymenoptera: Formicidae) in the southeastern United States. Midsouth Entomologist 6: 104–114.

Merchlinsky, A., P. T. Frankson, R. G. Christopher, A. Lepczyk, J. E. Mohan, and R. J. Warren II. (2023). Warming promotes non-native invasive ants while inhibiting native ant communities. Ecological Entomology. 2023;48:588–596.

Mo, Y. (2013). Temporal food preference and effectiveness of selected bait products against *Pachycondyla chinensis* (Emery) (Hymenoptera: Formicidae). (Masters Thesis) Retrieved from http://tigerprints.clemson.edu/cgi/viewcontent.cgi?article=2650&context=all_theses. Accessed 12 May 2016.

Nelder, M. B., E. S. Paysen, P. A. Zungoli, and E. B. Benson. (2006). The introduced ant *Pachycondyla chinensis* (Formicidae: Ponerinae) emerged as a public health threat in the southeastern United States. J. Med. Entomol. 43: 1094–1098.

Nyamukondiwa, C., and P. Addison. (2014). Food preference and foraging activity of ants: Recommendations for field applications of low-toxicity baits. Journal of Insect Science: Vol. 14 | Article 48.

Pecarevic, M. Z., J. Danoff-Burg, and R. R. Dunn. (2010). Biodiversity on broadway – enigmatic diversity of the societies of ants (Formicidae) on the streets of New York City. Plos One 5(10): e13222. Accessed 23 August 2015.

R Core Team. (2023). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org.

Rice, E. S. (2012). The species overlap of *Pachycondyla chinensis* and *Linepithema humile*: contributing mechanisms and consequences. Dissertation, North Carolina State University, Raleigh, NC.

Rice E. S., J. Silverman. (2013). Propagule Pressure and Climate Contribute to the Displacement of *Linepithema humile* by *Pachycondyla chinensis*. PLoS ONE 8(2): e56281.doi:10.1371/journal.pone.0056281**.**

Renyard, A., C. Gooding, J. M. Chalissery, J. Petrov and G. Gries. (2024). Effects of macro- and micro-nutrients on momentary and season-long feeding responses by select species of ants. https://doi.org/10.1038/s41598-024-56133-y.

Rodriguez-Cabal, M. A., K. L. Stuble, B. Guénard, R. R. Dunn, and N. J. Sanders. (2011). Disruption of ant-seed dispersal mutualisms by the invasive Asian needle ant (*Pachycondyla chinensis*). Biological Invasions. 14: 557–565.

Roulston, T'ai H., and J. Silverman. (2002). The Effect of Food Size and Dispersion Pattern on Retrieval Rate by the Argentine Ant, *Linepithema humile* (Hymenoptera: Formicidae). Journal of Insect Behavior, Vol. 15, No. 5.

Shik, J. Z. and J. Silverman. (2013). Towards a nutritional ecology of invasive establishment: Aphid mutualists provide better fuel for incipient Argentine ant colonies than insect prey. *Biol. Invasions* 15, 829–836 (2013).

Smith, M. R. (1934). Ponerine ants of the genus *Euponera* in the United States. Ann. Entomol. Soc. Am. 27: 558–564.

Smith M. R. (1947). A generic and subgeneric synopsis of the United States ants, based on the workers. Am Midl Nat 37: 521–647.

Traniello, J. R. (1989). Foraging strategies in ants. Ann. Rev. Entomol. 34: 191-210.

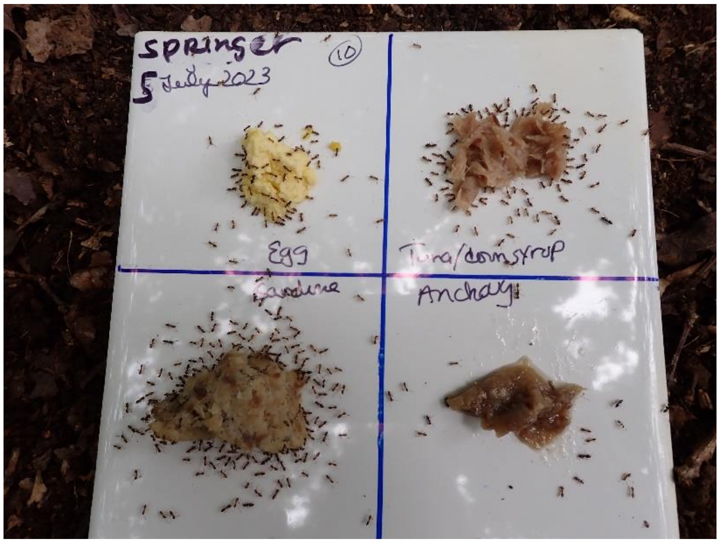
Tripp, Jason M., D. R. Suiter, G. W. Bennett, J. H. Klotz, and B. L. Reid. (2000). Evaluation of Control Measures for Black Carpenter Ant (Hymenoptera: Formicidae). J. Econ. Entomol. 93(5): 1493—1497.

Warren, R.J. II, A. McMillan, J.R. King, L. Chick, and M.A. Bradford, M.A. (2015). Forest invader replaces predation but not dispersal services by a keystone species. Biol Invasions (2015) 17:3153–3162 DOI 10.1007/s10530-015-0942-z.

Waters, J.S., N. W. Keough, J. Burt, J. D. Eckel, T. Hutchinson, J. Ewanchuk, M. Rock, and J.A. Markert, H. J. Axen, and D. Gregg. (2022). Survey of ants (Hymenoptera, Formicidae) in the city of Providence (Rhode Island, United States) and a new northernmost record for Br*achyponera chinensis* (Emery, 1895.). Check List 18 (6): 1347–1368. https://doi.org/10.15560/18.6.1347.

Zungoli, P. A., E. P. Benson, and B. Ellis. (2014). Challenges for successful management of *Pachycondyla chinensis* (Hymenoptera: Formicidae). Proceedings of the Eighth International Conference on Urban Pests. Https://cabidigitallibrary.org by 70.33.100.30. pp. 259–263.

A



B

Figure 5.1. Ceramic tile with the four food groups: egg (upper left), tuna/corn syrup (upper right), anchovy (lower right), and sardine (lower left). Each quadrant contained ≈ 4 gms of food, and the number of ants in each quadrant was counted one hour after the tiles were deployed.

(A) Non-*B. chinensis* workers were response to food types;

(B) *Brachyponera chinensis* worker ant response to food types.

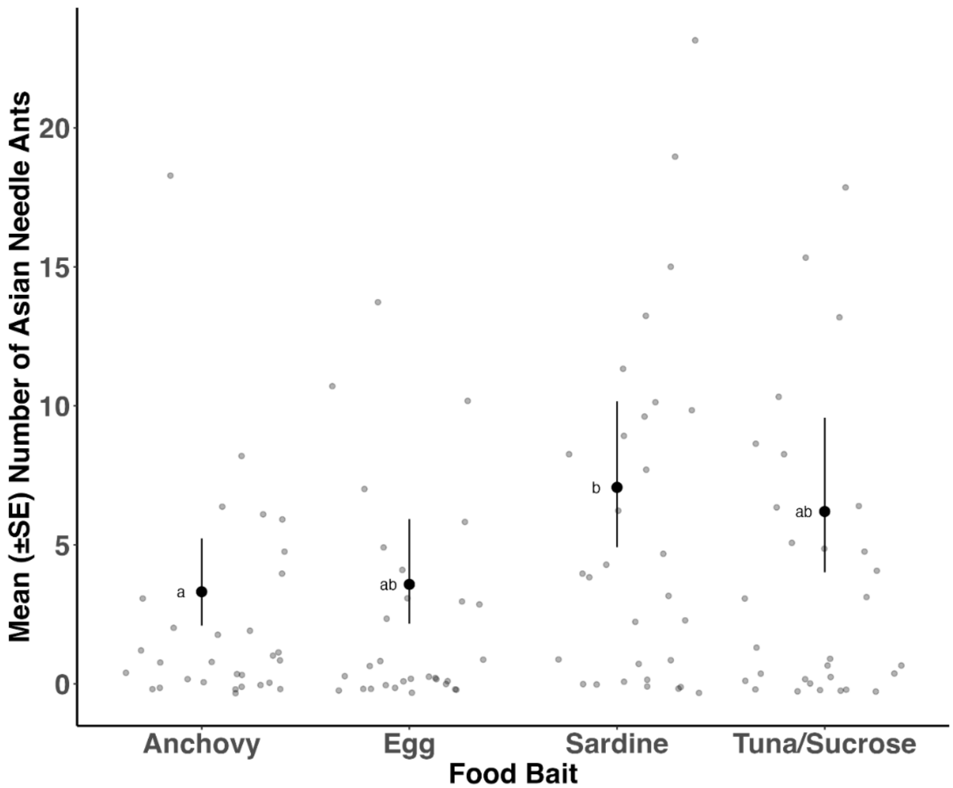


Figure. 5.2. Mean number of Asian needle ants feeding on various food baits 1 hour after foods were placed in areas where Asian needle ants were actively foraging in Griffin and Conyers, GA. Post-hoc multiple comparisons test. The circles indicate the number of ants found in each quadrant. Means followed by the same letter are not significantly different.

CHAPTER 6

CONCLUSION

There are no formal management strategies for the Asian needle ant. Continued research is needed to fully understand its biology and current journey in establishing colonies across large geographic areas. This ant is a threat to humans and the environment. They have a venomous sting that can cause severe allergic reactions; colonies have moved from forest habitats into suburban areas where colonies are found located around the perimeter of buildings, and their potential to devastate natural ecosystems by excluding seed-dispersing native ant species and taking over their nesting sites.

The research conducted in this thesis contributes vital information toward managing the Asian needle ant in the suburban environment. In chapter two, the results concluded that bait acceptance is essential. If the bait is not palatable to the worker, it will not accept it. The proper time to apply bait (season) is critical to the success of management. The ant is foraging activity increased in late May and early June, and their peak population increased from late July through late August.

Conventional granular insecticides should be the first line of defense since this ant species is a nuisance and a threat to human health. When the ants are actively foraging for food items, the time of day will ensure the workers will find and carry the bait to their colonies. Chapter 3 concluded that commercial granular baits containing indoxacarb and abamectin reduced foraging activity in field trials and produced a low survival percentage in laboratory trials. Chapter 4 results concluded with a small gap within the foraging activity during July and August; native ants are not actively foraging, whereas *B. chinensis* are actively foraging. Baiting during these months may decrease the foraging activity of Asian needle ants while limiting collateral damage to our native species.

Chapter 5 showed a significant difference between sardine and anchovy, with sardine as the preferred food choice.

The results and knowledge obtained from this research, including bait acceptance, efficacy trials, and seasonal activity, may provide significant environmental benefits to management techniques of the Asian needle ant, protect our native ant species, and reduce the amount of insecticides deployed into the environment.