ALTERNATIVE MANAGEMENT OF THE INVASIVE ARGENTINE ANT

by

JACOB BENJAMIN HOLLOWAY

(Under the Direction of Daniel R. Suiter)

ABSTRACT

The Argentine ant (Hymenoptera: Formicidae) is a major nuisance pest in urban environments. The objectives of this thesis were to expound upon existing research concerning monthly and seasonal foraging habits of the Argentine ant; further investigate the use of conventional insecticides as management options; and to assess the use of seven less-conventional products as repellents/deterrents against the Argentine ant. This research indicates that foraging activity in Barnesville, Georgia is highest from June to October; foraging activity, over 24 h, is highest during temperatures ranging from 20-30°C; continued use of conventional insecticides, namely fipronil, bifenthrin, and indoxacarb, against Argentine ants remains a viable strategy to reduce foraging activity around urban structures; and a few of the less-conventional products that are evaluated herein, namely peppermint oil, fresh rosemary, fresh spearmint, and Argentine ant trail pheromone, demonstrate repellency/deterrency to the Argentine ant in field and laboratory settings.

INDEX WORDS: *Linepithema humile*; foraging, seasonal foraging; natural pest control; urban pest management; integrated pest management; trail pheromone

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JACOB BENJAMIN HOLLOWAY

Major Professor:	Daniel R. Suiter
Committee:	Brian T. Forschler
	Wayne A. Gardner

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2014

DEDICATION

For Priscilla and Lanie Jane

&

In memory of my Dad, John William Holloway

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"No killing moths or putting boiling water on the ants." –Radiohead

"Go to the ant, O sluggard; consider her ways, and be wise. Without having any chief, officer, or ruler, she prepares her bread in summer and gathers her food in harvest." –Proverbs 6: 6-8, ESV

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CHAPTER 1

INTRODUCTION AND REVIEW OF THE SCIENTIFIC LITERATURE ASSOCIATED WITH THE MANAGEMENT OF ARGENTINE ANTS (HYMENOPTERA: FORMICIDAE) IN URBAN SETTINGS

Introduction

An aspect of developing and utilizing a successful pest management strategy is an understanding of the behavior of the target pest. Although frequently studied, the Argentine ant, *Linepithema humile*, continues to present challenges to effective pest control. One such challenge includes the development of effective, cost-efficient, and environmentally innocuous control methods. Part of this development process requires an understanding of the behavior of *L. humile*, and in particular, knowledge of its foraging activity and patterns. The research reported herein seeks to contribute to the knowledge of foraging behavior of *L. humile* in central Georgia and evaluate the efficacy of several alternative control methods, including essential oils and synthetic, microencapsulated Argentine ant trail pheromone. This thesis is divided into a literature review of management of Argentine ants in urban settings and subsequent chapters exploring the aforementioned research objectives.

Literature review

History

The Argentine ant, *Linepithema humile* (Mayr), is a significant agricultural and urban pest, prevalent in primarily Mediterranean climates, and is considered one of the most important non-termite urban pests in Georgia (Human and Gordon 1996, Kennedy 1998, Vega and Rust

2001, Guillebeau et al. 2008). It is an ecologically- and economically-important invasive nuisance pest (Newell and Barber 1913, Vega and Rust 2001). It was first described by Gustav Mayr in 1868 and it is native to the Paraná river drainage of Brazil, Paraguay, Argentina, and Uruguay (Wild 2004). Although native to South America, *L. humile* is now found on six continents (Suarez et al. 2001). It probably entered the United States (U.S.) by 1891 in coffee shipments from Brazil (Foster 1908) and Barber (1916) places the Argentine ant in Georgia by 1916. Agriculturally, Argentine ants primarily impact citrus and grapes by interfering with biological control of Hemiptera and through damage to irrigation systems and beehives (Newell and Barber 1913, Vega and Rust 2001). Conversely, a nuisance pest, Argentine ants invade buildings in search of more favorable living conditions, and/or food and water, and such infestations and foraging behavior are associated with weather conditions and positively correlated with temperature (Markin 1970a, Rust et al. 2000, Gordon et al. 2001).

A consequence of Argentine ant infestations in both agricultural and urban settings is the use of various control measures. Despite the rise of an integrated approach to managing the Argentine ant, insecticide use remains the most common strategy employed (Soeprono and Rust 2004a, Klotz et al. 2007). Although *L. humile* do not pose a direct health threat to humans, the continued reliance on chemical inputs poses significant environmental risks (Smith 1965, Silverman and Brightwell 2008, Greenberg et al. 2010, Jiang et al. 2014). In light of these concerns, alternative control methods have recently received increasing attention, with particular interest in the use of "natural" products, including essential oils as repellents and insecticides; pheromones, alone and in conjunction with other products; and other less conventional products including plants, detergents, and food items (Bader 2006, Isman 2006, Wiltz et al. 2007, Suckling et al. 2008, Tanaka et al. 2009, Sunamura et al. 2011, Scocco et al. 2012). Although

studies have explored the practical uses of natural products for the control of arthropods, much remains in their implementation as control agents for the Argentine ant, especially under field conditions (Isman 2006, Wiltz et al. 2007, Scocco et al. 2012).

The purpose of this review is to summarize the relevant literature associated with *L. humile* in regards to its importance as an urban pest and the associated efforts to manage it in the urban environment. In particular, this review will focus on integrated and alternative means to manage *L. humile* and the role foraging behavior plays in management efforts. Finally, the emergence of urban integrated pest management will be explored and what it means for management efforts of *L. humile*. In compiling this review, the following reviews were particularly helpful: Vega and Rust (2001), Holway et al. (2002), Ipser and Gardner (2004), Isman (2006), Rust and Su (2012), and Silverman and Brightwell (2008).

Integrated Pest Management

In the agricultural environment, the tenets of integrated pest management (IPM) were practiced in one way or another long before being formally delineated by Stern et al. in 1959 (Kogan 1998). Stern et al. (1959a) enumerated the bases for implementing an integrated approach to pest control as the following: increases in arthropod resistance to pesticides; increased usage and reliance on insecticides; secondary outbreaks of arthropods other than those against which control was originally directed; toxic insecticide residues on food and forage crops; hazards to insecticide handlers and to persons, livestock, and wildlife subjected to contamination by drift; and legal complications from suits and other actions pertaining to contamination by drift. Because of these concerns, a more thorough methodology was designed that includes integrating biological control with chemical control and the inclusion of additional practices such as monitoring pest levels through population sampling, pest identification,

establishing and utilizing pest and economic thresholds, cultural management techniques, and the use of more selective insecticides and pest-resistant plants (Stern et al. 1959a, Flint and Bosch 1981, Leslie and Cuperus 1993). Integrated pest management has historically dealt primarily with managing pests associated with crops in agricultural settings, with the first integrated control program being instituted for the management of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), on alfalfa grown for hay (Stern et al. 1959a, 1959b; Ehler 2006). Although historically and primarily associated with cropping systems, IPM practices have begun to expand into the urban environment with a push for establishing "urban IPM" programs (Walker 1981, Sawyer and Casagrande 1983, Bennett and Owens 1986, Hartman et al. 1986, Robinson 1996).

Urban IPM programs tend to focus on addressing an overreliance on chemicals by implementing many of the traditional IPM measures such as proper pest identification, cultural control methods, pest monitoring, and the use of more selective and alternative insecticides (Walker 1981). A key difference in the structure of agricultural IPM and urban IPM is an emphasis on sociological factors in urban IPM (Sawyer and Casagrande 1983). This is expected, of course, because in agricultural settings there are clear and definite economic thresholds. Whereas these thresholds, in urban environments—with notable exceptions such as commercial food production and food storage—tend to be associated with subjective perceptions rather than tangible economic factors (Sawyer and Casagrande 1983). Another key difference between agricultural IPM and urban IPM is that much of urban IPM is focused indoors and the work done outdoors in an urban setting is very different from that in a traditional cropping system (Robinson 1996). This is important because insects are rarely tolerated indoors, thus setting the pest threshold at or around zero or one for indoor urban environments (Potter and Bessin 1995, Robinson 1996). However, the outdoor thresholds can vary significantly depending on the

person, location, and nature of the environment (specifically whether it is a commercial or residential setting). Varying thresholds occur in agriculture, but the variability across a specific crop is often limited and is directly tied to clearly defined and acceptable pest damage levels (Horne and Page 2008). Varying thresholds in urban environments can complicate an effective treatment strategy because thresholds often indicate when to treat, usually with a chemical, and with no clear benchmark on when to or not to employ chemical treatments there can be significant variation in treatment prescriptions (Robinson 1996, Horne and Page 2008). However, in recent years, there has been a push to establish more clearly defined action thresholds. In particular, Maley et al. (2014) have defined the action threshold in affordable housing as "the maximum pest population that can be tolerated at a particular time and place without harming people, property, and the environment." This definition remains subjective as what one person can tolerate often defers from another person. Indicative of this subjectivity and often very low tolerance of pests indoors are results from a survey conducted by Potter and Bessin (1995). They found a strong intolerance for a single insect sighting indoors, with 62% of respondents indicating a single cockroach sighting would cause them to take action and 41% of respondents being just as intolerant of a single ladybug sighting (Potter and Bessin 1995). This intolerance in urban settings is very different than in agricultural settings and provides another contrast between the two environments. Despite concerns over directly transferring agricultural IPM practices in urban environments, IPM practices have become incorporated in recent years in urban pest management, sometimes without any direct relevance in an urban setting (Robinson 1996).

Importance of Argentine ants in urban versus agricultural settings. The implications of an infestation of *L. humile* depend largely on the environment where it occurs. In agricultural

settings, such as in citrus orchards and vineyards, *L. humile* interferes with control of economically-destructive insects by tending scale insects, mealybugs, and aphids and protecting them against their natural enemies such as parasites and predators (Vega and Rust 2001, Soeprono and Rust 2004a). In fields with drip irrigation, *L. humile* was one of four pest ant species known to cause damage to irrigation equipment by chewing through the plastic irrigation tubes (Vega and Rust 2001). Lastly, *L. humile* attack commercial beehives, which, in addition to the economic impact this has on honey production, interferes with crop pollination by forcing the bees to leave their nest (Newell and Barber 1913, Vega and Rust 2001). While an economic pest in agricultural settings, *L. humile* is a nuisance pest in urban settings. In the urban environment *L. humile* are often found in large numbers foraging in and around homes, warehouses, businesses, schools, food establishments, and healthcare facilities (Knight and Rust 1990b, Klotz et al. 1995, Vega and Rust 2001).

Despite success with other ant species, biological control for both agricultural and urban infestations of *L. humile* has not occurred (Soeprono and Rust 2004a, Wild 2004, Ward et al. 2010). And although the implications of an *L. humile* infestation differ based on where it occurs, treatment options for agricultural and urban settings are often similar and tend to rely heavily on the use of chemical applications including baits, contact and barrier sprays, and granulars (Rust et al. 2003, Soeprono and Rust 2004a, Klotz et al. 2007). Exceptions in urban settings include employing cultural management techniques, habitat exclusion, structural modification or repair, and reducing access to water sources (Reierson et al. 2001, Soeprono and Rust 2004a). Although the options are similar, the approach to and goals of managing *L. humile* differ widely depending on the setting. This is expected given the differences not only in geographical setting but also due to implications of the infestation. In agricultural and commercial environments, the impact

of not reducing or managing the infestation can have significantly different economic and health outcomes than in residential urban settings (Soeprono and Rust 2004a). Evident by the distinction of *L. humile* as an economic pest agriculturally and a nuisance pest in urban settings, the consequences of infestation vary considerably between the two locales.

Another important consideration of *L. humile* is its success as an invasive species. Genetic evidence suggests that introduced populations of *L. humile* in the U.S. originated from around the port city of Rosario in Argentina (Tsutsui et al. 2001). In the U.S., *L. humile* has become established because it directly or indirectly competes for resources with native fauna, including native ants, oftentimes resulting in displacing the native species (Human and Gordon 1997, Ipser and Gardner 2004). One such means of competition is for food resources. *Linepithema humile* workers are able to readily exploit resources due to the speed at which they are able to locate and collect food, which can promote high worker densities (Holway et al. 2002). This may be achieved several ways. One is by quick discovery of food and water, which is achieved by highly efficient "exploratory swarms" of worker ants that are able to "systematically sweep" a chemically unmarked area with "maximum economy" (Deneubourg et al. 1990). The quick discovery of food and water is further exploited by quick and efficient recruitment of additional workers to those sources (Flanagan et al. 2013). Additionally, *L. humile* are known to tend hempiterans, which allows for greater exploitation of honeydew production (Newell and Barber 1913, Markin 1970b, Holway et al. 2002). Efficient food acquisition, recruitment, and resource exploitation, coupled with often high worker density and colony size, combine to create a formidable and successful invasive force that can frequently out-compete other ant species for available resources.

Further adding to the success of *L. humile* as an invasive species is the lack of natural enemies in its introduced range (Holway et al. 2002, Kabashima et al. 2007). Although studies have shown *Solenopsis invicta* has displaced introduced populations of *L. humile* in the southeastern U.S., until recently there was little evidence of any other enemy (predator, parasite, or disease) in its introduced range that may pose a threat to *L. humile* (Ipser and Gardner 2004). However, Rice and Silverman (2013) have reported instances of small-scale displacement of *L. humile* by the Asian needle ant, *Pachycondyla chinensis* (Emery), which was first reported in Georgia in 1934 (Smith 1934). The ultimate effects *P. chinensis* will have on of *L. humile* remain to be seen.

Importance of foraging in an integrated pest management approach of the Argentine ant. An important feature of *L. humile* inherent in both agricultural and urban settings is that of foraging (Foster 1908). Management strategies of *L. humile* in both environments often seek to take advantage of this behavior, primarily through the use of baits (Soeprono and Rust 2004a). Ant foraging is, by its most deceptively simple definition, the movement of ants in response to colony hunger (Wheeler 1928, Vowles 1955). While this definition may seem elementary; the totality of what exactly qualifies as colony hunger, and which stimuli induce responses, has been heavily researched and debated over the years (Howard and Tschinkel 1981, Bonabeau et al. 1998, Halley and Elgar 2001). What is known for *L. humile* is that movement in and out of the nest is predicated on the colony's collective need for food, regardless of colony composition—that is, nest ingress and egress does not differ on the bases of whether the colony was comprised of only workers, workers and brood, or if the colony contained workers, brood and queens (Brightwell and Silverman 2007). Foraging by *L. humile* involves a portion of the colony's workers collectively and individually searching for food sources by continually

exploring and chemically marking previously unmarked areas (Deneubourg et al. 1990, Nonacs and Soriano 1998). This process prevents the ants from having to search the same area twice and is accomplished with the chemical aid of pheromonal communication (Van Vorhis Key and Baker 1982b, 1986; Deneubourg et al. 1990). Specifically, *L. humile* uses the trail pheromone (*Z*)-9-hexadecenal, and this pheromone both guides searching workers and recruits other workers once food is located (Van Vorhis Key and Baker 1982b, Deneubourg et al. 1990). Because of this constant and expedient exploration, *L. humile* foragers are able to cover an extensive area of previously unmarked territory in minimal time and without regard to the photoperiod (Deneubourg et al. 1990).

Although *L. humile* may forage at any time, regardless of the photoperiod, their foraging activity is strongly and positively correlated with air temperature (Markin 1970b). Foraging activity is greatest between 15°C (≈60°F) and 30°C (≈90°F), but also occurs at more extreme temperatures (5-35°C) (Markin 1970b, Vega and Rust 2001). The correlation between foraging and temperature is so strong that observations of the speed of trailing Argentine ants can be used to determine air temperature within one degree (Shapley 1924). Gordon et al. (2001) reported that Argentine ant abundance indoors was greatest in cold, rainy weather in the winter months and hot dry weather in the summer months. Rust et al. (2000) observed maximum foraging activity in California citrus groves between August and October. Sanders et al. (2001) have also observed *L. humile* to be more broadly distributed in the fall than in the spring in Northern California. Differences in diurnal foraging activity are also explained by air temperature, with activity continuous throughout the day and night in warmer months (May to September) and primarily during the daytime in the colder months (Markin 1970b, Abril et al. 2007). This is attributable to little fluctuation in the warmer temperatures over 24 h from May to September,

and due to it being warmer in the daytime in the colder months (December to February). Although the correlation between foraging activity and air temperature is clear, there is no conclusive, observable correlation between relative humidity and foraging behavior (J.B.H., unpublished data; Markin 1970b; Rust et al. 2000).

In addition to foraging intensity, information on foraging range is also crucial for the development of an informed management program. *Linepithema humile* foragers travel from 61 m (200 feet) up to 106 m (350 feet) away from their respective colonies (D.R.S., unpublished data; Vega and Rust 2003). This is important because *L. humile* is a unicolonial species, with workers from one colony often moving in and out of other colonies. This behavior can present management problems as there are not clearly defined colonial boundaries (Holway et al. 2002). Unicoloniality is made possible by a lack of, or reduced intraspecific competition within, multiple colonies (Holway 1998, Holway et al. 2002). This feature allows for large populations and multiple colonies (polydomy), or supercolonies, of *L. humile* that can extend over great geographic distances (Tsutsui et al. 2000, Giraud et al. 2002, Ipser and Gardner 2004). In addition to unicoloniality, *L. humile* are polygynous (multiple queens), a trait which can also contribute to increases in population size (Vargo and Fletcher 1989).

Knowledge of foraging behavior and patterns is inherently important to the development of an effective ant management program for several reasons. Foraging allows for direct access to a portion of the colony at any given time (Nonacs and Soriano 1998). In its simplest application, access to the ants allows for direct treatment, which is required by a number of treatment options including many spray-applied insecticides. More importantly than direct access is the ability to exploit the biology of foraging through the use of baits, which capitalize on both food and exploratory recruitment (Deneubourg et al. 1990, Klotz et al. 2002). Baits require less insecticide

per unit area, are more target-specific than sprays, and work due to foraging workers either bringing the bait into the nest directly (as is the case in granular bait) or by ingesting it (which is the case with liquid bait) and transferring by trophallaxis to the others in the nest (Knight and Rust 1991; Klotz et al. 2002; Soeprono and Rust 2004c, 2004a). Slow-acting perimeter sprays also take advantage of foraging activity. Instead of exploiting recruitment and exploration, the sprays work primarily through horizontal transfer of the insecticide (Klotz et al. 2002, Soeprono and Rust 2004c). This is accomplished by foraging ants coming into contact with the treatment and then returning to the nest where the treatment is either transferred through direct contact of the treated ant or corpse (necrophoresis) and others in the nest (Klotz et al. 2002, Soeprono and Rust 2004c, Wiltz et al. 2009). The sprays are only successful if they do not interrupt existing foraging patterns, which occurs only if the treatment is slow-acting (Soeprono and Rust 2004b).

Urban Integrated Pest Management of Linepithema humile

As *L. humile* has continued to cause economic and ecological concern, there have been many studies aimed at managing the ant (Rust et al. 2003, Soeprono and Rust 2004a, Klotz et al. 2007, Silverman and Brightwell 2008). In accordance with integrated pest management strategy, prior to any type of intervention, a proper inspection should be conducted and effective monitoring techniques should be employed throughout the treatment period (Silverman and Brightwell 2008). Monitoring techniques vary widely depending on available time and resources, and include the use of sticky traps, visual inspections by both pest control personnel and customers, measuring sucrose consumption, and pitfall trap collections (Alder and Silverman 2004). Despite a variety of available techniques, Alder and Silverman (2004) found worker counts at baits to be superior to the other monitoring techniques. Although there are a variety of treatment options available once an infestation has been confirmed, ranging from nonchemical

physical barriers and controls to chemical treatments, Soeprono and Rust (2004a) report that no one strategy has proven entirely successful in managing or controlling *L. humile*. Commonly, management of *L. humile* is attempted by employing several of these chemical and nonchemical methods in an integrated pest management approach (Klotz et al. 2002, Rust et al. 2003, Silverman and Brightwell 2008).

Conventional insecticides. Despite the recommendation to utilize all of the strategies involved in an integrated approach to manage *L. humile*, the use of insecticides remains the most common method utilized in attempts to manage *L. humile* (Soeprono and Rust 2004a, Klotz et al. 2007). Rust et al. (2003) have noted that insecticidal baits have been recommended for *L. humile* control for at least a century. Recently, there have been major improvements to ant baits and many applications have shown promise in effectively managing Argentine ants (Suiter et al. 1997, Greenberg and Klotz 2000, Rust et al. 2003). Specifically, perimeter applications of bait stations containing hydramethylnon and sulfluramid have demonstrated efficacy in reducing the number of foraging ants (Forschler and Evans 1994a, 1994b; Blachly and Forschler 1996; Forschler 1997). Similar treatments utilizing fipronil-treated sucrose bait have significantly reduced ant populations (Vega and Rust 2003).

Liquid, residual contact insecticides are, in addition to baits, another common method used in control efforts (Rust et al. 2003, Soeprono and Rust 2004a, Klotz et al. 2007, Wiltz et al. 2009). Chemical applications primarily are in the form of contact insecticides applied as residual perimeter, space, crack or crevice treatments with sprays or granulars (Soeprono and Rust 2004a). Contact treatments consist of either repellent or nonrepellent insecticides that are used as perimeter sprays applied to buildings, entry points to buildings, cracks and crevices of buildings, sprayed or banded around trees and other plants, and into nests and common nesting sites

(Knight and Rust 1990a, Soeprono and Rust 2004a, Klotz et al. 2007). Klotz et al. (2007) evaluated a variety of these treatments, including gel baits, granular baits, perimeter sprays, spot treatments and combinations of treatments, and found that a combined application of fipronil as a perimeter spray and broadcasted bifenthrin granules was the most successful in reducing ant activity around homes. The combination treatment of a fipronil perimeter spray and broadcasted bifenthrin granules resulted in a 96% reduction in ant activity at 1 week post-treatment and a 90% reduction at 8 weeks post-treatment compared to pretreatment levels (Klotz et al. 2007). In addition to this, Klotz et al. (2007) also observed that a perimeter treatment of fipronil alone was nearly as effective as the combined treatment, with a 93% reduction in ant activity at 1 week post-treatment and a 81% reduction at 8 weeks post-treatment. Although fipronil has shown consistent efficacy at reducing ant populations, a relatively new chemical formulation of indoxacarb has also shown potential as an alternative to formulations containing fipronil (Gallagher et al. 2012).

In addition to chemical applications, nonchemical options have been evaluated for managing *L. humile*. These methods range from practices aimed at excluding Argentine ants from structures—such as building construction modifications and improvements—to proper food storage and waste removal techniques (Soeprono and Rust 2004a). However practical and useful nonchemical control techniques may be, the use of chemical control remains prevalent. As chemical control use continues, most notably liquid sprays and granules, there is concern about the realized and potential drawbacks to their continued use (Soeprono and Rust 2004a). These concerns include secondary ecological effects, such as non-target and environmental impacts; insecticide resistance issues; improper use; insecticide run-off and water contamination, primarily driven by irrigation systems and application close to watershed sites; and costs (Rust et

al. 2003; Soeprono and Rust 2004a; Greenberg et al. 2010; Scocco et al. 2012; United States Environmental Protection Agency 2013b, 2013c; Jiang et al. 2014).

These concerns have led to major changes to regulations in recent years that have limited the type and use of chemical measures. In 2009, the U.S. Environmental Protection Agency, in an effort "to reduce ecological exposure from residential uses of pyrethroids and pyrethrins products", implemented an initiative to revise guidelines for pyrethroid and pyrethrins pesticide products used in non-agricultural outdoor settings (United States Environmental Protection Agency 2013c). This initiative specifically limits non-agricultural outdoor uses of pyrethroids and pyrethrins to spot or crack-and-crevice treatments only with a few exceptions (United States Environmental Protection Agency 2013c). In addition to expanding restrictions on pyrethroid and pyrethrin use, the U.S. E.P.A. in 2013 implemented a series of changes to restrict the use of neonicotinoid insecticides in order to limit exposure to pollinators (United States Environmental Protection Agency 2013b). These changes include changing pesticide labels to limit applications to protect bees, including limiting application of neonicotinoids while bees are foraging in non-agricultural settings (United States Environmental Protection Agency 2013a). In light of these concerns and changes, several alternative methods to conventional control have received attention as potential effective management techniques.

Alternative management measures. As previously mentioned, integrating several approaches appears promising in effectively managing *L. humile*. One approach is to utilize a synthetic version of the *L. humile* trail pheromone, (*Z*)-9-hexadecenal, either in conjunction with baiting or spraying techniques to improve efficacy or as a means in itself to disrupt normal foraging activity (Greenberg and Klotz 2000, Tanaka et al. 2009, Suckling et al. 2010, Sunamura et al. 2011, Choe et al. 2014). This is particularly appealing due to the potential this approach

may have on reducing the overall amount of applied conventional insecticides. As a standalone product, synthetic (*Z*)-9-hexadecenal has shown efficacy at disrupting Argentine ant trails when high concentrations of the pheromone are applied to paper discs and then the treated discs applied to trails (Suckling et al. 2010). However, one limiting factor of the use of synthetic pheromone is the longevity of the pheromone in the field, as it highly volatile in its natural form (Suckling et al. 2008). In light of this issue, Suckling et al. (2010) formulated the synthetic pheromone into a microencapsulated-sprayable product that can be readily dispersed in a field setting, which also allows the pheromone to more slowly volatilize over time.

One goal of an effective integrative pest management program is to reduce the overall use of chemical insecticides; as such the use of plant essential oils has also received growing attention within the pest management community (Isman 2006, Wiltz et al. 2007, Quarles 2009). One reason for wanting to reduce the use of chemical insecticides is prevent or reduce chemical runoff and environmental damage to waterways (Quarles 2009, United States Environmental Protection Agency 2013c, Choe et al. 2014, Jiang et al. 2014). In California, approximately 54,000 kg of the phenylpyrazole insecticide fipronil was used in 2006, primarily for structural pest control (Gunasekara and Troung 2007). This represents just a fraction of the overall insecticide use in the U.S., with approximately 4.1×10^7 kg of insecticides applied in the U.S. in 2006 (Grube et al. 2011). In the world, the amount of insecticide use is even higher, with approximately 4.3×10^8 kg of insecticides applied in 2006 (Grube et al. 2011).

Plant volatiles have been used in agriculture for pest management purposes for over two millennia (Isman 2006). Essential oils represent one of four major types of botanical products in use for pest control, with pyrethrum, rotenone, and neem being the other three (Isman 2006). Recent, increased attention to essential oils is partly due to their being exempted from

registration by the Environmental Protection Agency and classified as so-called "25(b)" products, owing to the name of the federal act that granted exemption (Drees 2005, Geiger and Tootelian 2005, Isman 2006). According to Isman (2006), this has caused an increase in the development and production of essential oil-based insecticides, fungicides, and herbicides for both commercial and residential use. Adding to the appeal of essential oil use and product development is that they possess low mammalian toxicity, which is in contrast with many of the conventional insecticides and other synthetically-derived insecticides (Rajendran and Sriranjini 2008, Scocco et al. 2012). Oils of particular interest are rosemary oil, clove oil, and thyme oil (Isman 2006). Rosemary, for example, has fumigant, repellent, and contact toxic properties against several insects, including stored product pests such as the bean weevil, *Acanthoscelides obtectus* (Papachristos and Stamopoulos 2002, Isman et al. 2008). Rosemary oil is toxic to adult turnip aphids, *Lipaphis pseudobrassicae*, the human head louse, *Pediculus humanus capitis*, the two-spotted spider mite, *Tetranychus urticae*, the armyworm, *Pseudaletia unipuncta*, and the cabbage looper, *Trichoplusia ni* (Yang et al. 2004, Sampson et al. 2005, Miresmailli et al. 2006, Isman et al. 2008). In addition to toxic characteristics, rosemary oil has shown promise as a viable repellent, demonstrating repellency against four mosquito species, *Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus*, and *Culex pipiens pallens* (Choi et al. 2002, Prajapati et al. 2005). Plant essential oils have been tested as repellents and insecticides against *L. humile*, however, there have only been a limited number of laboratory tests and no field studies to date (Wiltz et al. 2007, Scocco et al. 2012).

Given continued concern over the use of conventional chemical strategies and their environmental impact, safer management practices warrant further investigation. Further work, including field and laboratory testing, is needed in order to ascertain the viability of using

alternative control measures, including essential oils; fewer insecticides; and less active ingredient of conventional insecticides for *L. humile*.

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CHAPTER 2

RATING MONTHLY VARIATIONS IN FORAGING OF THE ARGENTINE ANT (HYMENOPTERA: FORMICIDAE) AROUND RESIDENTIAL STRUCTURES IN GEORGIA[1]

[1] Holloway, J.B., and D.R. Suiter. To be submitted to the *Journal of Economic Entomology*.

Abstract Foraging activity of *Linepithema humile* was monitored monthly for 24 mo in Barnesville, Georgia on the exterior foundational walls of residential buildings. Monthly inspections consisted of utilizing a novel monitoring technique by visually assigning an intensity rating of the foraging activity on each wall of the buildings. Ratings were compared with air temperature and relative humidity. Foraging activity was highest in the warmer months of June to October, peaking in July. Workers foraged every month despite cooler temperatures, although at lower intensities. Trails were also video recorded at a different site for 12 h in May and 24 h in June to ascertain fluctuations of trailing intensity throughout the day. Temperature significantly impacted (F = 13.10; df = 24, 2394; $P < 0.0001$) and was positively correlated (R = 0.257; P <0.0001) with foraging intensity. There was no correlation of relative humidity with foraging intensity. In the video study, Argentine ant foraging activity was highest during moderately warm temperatures (20-30°C) over 24 h and photoperiod did not appear to impact the foraging intensity.

Key Words *Linepithema humile*, foraging, monthly variation, seasonal variation, behavior

Introduction

The Argentine ant, *Linepithema humile* (Mayr), is a significant agricultural and urban pest, prevalent in primarily Mediterranean climates, and is considered one of the most important non-termite, nuisance pests in suburban and urban Georgia (Vega and Rust 2001, Guillebeau et al. 2008). It is an ecologically- and economically-important invasive nuisance pest (Newell and Barber 1913, Human and Gordon 1996, Human and Gordon 1997, Kennedy 1998, Vega and Rust 2001). Although native to South America, *L. humile* is now found on six continents (Suarez et al. 2001). It probably entered the United States by 1891 in coffee shipments from Brazil (Foster 1908); Barber (1916) places the Argentine ant in Georgia by 1916.

Over the past several decades there has been an increase in the use of combined approaches for managing pest insects in the urban environment. In the agricultural environment, this has been referred to as Integrated Pest Management (IPM) for decades. One aspect of IPM is the use of monitoring tools to properly identify the pest, accurately assess pest infestation levels, as well as gauge the impact of a particular intervention, be it chemical or non-chemical (Stern et al. 1959a, Walker 1981, Sawyer and Casagrande 1983, Bennett and Owens 1986, Alder and Silverman 2004, Silverman and Brightwell 2008).

There was found no instance in the literature of rating Argentine ant trails, particularly in urban settings. Rather, all measurements of ant activity have been concerned with estimating colony size, not activity, primarily through visual counts. Musgrove and Carman (1965) assigned ratings to Argentine ant activity on trees 14 mo after applying treatment, but the ratings were done to assess the average size of the colonies, not to assess foraging intensity in general. Andrew et al. (2013) ranked activity of *Iridomyrmex purpureus* (Formicidae) at nesting sites to determine peak foraging activity relative to thermal limits using a four point nominal scale.

Activity was ranked between 0 (no activity) and 3 (full activity). According to Andrew et al. (2013), an activity ranking of 1 indicated a few individuals on the nest surface with lethargic movement; a ranking of 2 indicated a number of ants on the nest surface but none foraging and moving away from the nest; and a ranking of three was high number of ants vigorously moving around on the top of the nest and also leaving the nest surface for foraging.

Although there are no known instances of rating Argentine ant foraging intensity, there is a well-known method used to evaluate colony size for the red imported fire ant, *Solenopsis invicta*. Harlan et al. (1981) developed a colony rating system, which was later modified by Lofgren and Williams (1982) and is referred to as the colony index system (Fuxa et al. 2005). This system is a visual rating of colonies that is used to estimate the number of ants in the colony by the number of ants and presence of worker-caste brood observed when the mound is disturbed, often by removing a shovel-full of dirt to expose ants within the nest (Lofgren and Williams 1982, Fuxa et al. 2005).

When monitoring the activity of Argentine ants, techniques commonly used are bait monitoring (visual counts of workers at baits) with either jelly, honey, or a protein source such as tuna; consumption of sucrose solution; pitfall trapping, although not commonly employed in urban settings due to aesthetic considerations; and visual counts of ants crossing either a real or imaginary line on a tree, wall, or some other location (Baker et al. 1985, Moreno et al. 1987, Forschler and Evans 1994a, Blachly and Forschler 1996, Human and Gordon 1996, Shorey et al. 1996, Holway 1998, Rust et al. 2000, Vega and Rust 2003, Alder and Silverman 2004, Stanley et al. 2008). These techniques often require multiple visits to the infestation site, often 24 h after placement of the material (sucrose consumption); are often time-consuming (particularly pitfall trapping and bait monitoring); and depend on a response to the baiting material or entrapment

device (Alder and Silverman 2004). Although pitfall trapping is rarely used in urban settings, bait monitoring and visual counts are often used. When gauging the impact of an insecticide-treatment for Argentine ants, Alder and Silverman (2004) found that worker counts at baits was the most efficient and consistent method available to determine infestation intensity, compared to the use of visual counts, sucrose consumption, and pitfall trap collections. Although Alder and Silverman (2004) demonstrated the most relatively accurate and efficient method for monitoring Argentine ant infestations, the method used to determine efficiency was not entirely indicative of the actual time involved in performing a visual inspection—in their case visual counts—and over-estimates the time required to perform a visual inspection (J.B.H., unpublished data). Furthermore, ant counts at baits can be either under- or over-representative of actual ant presence as either the ants do not respond adequately or at all to the baiting material and continue foraging past the bait, or counts may be constrained by the physical dimension of the bait itself (i.e. only a certain amount of ants can feed on a drop of jelly at a time) (D.R.S., personal observation).

As an alternative to monitoring with sweet foods, my study proposes that visual inspections are an efficient and effective means of monitoring Argentine ants throughout the year, and in particular, the use of a foraging intensity scale provides a means to rate foraging activity and track monthly fluctuations. In addition to offering an alternative monitoring technique, another purpose of this study was to rate monthly variations in foraging activity of Argentine ants and to assess possible correlations of temperature and relative humidity with foraging activity. Additionally, trails were video-recorded and an intensity rating was assigned to the trails at 15 min intervals to determine changes in foraging over the course of 24 h.

Materials and Methods

Field Foraging Study.

Research site and schedule. Argentine ant foraging activity on the exterior foundation walls of eight buildings (Towaliga Village, Barnesville, Georgia, 33°3'20.10"N, -84°10'0.96"W) was monitored every 3 to 5 wk for 24 mo beginning in June 2011; there were a total of 26 inspections over 24 mo of this study. The buildings consisted of two architectural styles (Figures 2.1 to 2.3). Visual inspections were performed on each building wherein an ant trailing intensity rating was assigned to each of the buildings based on foraging activity on the outside perimeter walls of each building. There were either 63.1 (style A) or 72.5 (style B) linear meters around each building.

The ratings for each wall section ranged from 0 to 3, where 0 was no visible ant foraging activity; 1 was light foraging activity; 2 was medium foraging activity; and 3 was high foraging activity. Each building consisted of 12 wall sections and the entire length of each wall section was independently rated and assigned a single value, 0 to 3. The 12 individual wall section ratings were summed to provide a total rating for each building, which ranged from 0 to 36. A total of 96 wall sections were included in the study. The cardinal direction of each wall (the direction the wall faced) was identified and compared with trail ratings.

Ant foraging activity was qualitatively rated on an intensity scale of zero, low, medium, and high. Low ratings consisted of at least more than two ants moving in a manner consistent and in the same direction as the others. One ant moving in a direction opposite of another ant did not constitute a rating of one because this was not a consistent trail. Medium ratings consisted of trails at least 2 ants wide, and typically involved movement in two directions (i.e. left and right

on the foundations). High ratings consisted of trails >4 ants wide, and typically involved movement in two directions.

In addition to trail intensity, time of day, air temperature, and relative humidity were recorded at each inspection. All inspections were conducted between 0900-1200 h. Each inspection lasted an average of an hour, with an average time of 7.5 min per building (J.B.H., unpublished data). Air temperature and relative humidity were recorded using a combination digital thermometer and humidity reader (Model 1523, Taylor Precision Products, Oak Brook, IL), and recorded at the beginning and ending of each observation period.

Response variables. The mean temperature of each inspection, calculated as the following: $\frac{\text{beginning temperature} + \text{ending temperature}}{2}$, as well as the mean relative humidity, were used in comparison with the trail intensity ratings for that inspection.

Video Foraging Study.

Research site and schedule. Argentine ant trailing activity was monitored during May and June at a single trail location (University of Georgia, Griffin, Georgia, 33°15'54.29", -84°16'57.16). Trailing activity was video recorded (Sony XR150; Sony Corporation, Tokyo, Japan) for a continuous 24 h period, on June 15 and June 20, 2012; and a continuous 12 h period on May 25, 2012. A digital thermometer was included in the frame of the video for comparison of ratings (described above) against air temperature. The video camera was placed approximately 30 cm to the side and above an active ant trail. The trail was either on a tree (June readings) or on the ground next to the tree (May reading). A lamp was used to illuminate the trail during the night hours. Markin (1970b) has noted that generally the Argentine ant does not show a change in foraging behavior due to light.

Response variable. The video was analyzed for each date (12 or 24 h period), at 15 min intervals, and the trail was given an intensity rating as previously described, and the air temperature recorded.

Statistical analysis. For the seasonal foraging study, trail intensity rating data, by wall, were analyzed and compared with temperature, humidity, cardinal direction, temperature*cardinal direction interaction, and temperature*humidity interaction by mixed model analysis of variance (PROC GLIMMIX [SAS Institute, Inc. 2010]). The correlation of rating and temperature was also subjected to correlation analysis (PROC CORR [SAS Institute, Inc. 2010]).

For the video foraging study, trail intensity rating data were analyzed and compared with time, temperature, and the time*temperature interaction by mixed model analysis of variance (PROC GLIMMIX [SAS Institute, Inc. 2010]).

Results

Field foraging study. Temperature significantly impacted (F = 13.10; df = 24, 2394; P < 0.0001) and was positively correlated (R = 0.257; P < 0.0001) with foraging intensity. Wall cardinal direction (F = 0.00; df = 3, 2394; P = 1.000) and the interaction of temperature*wall cardinal direction (F = 1.20; df = 72, 2394; P = 0.1281) were not significant.

Summing all of the wall ratings for each inspection provides a total rating for an inspection. Over the course of the study, most months received at least two inspections. Taking a mean total rating for each month (using the formula: $\frac{\text{Month A inspection 1} + \text{Month A inspection 2}}{\text{n}}$, where n = the total number of inspections done for that month (if June was inspected once in 2011 and once in 2012 [a total of two times], then the equation for June would be $\frac{\text{June 2011 Total Rating} + \text{June 2012 Total Rating}}{2}$. Taking a mean total for the entire study, that is

summing all of the total ratings for each inspection and dividing it by the total number of inspections, the mean rating for the entire study was 79 (out of 288). The mean temperature for the entire study was 21°C. This is an average rating of less than a low intensity for every wall. Argentine ant activity was highest from June through October, peaking in July (Figure 2.4), with July having an average rating of 142—which is nearly double the overall yearly average rating for this study—and an average temperature of 30°C (Figure 2.4). January had the lowest average rating, with a rating (27) and an average temperature of 8°C. Overall, there were 26 inspections, with 2,496 unique observations of wall sections. Out of 2,496 observations, there were 1,218 ratings of zero, 742 of one, 178 of two, and 358 of three.

Each wall section cardinal direction (north, south, east, west) had an average rating for the entire study nearly identical as the others—that is, when wall sections are sorted by cardinal direction and averaged for the entire study by direction, the ratings are nearly the same for each direction (Figure 2.5). When separating the number of wall sections per mean rating, on average there were 60.4% of wall sections receiving an average low rating; 25% of wall sections received an average rating of zero; 14.6% of wall sections received an average rating of medium; and 0% of the wall sections averaged a high rating (Figure 2.6). During peak months (June through October), 40.6% of wall sections averaged a low rating; 32.3% of wall sections averaged a medium rating; 25% of wall sections average a rating of zero; and 2.1% of walls averaged a high rating (Figure 2.6).

When comparing intensity ratings among buildings (12 wall sections per building), there was considerable variability both among buildings and within (Figure 2.7). This variability is noted for instance in building 1, where a majority of wall sections averaged an intensity rating of ≈1.5, while a few wall sections averaged near zero.

Video foraging study. Temperature (F = 0.05; df = 126, 2; P = 1.0000); time (F = 0.05; df = 89, 2; P = 1.0000); and their interaction (F = 0.05; df = 44, 2; P = 1.0000) did not significantly impact trailing intensity. This is likely due to limitation in data recording, in that Argentine ant activity at this trail was particularly active, with mostly high activity throughout the 24 h period (≈82% of all observations rated were rated as high) (Figure 2.8). Despite this limitation, visual observations showed higher activity in temperatures ranging from 19.5 to 30 °C (lowest recorded temperature was 19.5 °C and the highest recorded temperature was 51.8 °C).

Discussion

Monitoring is an integral part of a complete integrated pest management program. This study demonstrates that Argentine ant activity can be monitored quickly and effectively throughout the year without having to rely on baiting or other often time-consuming methods (Alder and Silverman 2004). This study serves as a starting point for further investigations into both the use of visual monitoring techniques as an effective part of an IPM program and as a means to accurately track Argentine ant foraging activity outdoors throughout the year. And given that indoor infestations may be a result of foragers travelling to and from an outdoor nest site, this method may also contribute to knowledge of indoor infestations as well (Gordon et al. 2001).

This study had nearly identical results as (Rust et al. 2000) in that peak foraging activity was observed from June to October in both studies and Argentine ants showed a seasonal pattern of foraging observed by Markin (1970b), Krushelnycky and Reimer (1998), Rust et al. (2000). , and Heller and Gordon (2006). Contrary to both Markin (1970b) and Rust et al. (2000), this study noted marked declines in foraging activity by October rather than later in the year. Rust et al. (2000) surmised mild winters may have resulted in brood being produced earlier in the year in

their observation period, thus resulting in an earlier worker die-off. This may also be the case in this study, as the average temperature throughout the winter month was relatively higher than expected (Figure 2.4). This study noted foraging activity highest between temperatures of 20 to 30°C. This is similar to results obtained by Markin (1970b), Rust et al. (2000), and Vega and Rust (2001), with each study observing peak activity to occur between 10-30°C.

Seasonality, namely temperature, as an indicator of activity is not limited to Argentine ants. Porter and Tschinkel (1987) noted that temperature, namely soil temperature, was the primary abiotic factor affecting foraging rates of the red imported fire ant. Vogt et al. (2003) noted that season, not necessarily air or soil temperature, was the best predictor of red imported fire ant foraging, but they did note foraging was dependent on suitable temperatures. Suitable temperatures for red imported fire ant foraging activity are similar to that for Argentine ants, with optimal foraging occurring between temperatures of 22-36°C (Porter and Tschinkel 1987).

In addition to further support for seasonality of Argentine ant foraging, another important finding in this study was that there is considerable variability of foraging intensity and activity among wall sections on the same building. This can have implications for treatment strategies, in that you might only spot treat the visible ant trails instead of applying the treatment to the entire structure. Klotz et al. (2007) had success with spot treating visible trails with fipronil and in light of new restrictions on pyrethroids and pyrethrins, this may become even viable solution for treatments to the exteriors of structures and reducing the overall amount of applied chemical (United States Environmental Protection Agency 2013). This was observed throughout the study with some walls on average having around a zero rating for the study (Figure 2.7). The variability among foraging activity on a single building was also indicated by Alder and Silverman (2004), but not explicitly pointed out, by their demonstrating it taking more time to

locate at least five trails on a building to conduct visual counts of foragers. The variability and lack of activity on some of the walls was one of the primary reasons the visual counting method was deemed to be most time consuming than bait monitoring in their study. Given that there is no specific need to actually count ants to determine activity around the perimeter of a structure, as indicated by this study, and the average time of 7.5 min to conduct an inspection, it is offered that visual monitoring and using an intensity rating scale is less time consuming and offers an effective means of ascertaining Argentine ant activity on buildings.

In terms of scheduling, we held the time of the inspections constant to control for time of day, but temperatures fluctuated greatly at times. It would be important to investigate the possibility of doing multiple inspections per day, multiple inspections during the week, and possible weekly inspections for future studies. In light of concerns of only inspecting in the morning, we decided to also include 24 h monitoring. However, our response variable proved to be insufficient for this project due to the high activity of the trail we selected. The 24 h monitoring could address multiple issues, including fluctuations over time that even multiple inspections in one day cannot. In addition to this, although we recorded air temperature, and there was a relationship between air and surface temperatures, a more robust study would include surface temperature. We did perform 192 observations of wall temperature using a traceable, infrared thermometer (Fisher Scientific Traceable Infrared Thermometer with Trigger Grip, Thermo Fisher Scientific, Waltham, MA) on walls at the conclusion of the study and compared the temperature with rating and found that it was not significant ($F = 0.29$; df = 1, 185; $P = 0.5911$). Although this was similar to results by Enzmann et al. (2012) that showed no direct relationship between Argentine ant activity and ground temperature, we would suggest future studies fully incorporate surface temperatures with all inspections and intensity ratings.

In conclusion, through the use of visual monitoring, this study demonstrates that Argentine ant activity in Barnesville, Georgia is highest in the warmer months (June-October); peaking in July. Furthermore, activity is strongly correlated with air temperature. Results indicate the need to inspect the entire structure before implementing any treatment protocol, and from this future studies could focus on spot treatments as an alternative or replacement for full perimeter treatment.

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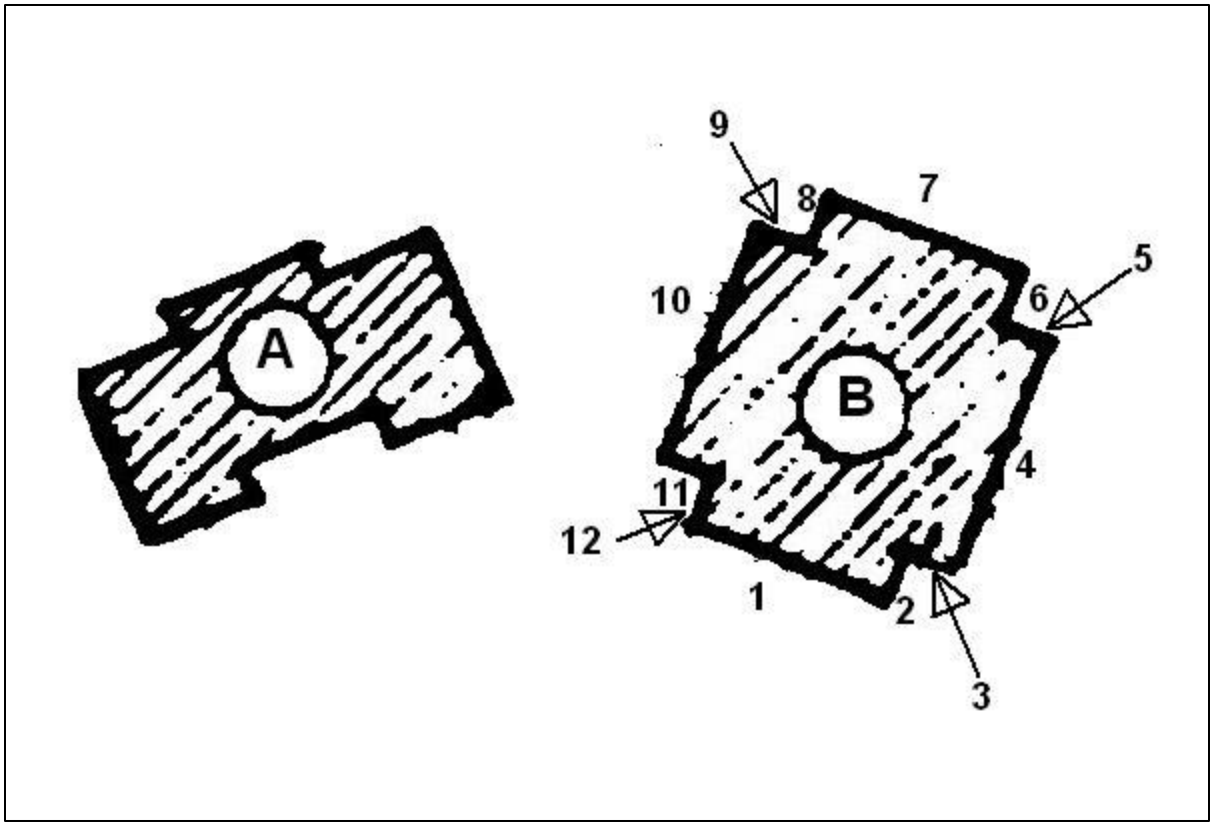

Figure 2.1. A sketch representing the two different architectural building styles (A and B) included in the field foraging study and the division of walls into numerical sections on building style B.

Figure 2.2. Picture of a building used in the field foraging study (building style A).

Figure 2.3. Picture of a building used in the field foraging study (building style B).

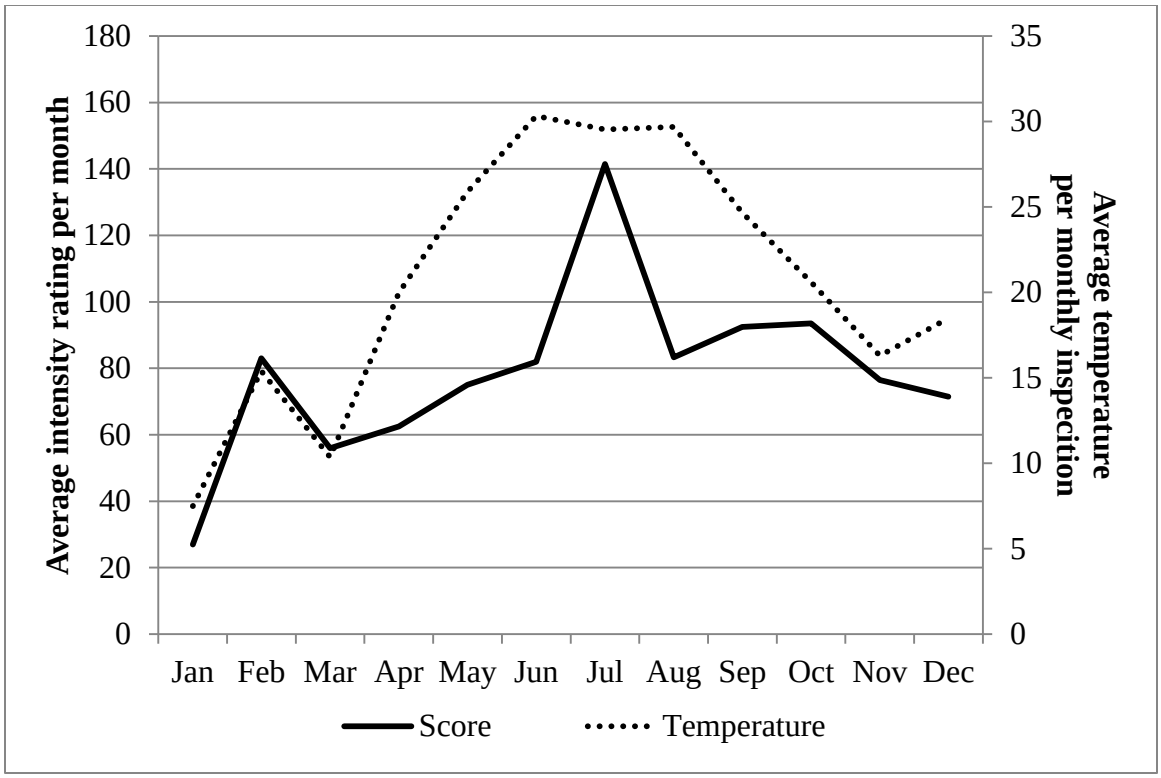

Figure 2.4. Average total monthly intensity ratings (sum of all wall section ratings) versus average monthly temperature readings for the field foraging study (n = 26).

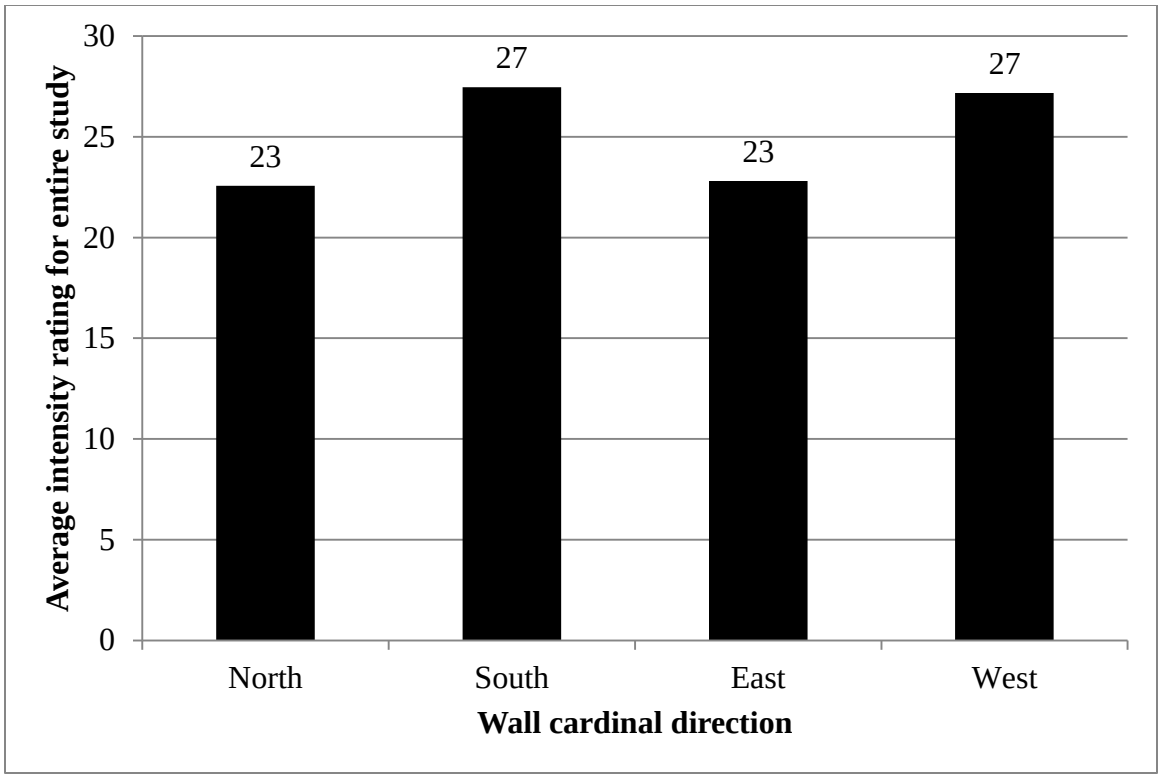

Figure 2.5. Average wall section intensity ratings (out of 72 for each direction) by cardinal direction (the direction the wall faces) for the field foraging study.

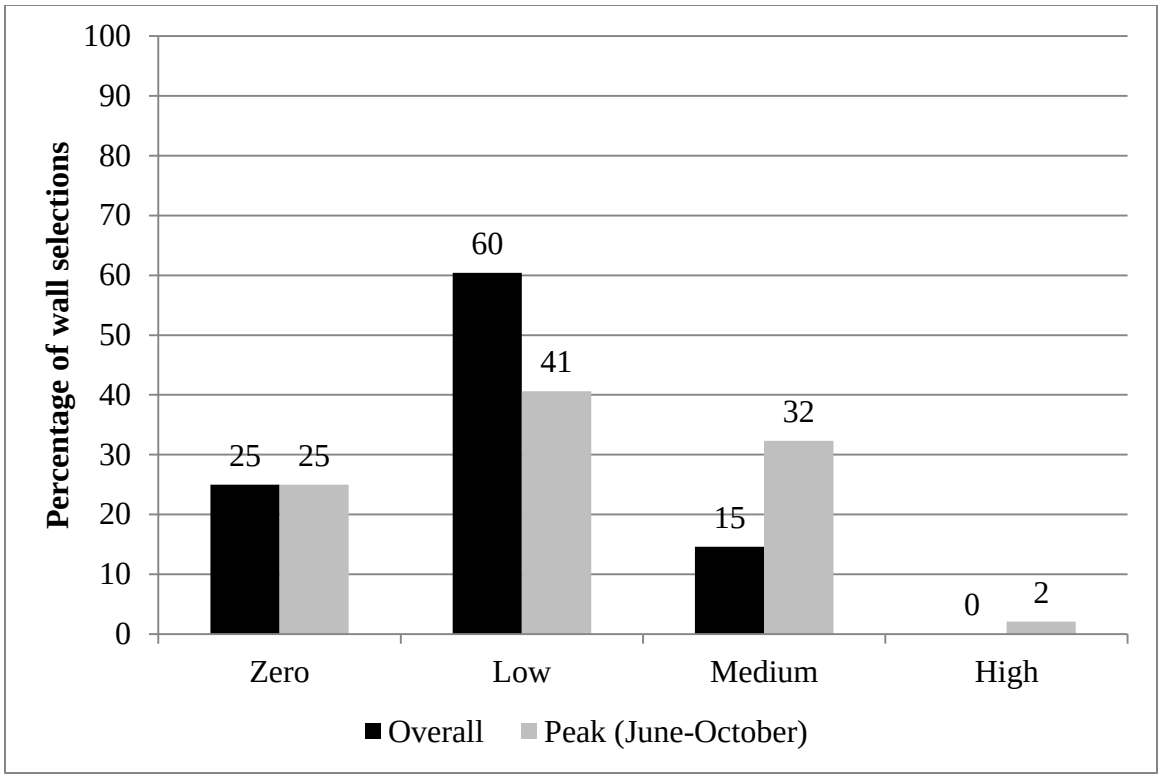

Figure 2.6. Percentage of wall sections sorted by average intensity rating both for the entire study and by peak months for the field foraging study.

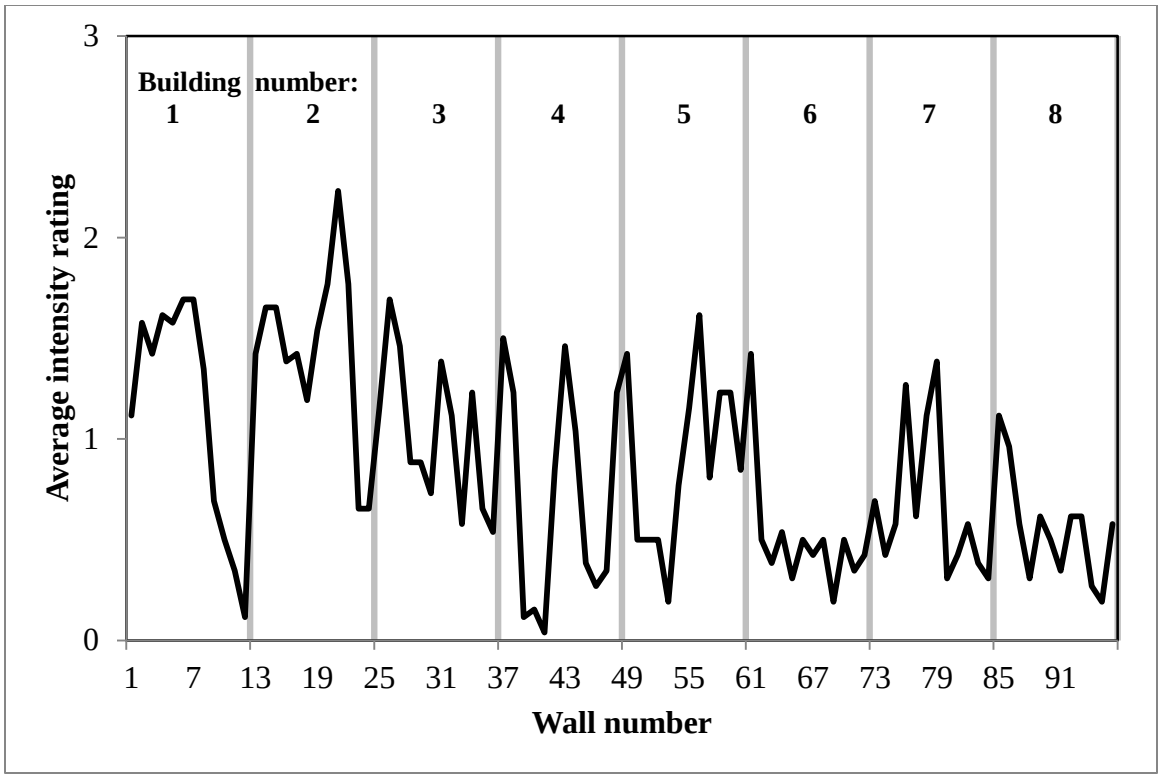

Figure 2.7. Average intensity rating by wall section (number) and separated by building for the field foraging study.

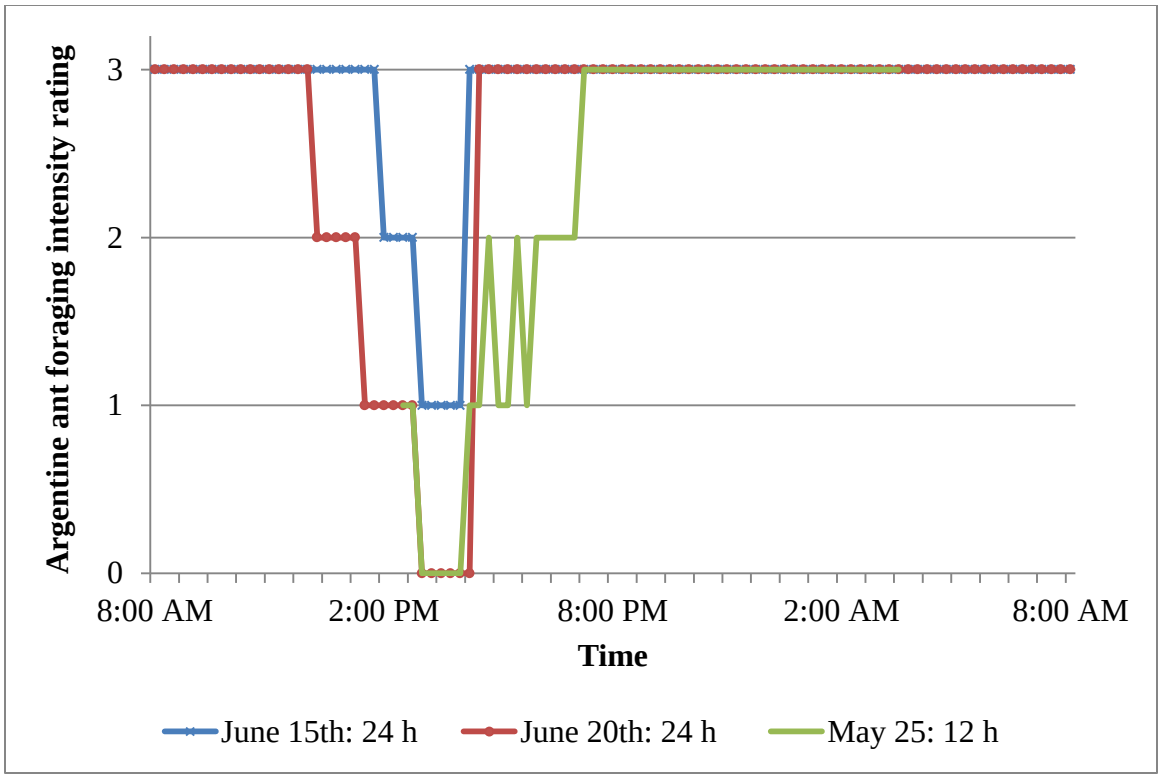

Figure 2.8. Argentine ant intensity rating, by time of day, for each 15 minute interval by date for the video foraging study.

CHAPTER 3

EFFICACY, RESIDUAL ACTIVITY, AND SECONDARY KILL OF INDOXACARB-BASED INSECTICIDE, ARILON®, AGAINST FORAGING ARGENTINE ANTS (HYMENOPTERA: FORMICIDAE)[1]

[1] Holloway, J.B., and D.R. Suiter. To be submitted to the *Journal of Economic Entomology*.

Abstract An insecticidal formulation of indoxacarb (Arilon®, Syngenta Crop Protection LLC, Greensboro, NC, USA) was assessed in laboratory assays and field trials as a possible alternative to bifenthrin and fipronil in reducing *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) worker ant foraging activity in urban settings. The field efficacy trials conducted in a residential setting yielded significant ($F = 33.8$; df = 4, 207; $P < 0.0001$) reductions of foraging activity following perimeter applications with the four insecticide treatments (two rates of indoxacarb and one rate each of bifenthrin and fipronil) in comparison to the untreated controls. Reductions in foraging activity did not differ significantly between the fipronil and bifenthrin treatments, but both insecticides provided significantly greater reductions in foraging than achieved with either of the two rates of indoxacarb. Worker ants exhibiting necrophoretic behavior by moving ants killed by exposure to topical sprays or contact with treated surfaces with indoxacarb further increased the potential for management of *L. humile* with indoxacarb in urban settings through secondary kill.

Key Words *Linepithema humile*,insecticide, arilon, talstar, termidor, indoxacarb, bifenthrin, fipronil, secondary kill

Introduction

The Argentine ant, *Linepithema humile* (Mayr), is a significant agricultural and urban pest and is considered one of the most important non-termite urban pests in Georgia (Vega and Rust 2001, Guillebeau et al. 2008). It is an ecologically- and economically-important invasive nuisance pest, often displacing native ants (Newell and Barber 1913, Human and Gordon 1996, Kennedy 1998, Vega and Rust 2001). Barber (1916) places the Argentine ant in Georgia by 1916. A nuisance pest in urban settings, Argentine ants invade buildings in search of more favorable living conditions, and/or food and water (Markin 1970a, Rust et al. 2000, Gordon et al. 2001).

Conventional control measures for Argentine ants consist of baits, contact insecticides, barrier sprays, or granulars (Klotz et al. 1995, Suiter et al. 1997, Greenberg and Klotz 2000, Rust et al. 2003, Klotz et al. 2007, Choe et al. 2014). Although sprays containing pyrethroids or fipronil are most commonly used to manage Argentine ants, a relatively new chemical formulation of indoxacarb (Arilon® Insecticide, Syngenta Crop Protection, LLC, Greensboro, NC) has also shown potential as an alternative to products containing pyrethroids or fipronil (Gallagher et al. 2012). In light of this new product, studies were conducted to determine the comparative efficacy in an urban setting with two conventional, commercially available insecticides (Termidor® SC termiticide/insecticide, BASF Corporation, Research Triangle Park, NC; and Talstar® Professional Insecticide, FMC Professional Solutions, Philadelphia, PA). In addition to comparing efficacy of these products, the potential for secondary kill of Arilon for control of Argentine ants was also investigated in laboratory assays.

Materials and Methods

Laboratory trial.

Ants. Argentine ants used in these bioassays were collected from property adjacent to the UGA Griffin campus in Griffin, Georgia, U.S.A. (N 33° 15'58.41", W 84° 17'19.98"). Ants, including brood and queens, were collected along with accompanying soil, leaf litter, and other debris. The collected ants and material were placed in a plastic tub (≈57 x 45 x 13 cm) (Model 400-5N, Del-Tec/Panel Controls Corporation, Greenville, SC). The tub was prepared in advance of ant collection by coating the inside walls with Fluon™ (Northern Products Inc., Woonsocket, RI) to prevent ant escape. In the lab, ants were provided harborage to prevent desiccation but were not provided food. Ants used in bioassays were used within 7 d of being collected.

Ant harborage. Harborage for the collected ants was prepared using polystyrene culture dishes (100 x 25 mm; NalgeNunc International, Rochester, NY) half-filled with Castone™ (Model 99044, Dentsply International Inc., York, PA), a high-strength, and water absorbent dental molding material. For each dish preparation, Castone powder was mixed with water at a ratio of 120 g to 40 ml, after which all of the mixture was placed in the dish. Before the Castone hardened, dishes were gently and repeatedly tapped on a horizontal surface to ensure even distribution of the material and to remove air bubbles. After air drying for ≈24 h, two holes (1.6 mm diam and 180° apart) were drilled through the side of the dish, just above the surface of the dried Castone, to provide entrance and exit holes for the ants. A third hole was drilled in the center of the accompanying lid of each dish. All dishes and lids were rinsed under running tap water to remove plastic debris and Castone dust prior to use. After rinsing, dishes and lids were placed in an oven (60°C) for 1-3 d to ensure complete drying of the Castone. After drying, and just prior to being used, dishes were filled with water to ensure complete saturation of the

Castone. Excess water was decanted and the Castone surface was wiped dry with a paper towel, ensuring that no standing water remained in the dish.

To separate ants from the leaf litter debris, four moistened dishes were placed in the corners of a tub that contained ants and debris, and 1 ml of water applied to each dish daily to maintain a moist harborage. After ≈72 h, as the leaf litter dried, the ants (workers, brood, and queens) moved into the moistened dishes. Argentine ants are susceptible to desiccation, and readily move from dry/drying habitats to moist habitats. Therefore, with this technique the ants moved from their dirt and leaf litter debris into clean, debris-free harborages that facilitated collection of ants for bioassays. The ants were held at ambient humidity and temperature (20-23 °C).

Test treatments. Six treatments of Arilon, and an untreated control were evaluated for their repellency to and secondary kill of Argentine ants. Arilon was applied at 0.50% (high), 0.05% (medium), and 0.005% (low) indoxacarb at a rate of 18.9 liters / 92.9 m^2 to ceramic tile or as a direct topical spray applied to live ants using a handheld sprayer. For the residual application, 1 ml of Arilon was applied to a ceramic tile (232.3 cm^2) using a disposable pipette. The treatment was allowed to air dry for 2 h before live ants were placed on the tile. Before ants were placed on the tile, a cut piece of PVC (11.4 cm diam x 7.6 cm length) pipe, with inside walls lined with Fluon was placed on the tile to prevent ant escape (Figures 3.1 and 3.2).

For the topical application, several hundred live ants were placed in a plastic box (31.3 x 23 x 10.2 cm; Pioneer Plastics, Inc., Dixon, KY), the bottom lined with paper towels (to absorb excess spray) and were then misted several times to ensure adequate application was made to the ants. Treated ants were kept in their respective treatment arenas for 24 h in order to ensure exposure. After 24 h, the sprayed, dead ants were transferred with forceps to weigh boats (Figure

3.3). For the untreated control, ants were placed in a plastic box (31.3 x 23 x 10.2 cm; Pioneer Plastics, Inc., Dixon, KY) with the inside walls coated with Fluon and placed in a freezer (≈0°C) for 24 h to kill the ants. After 24 h, the freezer-killed ants were removed from the freezer and allowed to age for 24 h before being added to bioassays.

Bioassay. Test arenas were glass dishes (216 x 165 x 63.5 mm; PYREX, Corning, Inc., Corning, NY) with the inside walls coated with Fluon to prevent ant escape (Figure 3.4). Harborages were prepared using Castone-filled polystyrene culture dishes (35 X 10 mm; Corning Inc., Corning, NY) as previously described. However, Castone powder was mixed with water at a ratio of 30 g to 10 ml, after which, approximately 11 g of the mixture was placed into a polystyrene culture dish, so that each dish was approximately half-filled with Castone. Newly prepared dishes were prepared as previously described.

To conduct the bioassay, 40 untreated live worker ants from the newly-collected colony, were placed in each test arena, along with a cotton ball soaked in 25% sugar water, and allowed 1-2 h to acclimate to the moistened harborage and glass dish arena. Following this acclimation period, 20 dead ants from each treatment, placed on top of a modified weigh boat, were added to the floor of each test arena (Figure 3.4). Tests were conducted at room temperature, and each treatment was replicated 12 times. After 72 h, the total number of dead ants (calculated by counting the total number of live ants and subtracting this number from 40) and the total number of ants removed from the weigh boat were counted for each replicate.

Field trial.

Research site. A field trial was conducted to compare the efficacy of three conventional insecticides: Arilon insecticide, Termidor SC termiticide/insecticide, and Talstar Professional Insecticide at their labeled rate. The trial was performed on 28 Argentine ant-infested buildings

in Towaliga Village in Barnesville, Georgia, (N 33° 3'17.11", W 84° 9'59.16"). The buildings consisted of two architectural styles; with either 63.1 or 72.5 linear meters around each building (Figure 3.5).

Test treatments. Two applications of Arilon (applied at 0.05% indoxacarb at 7.57 liters / 92.9 m^2 [low] and 0.005% indoxacarb at 18.9 liters / 92.9 m^2 [high]), Talstar (applied at 0.004% bifenthrin at 18.9 liters / 92.9 m^2), and Termidor (applied at 0.06% fipronil at 5.68 liters / 92.9 m^2) were each applied to each of five buildings. Eight buildings were left untreated and served as controls. The treatments were applied using a using a recently purchased, unused compressed air sprayer (11.4 liter, Model 430-3G, Solo, Newport News, VA). Arilon (0.05%) and Termidor were applied in a 61 cm wide band (30.5 cm up the structure's wall, and 30.5 cm out on the grass from the building's outside wall) to the building's exterior perimeter walls; Arilon (0.005%) and Talstar were applied in a 152 cm wide band (60.8 cm up, 91.2 cm out).

Response variable. Prior to treatment, a visual inspection was performed on each building wherein an ant trailing intensity rating was assigned to each of the buildings based on foraging activity on the outside perimeter walls of each building. The ratings for each wall section ranged from 0 to 3, where 0 was no visible ant foraging activity; 1 was light foraging activity; 2 was medium foraging activity; and 3 was high foraging activity. Each building consisted of 12 wall sections and the entire length of each wall section was independently rated and assigned a single value, 0 to 3. The 12 individual wall section ratings were summed to provide a total rating for each building, which ranged from 0 to 36.

Ant foraging activity was qualitatively rated on an intensity scale of zero, low, medium, and high. Low ratings consisted of at least more than two ants moving in a manner consistent and in the same direction as the others. One ant moving in a direction opposite of another ant did not

constitute a rating of one because this was not a consistent trail. Medium ratings consisted of trails at least 2 ants wide, and typically involved movement in two directions (i.e. left and right on the foundations). High ratings consisted of trails >4 ants wide, and typically involved movement in two directions.

Following treatment, visual inspections and ratings of the buildings were made at 1, 3, 4, 5, 8, 12, 16, 20, 24, 29, 35, 41, 46, and 50-weeks post-treatment. Percent reductions in ant activity, for each post-treatment sampling date, were calculated for each building with the following formula: $\frac{\text{pretreatment building rating} - \text{post treatment building rating}}{\text{pretreatment building rating}} \times 100.$

Laboratory trial statistical analysis. The numbers of dead ants for each treatment were analyzed by mixed model analysis of variance, utilizing the negative binomial distribution for the count data (PROC GLIMMIX [SAS Institute, Inc. 2010]). Following analysis, differences between least square means, for and among each treatment, were determined using pairwise t-tests (Table 3.1).

The number of ants removed from the weigh boats was further analyzed by mixed model analysis of variance (PROC GLIMMIX [SAS Institute, Inc. 2010]). Following analysis, differences between least square means, for each treatment combination, were determined using pairwise t-tests (Table 3.2). The correlation of dead with number removed from the weigh boats was subjected to correlation analysis, by treatment combination (PROC CORR [SAS Institute, Inc. 2010]) (Table 3.3).

Residual plots of all data can be found in Figures 3.6 and 3.7. Visual observation of residual plots provides a good indication that the data are normally distributed and analysis of variance is appropriate. Residuals should be uniformly distributed above and below a horizontal

reference with a mean of zero. Visual observation of our conditional residual plots suggests that our data appear normalized, and thus justify the use of ANOVA.

Field trial statistical analysis. For the field efficacy trial, treatment, week post-treatment, and the treatment*week post-treatment interaction were analyzed (PROC GLM [SAS Institute, Inc. 2010]). At each week post-treatment, percentage reduction in ant foraging activity for each treatment was then analyzed by one-way analysis of variance (PROC GLM [SAS Institute, Inc. 2010]). Following each one-way ANOVA, differences between least square means were determined using pairwise t-tests (Table 3.4). A residual plot of the data can be found in Figure 3.8. Visual observation of the residual plot suggests that our data appear normalized, and thus justify the use of ANOVA.

Results

Laboratory study. Regardless of how they were killed (sprayed or exposed to treated tiles), more live ants exposed to ant cadavers killed by the two highest concentrations of Arilon (high and medium) died than live ants exposed to the lowest concentration of Arilon (F = 13.05; df = 6, 70.83; $P < 0.0001$; Table 3.1, Figure 3.9). Ant mortality in the medium and high treatments (both spray and treated tile) was significantly greater than mortality in both low treatments and the freeze-killed control (Table 3.1); mortality in the low treatments (both spray and treated tile) was not significantly different than controls (Table 3.1, Figure 3.9).

The mean number of dead ants removed from the weigh boats was also significant (F = 3.82; df = 6, 59.02; P = 0.0028). With the exception of Arilon medium tile (P = 0.2863), significantly more treated cadavers were removed from weigh boats than were removed for the controls (Table 3.2, Figure 3.9).

The correlation of dead with removed was significant only for spray high (0.50% concentration; $P = 0.008$) and tile medium (0.05% concentration; $P = 0.001$) (Table 3.3). All treatments achieved higher mean dead ant removal than the control, and treatment method or concentration does not appear to affect necrophoresis.

Field study. Treatment ($F = 28.9$; df = 4, 322; $P < 0.0001$), week post-treatment ($F = 26.8$; df = 13, 322; $P < 0.0001$), and their interaction ($F = 3.28$; df = 52, 322; $P < 0.0001$), were highly significant. However, given no significant differences among treatments and controls after 24 weeks post-treatment, we halted the analysis. When data from weeks 1-24 were combined and analyzed by two-way ANOVA, treatment ($F = 33.8$; df = 4, 207; $P < 0.0001$); week-post treatment ($F = 10.14$; df = 8, 207; $P < 0.0001$); and their interaction ($F = 2.76$; df = 32, 207; $P < 0.0001$) were highly significant. Overall, Arilon 0.05% was comparable with Talstar and Termidor at 1, 4, and 5 weeks post-treatment (Table 3.4). Talstar, at 1-week post-treatment (mean = 84.3% ±13.6%; $P < 0.0001$), and Termidor, at 24-weeks post-treatment (mean = 84.5% ±8.74%; $P < .0001$), achieved the highest overall significant reductions. For weeks 1 through 24 post-treatment, Termidor and Talstar were most effective, followed by Arilon 0.05%, and Arilon 0.005% (Figure 3.10). The untreated control buildings generally had higher, but not significantly, ratings after treatment compared to the treated buildings (mean = -10.5% ± 13.2%; $P = 0.177$).

Discussion

Conventional approaches to insect management continually rely on the use of proven, effective insecticides. Among these for Argentine ants are pyrethroids and the phenylpyrazole insecticide, fipronil. Given recent, growing restrictions on the uses of pyrethroids and pyrethrins, continued reliance on products containing these active ingredients will likely wane and effective alternatives to fill the gap will be needed (United States Environmental Protection Agency 2013).

Given these issues, evaluations of different rates, concentrations, and combinations of products provide opportunity to explore the use or uses of conventional active ingredients in innovative ways. Two such ways, both involving the use of conventional methods, involve combining conventional spray applications or baits with an application of pheromone to improve efficacy (Greenberg and Klotz 2000, Choe et al. 2014). Another approach is to use conventional products at different rates and concentrations. In this study, we evaluated the use of Arilon at two different rates and concentrations. The 0.05% concentration was applied at a lower volume than the 0.005% concentration. What is interesting is that the lower concentration (0.005%), higher application rate, despite having more than five times the total amount of solution applied (Table 3.5), was significantly less effective than the higher concentration (0.05%), lower volume treatment. This indicates that more active ingredient should be applied and is significantly more effective despite higher application rate presumably achieving greater saturation of the treated area due to more water being applied. None of our treatments were extremely effective at reducing and sustaining that reduction for more than 24 weeks. Furthermore, our results for fipronil differed significantly from those reported by Klotz et al. (2007) at 1 and 8-weeks post-treatment. They noted a 93% reduction in ant activity at 1 wk post-treatment and 81% reduction in ant activity 8 wk post-treatment (response variable indicated by estimates of ant activity by consumption of 50% sucrose-water monitoring). We observed mean percentage reductions of 72% (±13%) at 1 wk post-treatment and 61% (±14%) at 8 wk post-treatment. This may be explained by our study encountering higher than normal ant activity during the duration of the study (J.B.H., personal observation). Or it may be the result of other, numerous unknown factors. Our results were not entirely what we expected, all products evaluated—Arilon (indoxacarb), Termidor (fipronil), and Talstar (bifenthrin)—significantly reduced ant activity in this study.

Additionally, Arilon is not repellent to Argentine ants and achieves secondary kill. Non-repellency is important in achieving interaction of live, untreated ants with dead, possibly treated ants, which occurs through necrophoresis. It is also important for achieving interaction of live, untreated ants with live, treated ants—which occurs through grooming and trophallaxis, and with the chemical itself—which is required of a contact insecticide to be effective (Knight and Rust 1990a, Wiltz et al. 2009). While comparative studies evaluating differences of secondary kill incorporating all of the products we evaluated have not been done, Choe and Rust (2008) found that fipronil was the only one of eight insecticides evaluated, including bifenthrin, that exhibited secondary kill of Argentine ants. Additionally, Soeprono and Rust (2004c) evaluated two of the active ingredients we used—fipronil and bifenthrin—and found that fipronil caused the highest rate of mortality, followed by bifenthrin. These results follow the trend of our field study where fipronil resulted in the highest mortality, followed by bifenthrin, and by indoxacarb.

Lastly, both the laboratory and field study confirm that indoxacarb is an effective means of reducing Argentine ant activity around structures in urban settings. Although not as effective as fipronil and bifenthrin, it has a similar LD_{50} as both products (Table 3.6) and, given changes in regulations on the uses of pyrethroids, it provides an alternative for pest management personnel to increasingly restricted products.

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Table 3.1. Differences of treatment least square means for number of ants dead at conclusion of the Arilon laboratory study.

Treatment*	Treatment	df	t-value	P-value
Control	Spray High	77	-4.96	<.0001
Control	Spray Low	77	-1.54	0.1266
Control	Spray Med	77	-6.39	<.0001
Control	Tile High	77	-6.13	<.0001
Control	Tile Low	77	-1.81	0.0745
Control	Tile Med	77	-5.21	<.0001
Spray High	Spray Low	75.39	3.51	0.0008
Spray High	Spray Med	55.7	-1.54	0.1283
Spray High	Tile High	56.2	-1.26	0.2133
Spray High	Tile Low	73.36	3.25	0.0017
Spray High	Tile Med	58.26	-0.27	0.7904
Spray Low	Spray Med	71.75	-5.01	<.0001
Spray Low	Tile High	72.31	-4.73	<.0001
Spray Low	Tile Low	77	-0.27	0.7898
Spray Low	Tile Med	74.64	-3.77	0.0003
Spray Med	Tile High	53.06	0.29	0.7763
Spray Med	Tile Low	69.77	4.75	<.0001
Spray Med	Tile Med	55.06	1.28	0.2068
Tile High	Tile Low	70.33	4.48	<.0001
Tile High	Tile Med	55.56	0.99	0.3254
Tile Low	Tile Med	72.62	-3.51	0.0008

*Low is the 0.005% concentration; Med is the 0.05% concentration; and High is the 0.50% concentration of Arilon.

Table 3.2. Differences of treatment least square means of number of dead ants removed from weight boats at the conclusion of the Arilon laboratory study.

Treatment	Treatment	df	t-value	P-value
Control	Spray High	70.96	-2.36	0.0212
Control	Spray Low	68.55	-3.01	0.0036
Control	Spray Med	66.1	-3.81	0.0003
Control	Tile High	68.62	-2.99	0.0038
Control	Tile Low	66.62	-3.63	0.0006
Control	Tile Med	77	-1.07	0.2863
Spray High	Spray Low	56.54	-0.67	0.5068
Spray High	Spray Med	54.3	-1.49	0.1429
Spray High	Tile High	56.6	-0.65	0.5204
Spray High	Tile Low	54.78	-1.30	0.2001
Spray High	Tile Med	64.35	1.29	0.2009
Spray Low	Spray Med	52.18	-0.82	0.4161
Spray Low	Tile High	54.44	0.02	0.9831
Spray Low	Tile Low	52.65	-0.63	0.5315
Spray Low	Tile Med	62.05	1.96	0.0549
Spray Med	Tile High	52.24	0.84	0.4042
Spray Med	Tile Low	50.49	0.19	0.8500
Spray Med	Tile Med	59.71	2.77	0.0075
Tile High	Tile Low	52.71	-0.65	0.5178
Tile High	Tile Med	62.11	1.94	0.0575
Tile Low	Tile Med	60.21	2.58	0.0123

*Low is the 0.005% concentration; Med is the 0.05% concentration; and High is the 0.50% concentration of Arilon.

Table 3.3. The CORR procedure simple statistics and Pearson Correlation Coefficients (Prob > |r| under H0: Rho=0) for dead with removed from weigh boats for the Arilon laboratory study.

Treatment	**Pearson Correlation Coefficients (*R* values)**	**Prob > |r| under H0**
Control	-0.51239	0.0885
Spray 0.50% (High)	0.72232	0.0080
Spray 0.05% (Medium)	0.33240	0.2911
Spray 0.005% (Low)	0.14028	0.6637
Tile 0.50% (High)	0.55435	0.0614
Tile 0.05% (Medium)	0.82112	0.0011
Tile 0.005% (Low)	-0.11816	0.7145

Table 3.4. Percentage reduction (mean ± S.E.) in foraging activity, given by changes from pretreatment rating, of ants around structures after being treated with various products at week post-treatment. Negative means denote an increase in activity.

Treatment	Week 1	Week 3	Week 4	Week 5	Week 8	Week 12	Week 16	Week 20
Arilon 0.05% (Low)	66.8 ± 13.6 A	-7.8 ± 26.6 B,C	53.3 ± 14.1 A	58.0 ± 14.3 A	10.4 ± 14.4 B	0.02 ± 17.0 B	12.2 ± 16.8 B	10.3 ± 16.9 B
Arilon 0.005% (High)	6.52 ± 13.6 B	-60.9 ± 26.6 C,D	11.3 ± 14.1 B,C	13.2 ± 14.3 B,C	37.9 ± 14.4 A,B	-13.4 ± 17.0 B	20.0 ± 16.8 B	36.7 ± 16.9 A,B
Control	-18.1 ± 10.8 B	-94.3 ± 21.0 D	-24.9 ± 11.2 C	-14.4 ± 11.3 C	23.4 ± 11.4 A,B	15.7 ± 13.4 B	22.9 ± 13.3 B	6.0 ± 13.3 B
Talstar	84.3 ± 13.6 A	70.2 ± 26.6 A	40.6 ± 14.1 A,B	35.0 ± 14.3 A,B	61.2 ± 14.4 A	71.7 ± 17.0 A	46.6 ± 16.8 A, B	40.9 ± 16.9 A,B
Termidor	71.7 ± 13.6 A	60.0 ± 26.6 A,B	55.8 ± 14.1 A	38.0 ± 14.3 A,B	61.0 ± 14.4 A	76.5 ± 17.0 A	74.8 ± 16.8 A	62.2 ± 16.9 A
	F = 13.67 df = 4, 23 P < 0.0001	F = 8.86 df = 4, 23 P = 0.0002	F = 7.63 df = 4, 23 P = 0.0005	F = 4.78 df = 4, 23 P = 0.0059	F = 2.61 df = 4, 23 P = 0.0619	F = 6.07 df = 4, 23 P = 0.0017	F = 2.37 df = 4, 23 P =0.0818	F = 2.19 df = 4, 23 P = 0.1018

Treatment	Week 24	Week 29	Week 35	Week 41	Week 46	Week 50
Arilon 0.05% (Low)	77.4 ± 8.74 A	92.9 ± 9.07 A		90.1 ± 13.0 A	92.2 ± 12.8 A	18.2 ± 15.2 A
Arilon 0.005% (High)	78.9 ± 8.74 A	74.6 ± 9.07 A	No ant activity	91.7 ± 13.0 A	87.2 ± 12.8 A	41.2 ± 15.2 A
Control	49.1 ± 6.91 B	71.2 ± 7.17 A	on this day	67.4 ± 10.3 A	81.5 ± 10.1 A	9.91 ± 12.1 A
Talstar	71.3 ± 8.74 A,B	84.7 ± 9.07 A	for all buildings	80.1 ± 14.3 A	71.4 ± 12.8 A	16.1 ± 15.2 A
Termidor	84.5 ± 8.74 A	93.3 ± 9.07 A		57.5 ± 14.3 A	89.4 ± 12.8 A	28.6 ± 15.2 A
	F = 3.41 df = 4, 23 P = 0.0249	F = 1.51 df = 4, 23 P = 0.2331		F = 1.40 df = 4, 23 P = 0.2639	F = 0.42 df = 4, 23 P = 0.7906	F = 0.75 df = 4, 23 P = 0.5702

Following ANOVA (PROC GLM), differences between least square means, within each week, were determined using pairwise t-tests; means within a column followed by the same letter are not significantly different.

Table 3.5. Total amount of product applied by building style and treatment in the field trial.

Treatment*	Bandwidth (in cm)	Building Style	Application Concentration	Volume Rate	Liters Applied	Total Amount of Product Applied
Arilon-Low	61 (30.5 up x 30.5 out)	A	.05%	7.57 liters / 92.9 m^2	3.13	7.7463 grams
Arilon-Low	61 (30.5 up x 30.5 out)	B	.05%	7.57 liters / 92.9 m^2	3.6	8.906 grams
Arilon-Low	61 (30.5 up x 30.5 out)	B	.05%	7.57 liters / 92.9 m^2	3.6	8.906 grams
Arilon-Low	61 (30.5 up x 30.5 out)	B	.05%	7.57 liters / 92.9 m^2	3.6	8.906 grams
Arilon-Low	61 (30.5 up x 30.5 out)	B	.05%	7.57 liters / 92.9 m^2	3.6	8.906 grams
Arilon-High	152 (60.8 up x 91.2 out)	A	.005%	18.9 liters / 92.9 m^2	19.6	4.8414 grams
Arilon-High	152 (60.8 up x 91.2 out)	A	.005%	18.9 liters / 92.9 m^2	19.6	4.8414 grams
Arilon-High	152 (60.8 up x 91.2 out)	A	.005%	18.9 liters / 92.9 m^2	19.6	4.8414 grams
Arilon-High	152 (60.8 up x 91.2 out)	A	.005%	18.9 liters / 92.9 m^2	19.6	4.8414 grams
Arilon-High	152 (60.8 up x 91.2 out)	A	.005%	18.9 liters / 92.9 m^2	19.6	4.8414 grams
Talstar	152 (60.8 up x 91.2 out)	A	0.004%	18.9 liters / 92.9 m^2	19.6	10.10 mL
Talstar	152 (60.8 up x 91.2 out)	A	0.004%	18.9 liters / 92.9 m^2	19.6	10.10 mL
Talstar	152 (60.8 up x 91.2 out)	B	0.004%	18.9 liters / 92.9 m^2	22.5	11.613 mL
Talstar	152 (60.8 up x 91.2 out)	A	0.004%	18.9 liters / 92.9 m^2	19.6	10.10 mL
Talstar	152 (60.8 up x 91.2 out)	B	0.004%	18.9 liters / 92.9 m^2	22.5	11.613 mL
Termidor	61 (30.5 up x 30.5 out)	A	.06%	5.68 liters / 92.9 m^2	2.35	14.4 mL
Termidor	61 (30.5 up x 30.5 out)	A	.06%	5.68 liters / 92.9 m^2	2.35	14.4 mL
Termidor	61 (30.5 up x 30.5 out)	Modified A	.06%	5.68 liters / 92.9 m^2	4.1	25.5 mL
Termidor	61 (30.5 up x 30.5 out)	A	.06%	5.68 liters / 92.9 m^2	2.35	14.4 mL
Termidor	61 (30.5 up x 30.5 out)	A	.06%	5.68 liters / 92.9 m^2	2.35	14.4 mL

*Arilon-Low is the Arilon 0.05% treatment; Arilon-High is the Arilon 0.005% treatment.

Table 3.6. LD_{50} (acute oral toxicity—combined male and female rat) for various active ingredients in pure form.

Product	**LD_{50} (mg/kg)**	**Source**
Rosemary Oil	5000	Sigma-Aldrich (2014)
Bifenthrin	53.4 to 210.4	National Pesticide Information Center (2014a)
Indoxacarb	< 1,000	United States Environmental Protection Agency (2000)
Fipronil	97	National Pesticide Information Center (2014b)

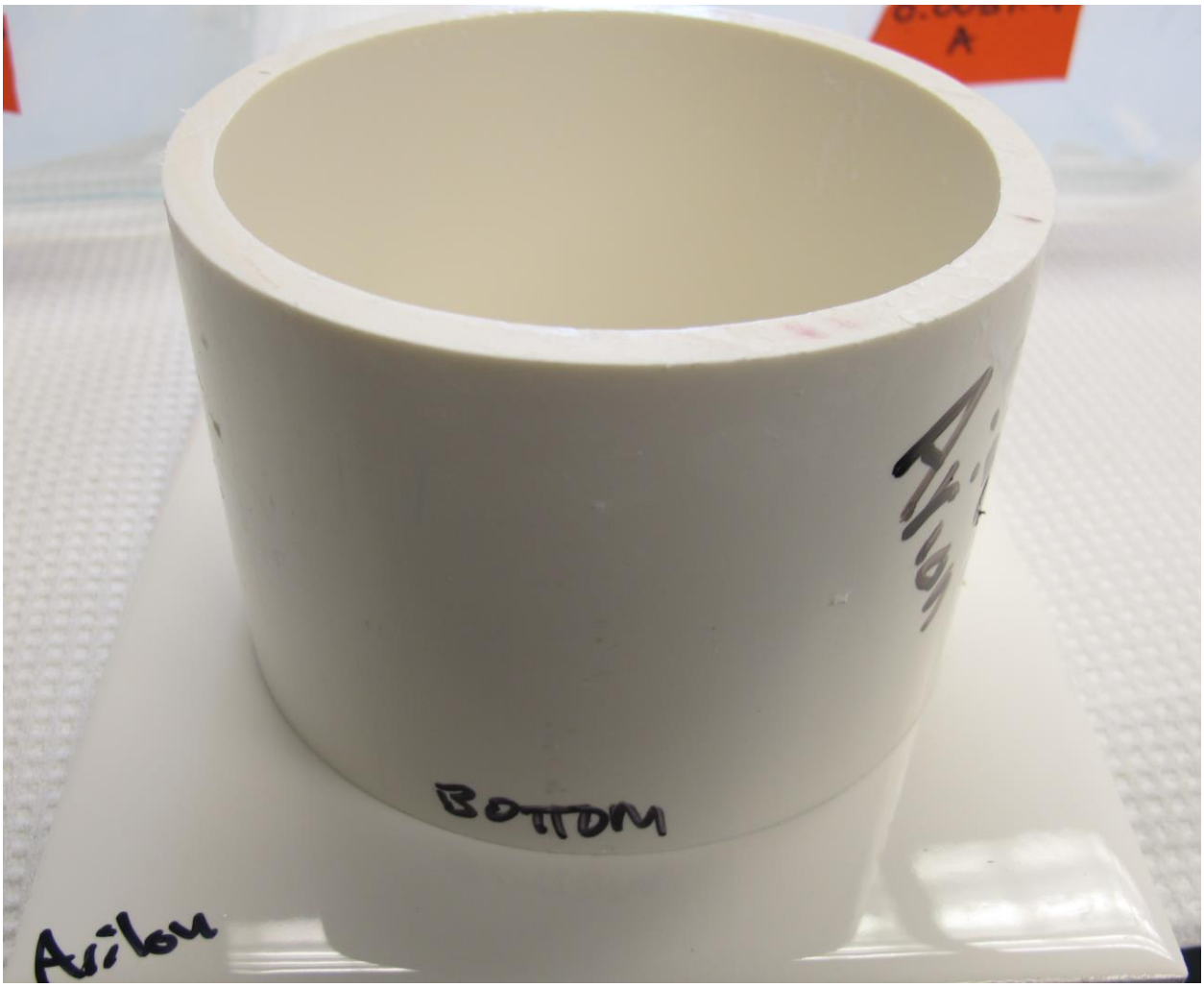

Figure 3.1. Side view of tile treatment design.

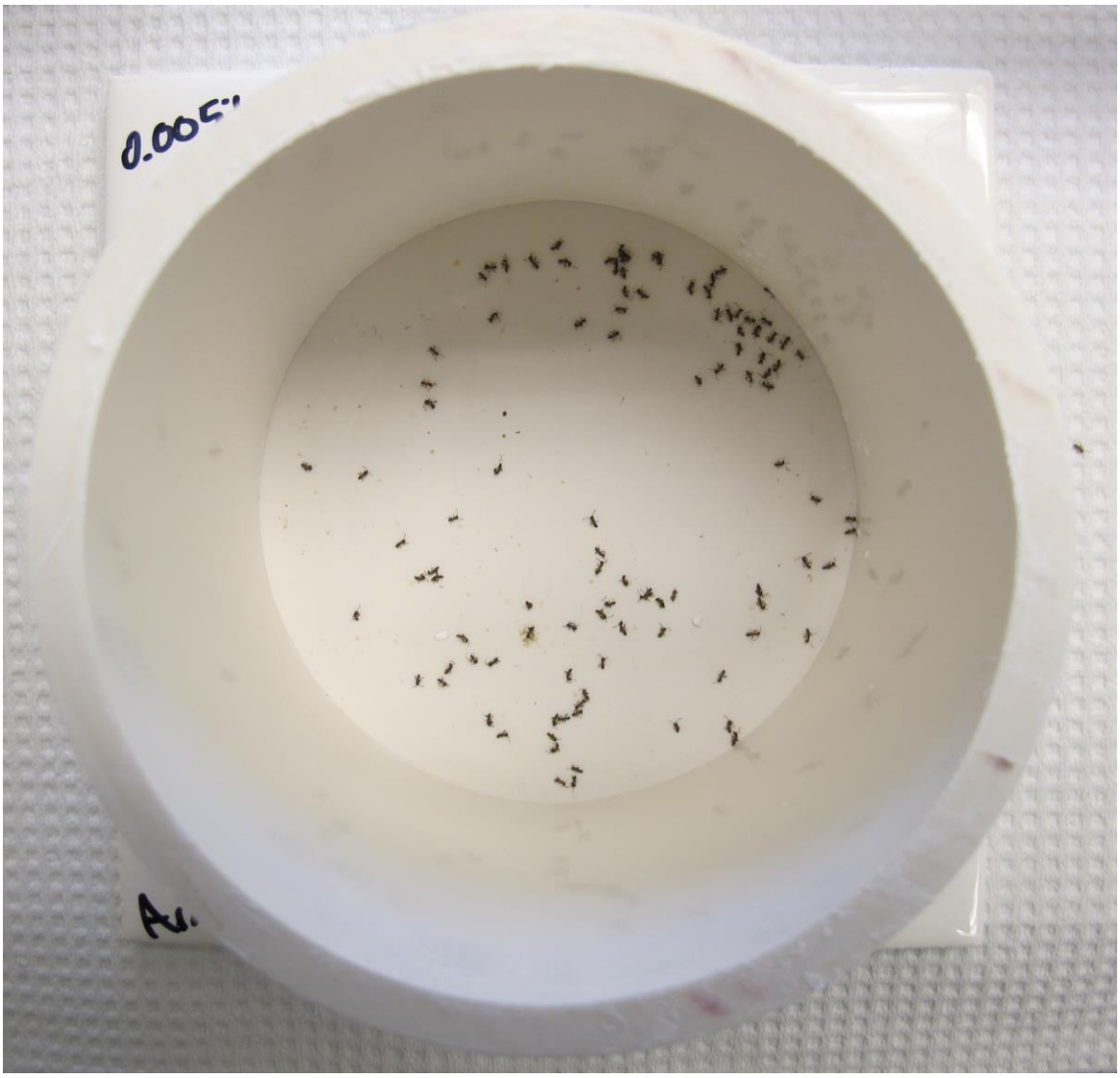

Figure 3.2. Top view of tile treatment design. Dead ants are visible on the tile.

Figure 3.3. Top view of spray treatment dish following transfer from plastic container. Dead ants are visible on dish floor.

Figure 3.4. Test arena for laboratory study, containing worker ants, harborage, and modified weigh boat.

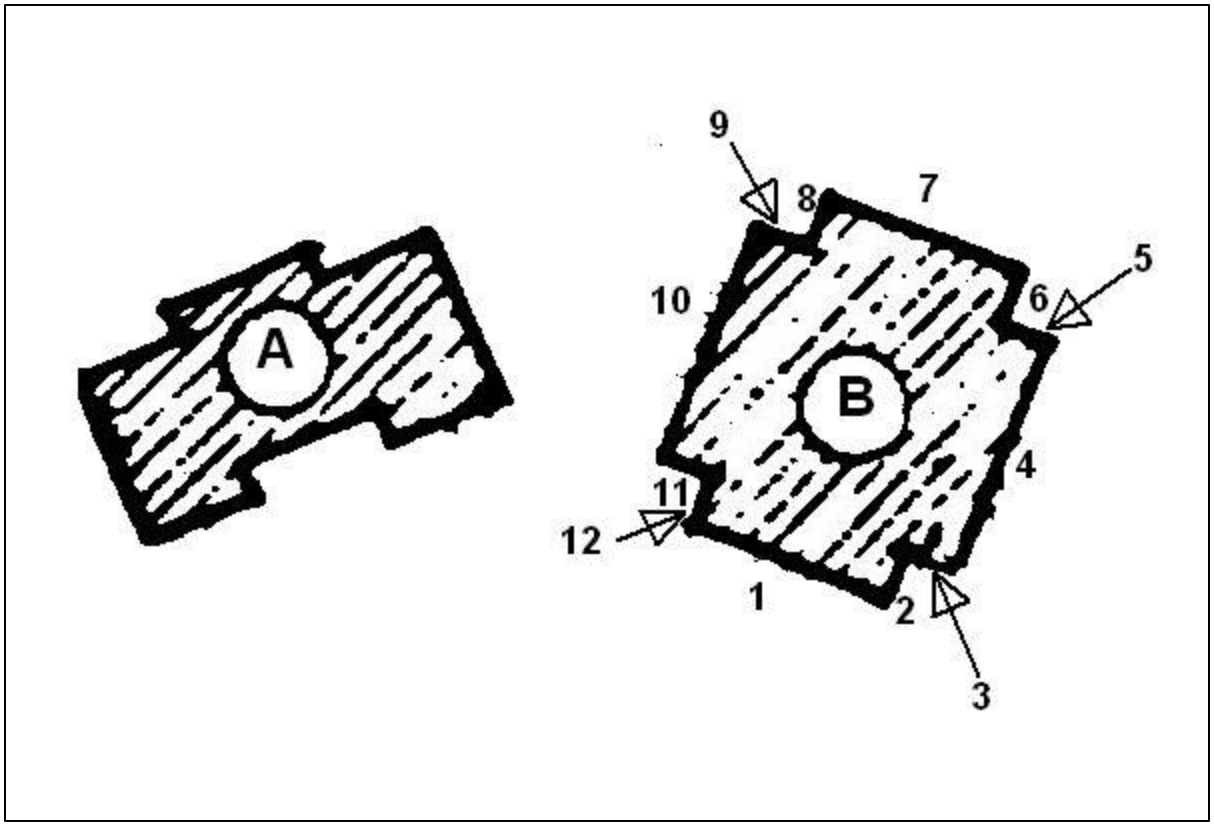

Figure 3.5. A sketch representing the two different architectural building styles (A and B) included in the field trial and the division of walls into numerical sections on building style B.

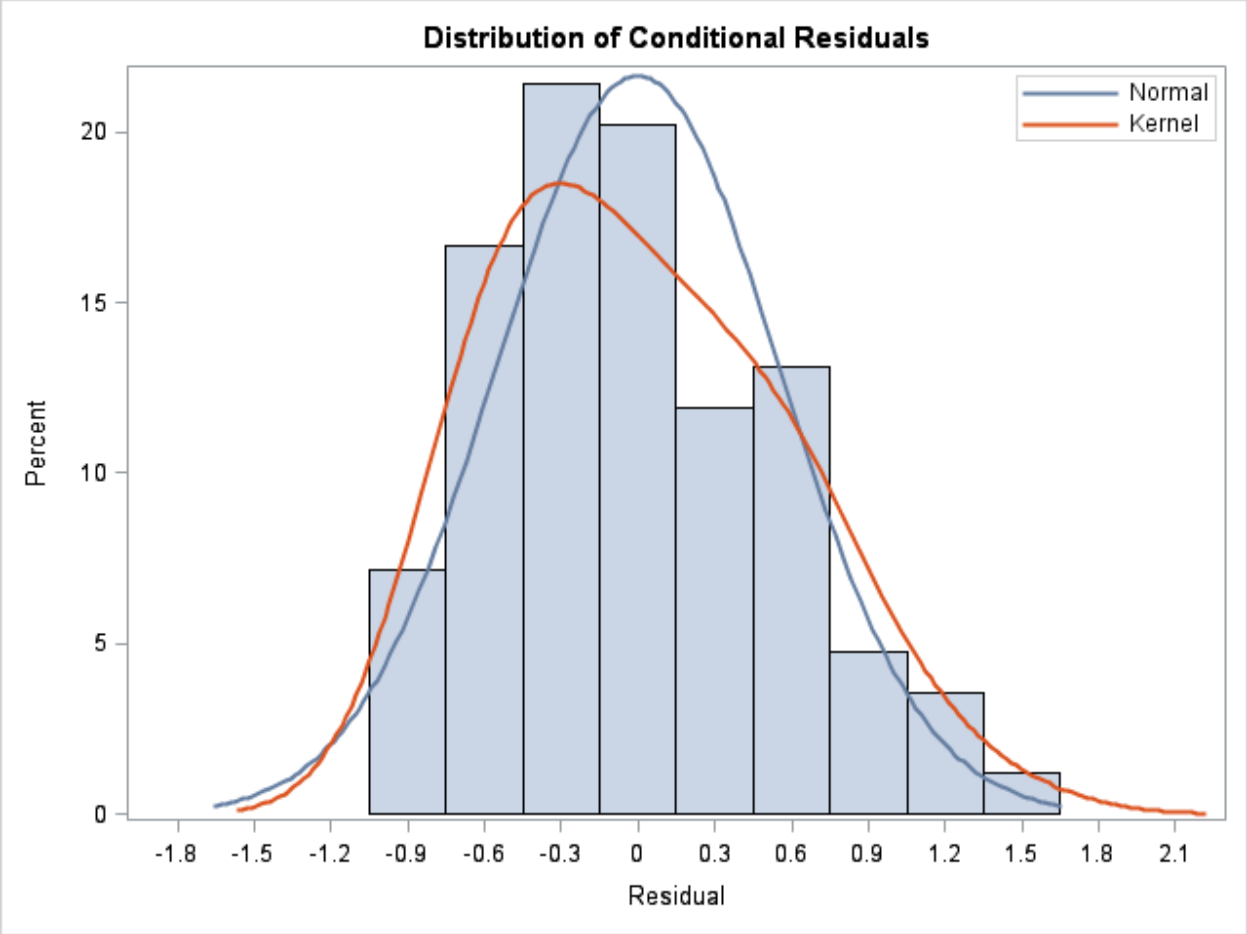

Figure 3.6. Residual plot for dead ants for laboratory study.

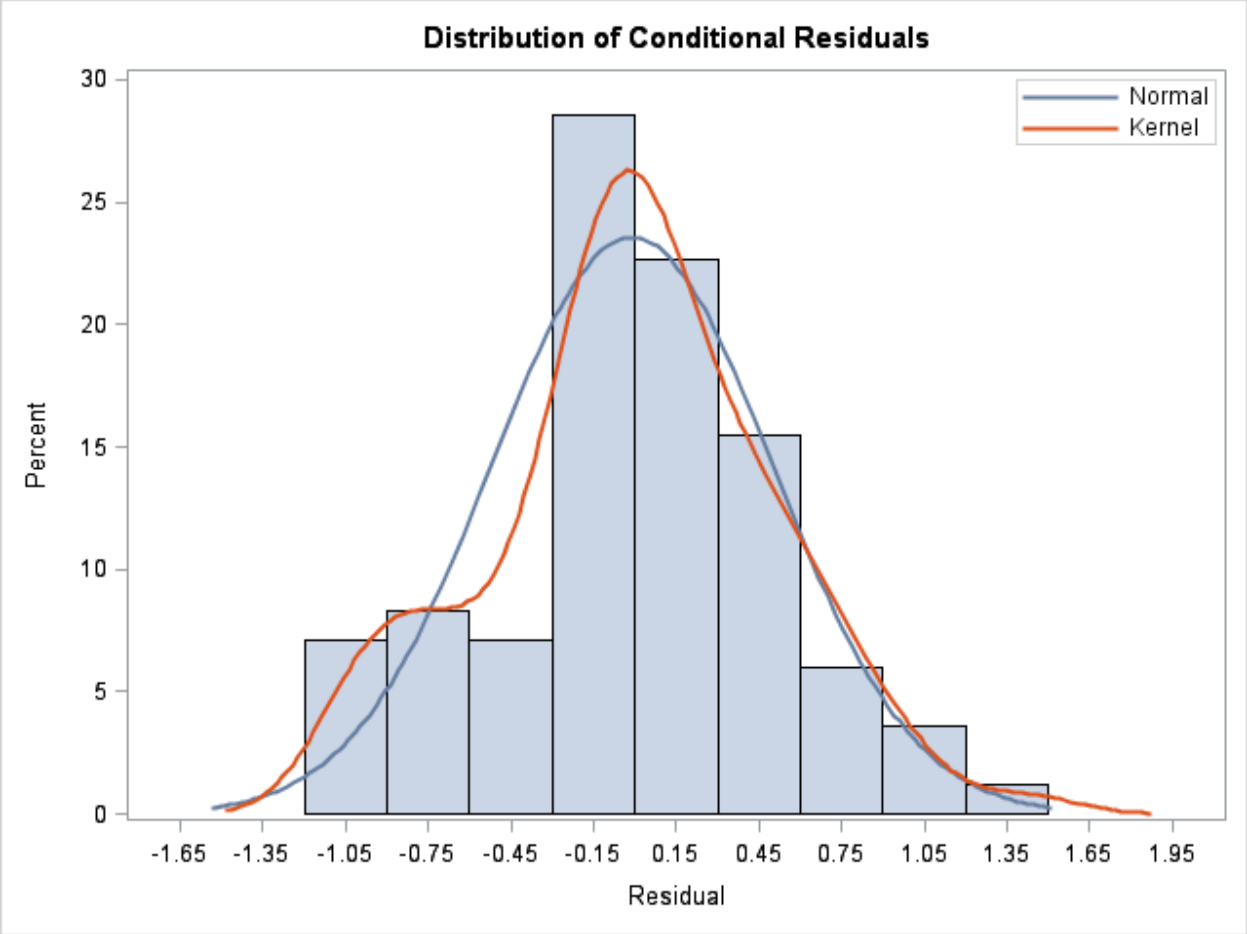

Figure 3.7. Residual plot for ants removed from weigh boats for laboratory study.

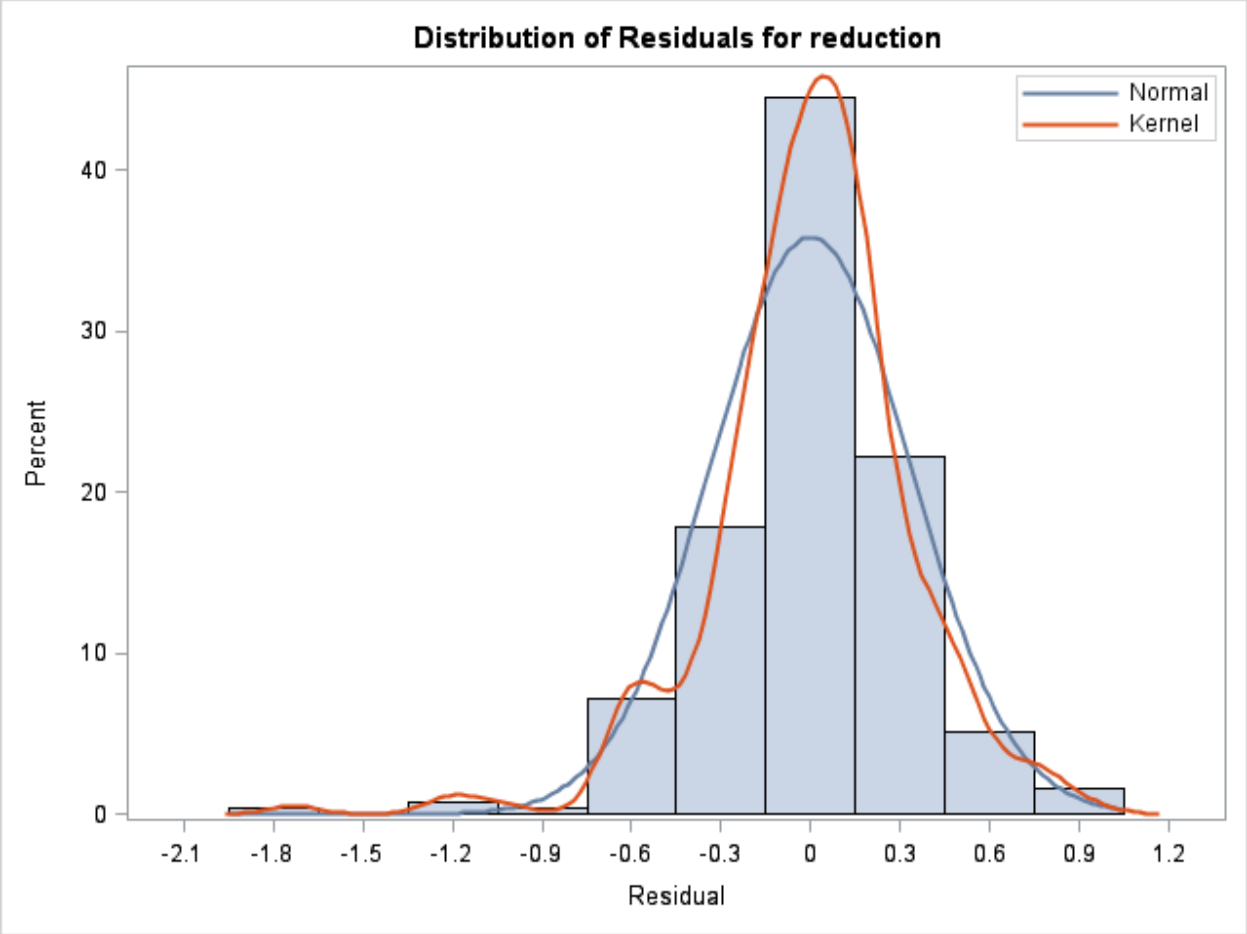

Figure 3.8. Residual plot for percentage reduction from pretreatment rating for weeks 1 through 24 post-treatment for the field study.

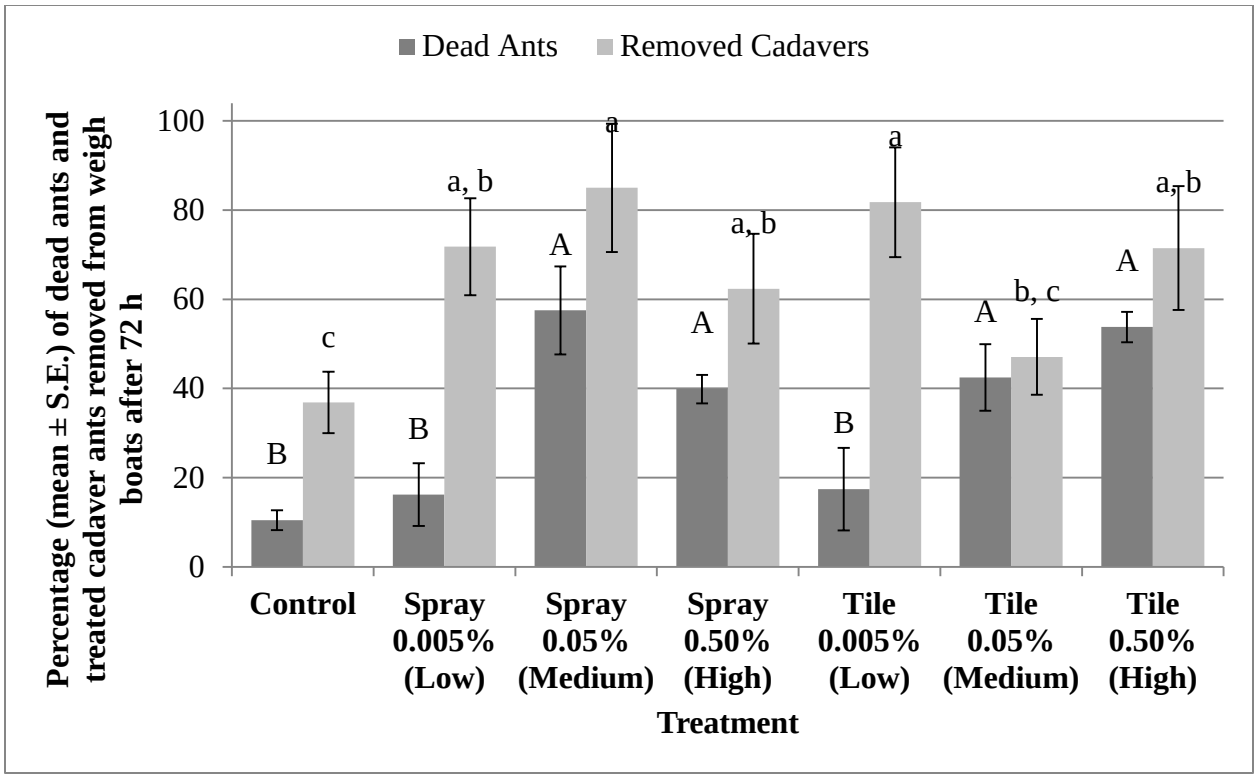

Figure 3.9. Percentage (mean ± S.E.) of dead ants (out of 40 ants total) and treated cadaver ants removed from weigh boats (out of 20 ants) after 72 h by treatment type for laboratory study. Percentages by column and type face that are the same are not significantly different.

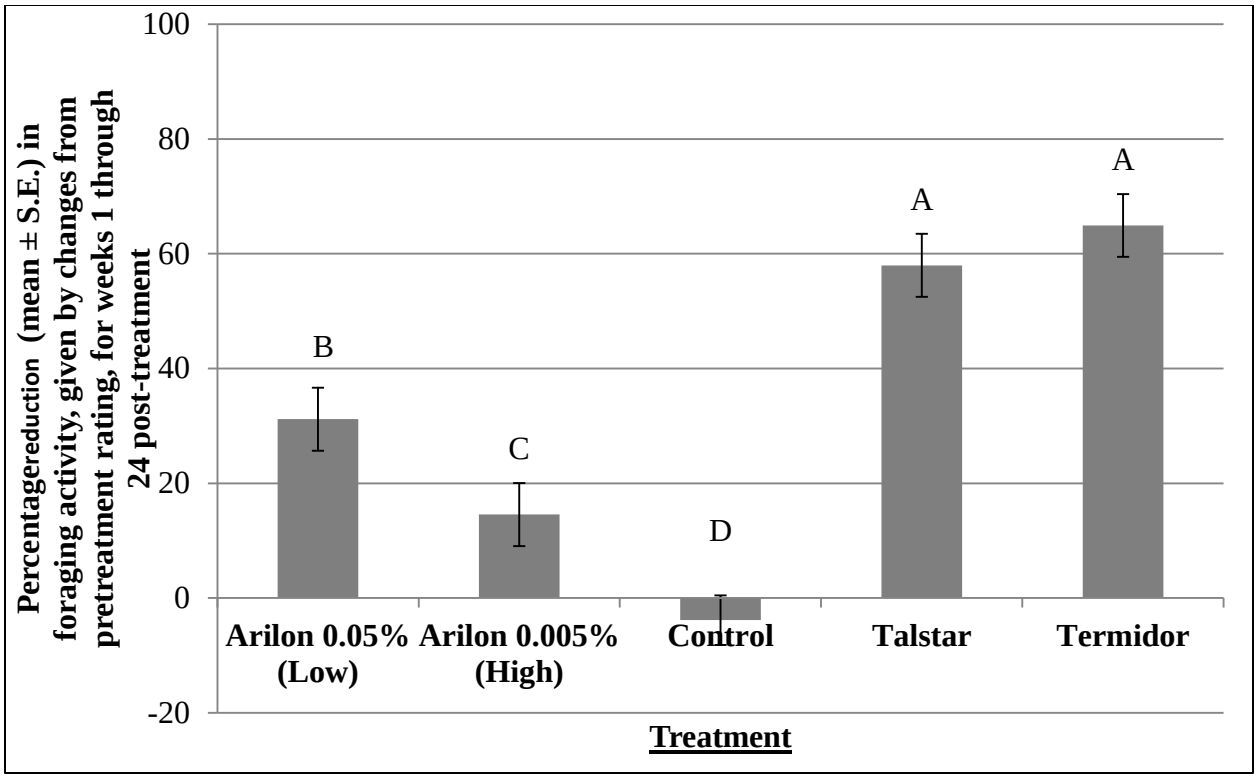

Figure 3.10. Percentage reduction (mean ± S.E.) in foraging activity, given by changes from pretreatment rating, for weeks 1 through 24 post-treatment for the field study. Means by column that have the same letter are not significantly different.

CHAPTER 4

REPELLENT AND DETERRENT ACTIVITY OF SELECTED PRODUCTS ON ARGENTINE ANT (HYMENOPTERA: FORMICIDAE) WORKERS[1]

[1] Holloway, J.B., and D.R. Suiter. To be submitted to *Environmental Entomology*.

Abstract Repellent and deterrent activity of various plant materials and a microencapsulated Argentine ant, *Linepithema humile*, trail pheromone, (*Z*)-9-hexadecenal, product on Argentine ant workers was evaluated in laboratory assays and field trials. A soybean tea, rosemary leaves, cucumber peels, tansy leaves, spearmint leaves, and a 1.0% peppermint oil solution were evaluated for repellency/deterrency in laboratory assays. The peppermint oil solution was highly repellent; rosemary was repellent; spearmint leaves were deterrent; and the soybean tea, cucumber peels, and tansy leaves were not repellent or deterrent. The microencapsulated, synthetic Argentine ant trail pheromone product was evaluated at three concentrations for impact on foraging activity in field trials and laboratory choice assays. The pheromone product applications resulted in significantly ($F = 8.95$; df = 3, 84; $P < 0.0001$) lower foraging activity than controls, and were significantly ($F = 10.69$; df = 7, 86; $P < 0.0001$) attractive in the laboratory choice assays.

Key Words *Linepithema humile*, natural pest control, repellency, essential oil, plant-based pest control, pheromone, (*Z*)-9-hexadecenal

Introduction

The Argentine ant, *Linepithema humile* (Mayr), is a significant agricultural and urban pest, is prevalent in primarily Mediterranean climates, and is considered one of the most important non-termite pests in the urban and suburban Southeastern United States (U.S.) (Vega and Rust 2001, Guillebeau et al. 2008). It is an ecologically- and economically-important invasive nuisance pest (Newell and Barber 1913, Vega and Rust 2001). Although native to South America, *L. humile* is now found on six continents (Suarez et al. 2001). It probably entered the U.S. by 1891 in coffee shipments from Brazil (Foster 1908); Barber (1916) places the Argentine ant in Georgia by 1916.

A consequence of Argentine ant infestations in both agricultural and urban settings is the use of various control measures, and insecticide use remains the most common strategy employed (Soeprono and Rust 2004a, Klotz et al. 2007). Although *L. humile* do not pose a direct health threat to humans, the continued reliance on chemical inputs poses environmental risks (Greenberg et al. 2010, Jiang et al. 2014). In light of these concerns, alternative control methods have recently received increasing attention, with particular interest in the use of "natural" products, including essential oils as repellents and insecticides; pheromones, as stand-alone applications and in conjunction with other products; and other less conventional products including plants, detergents, and food items (Bader 2006, Isman 2006, Wiltz et al. 2007, Suckling et al. 2008, Tanaka et al. 2009, Nishisue et al. 2010, Suckling et al. 2010, Sunamura et al. 2011, Scocco et al. 2012, Choe et al. 2014). Although studies have explored the practical use of natural products for the control of arthropods, much remains in their implementation as control agents for the Argentine ant, especially under field conditions (Isman 2006, Wiltz et al. 2007, Scocco et al. 2012).

Concerns over the environmental impact of chemical inputs have led to major changes to regulations in recent years that have limited the type and use of chemical insecticides for Argentine ant control. In 2009, the U.S. Environmental Protection Agency (E.P.A.), in an effort to reduce ecological exposure from residential uses of pyrethroid and pyrethrin products, implemented an initiative to revise guidelines for pyrethroid and pyrethrin pesticide products used in non-agricultural outdoor settings (United States Environmental Protection Agency 2013c). This initiative specifically limits non-agricultural outdoor uses of pyrethroids and pyrethrins to spot or crack-and-crevice treatments only with a few exceptions (United States Environmental Protection Agency 2013c). In addition to expanding restrictions on pyrethroid and pyrethrin use, the U.S. E.P.A. in 2013 implemented a series of changes to restrict the use of neonicotinoid insecticides in order to limit exposure to pollinators (United States Environmental Protection Agency 2013b). These changes include changing pesticide labels to limit applications to protect bees, including limiting application of neonicotinoids while bees are foraging in non-agricultural settings (United States Environmental Protection Agency 2013a). In light of these concerns and changes, several alternative methods to conventional control have received attention as potential management options.

Integrating several approaches appears promising in effectively managing *L. humile*. One approach is to utilize a synthetic version of the *L. humile* trail pheromone, (*Z*)-9-hexadecenal, either in conjunction with baiting or spraying techniques to improve efficacy or as a means to disrupt normal foraging activity (Greenberg and Klotz 2000, Tanaka et al. 2009, Suckling et al. 2010, Sunamura et al. 2011, Choe et al. 2014). This is particularly appealing given the potential this approach may have on reducing the overall amount of applied conventional insecticides. First identified as a major component of the Argentine ant trail-pheromone complex by Cavill et

al. (1979), synthetic versions of (*Z*)-9-hexadecenal have since been shown to demonstrate both attractant and repellent properties, depending on the applied concentration (Van Vorhis Key and Baker 1982a, 1982b; Suckling et al. 2008; Tanaka et al. 2009; Nishisue et al. 2010; Suckling et al. 2010; Suckling et al. 2011). (*Z*)-9-hexadecenal is also known to be a component of some moth sex pheromones and is widely available commercially (Suckling et al. 2011, El-Sayed 2014). Utilizing synthetic (*Z*)-9-hexadecenal to reduce Argentine ant foraging has been recently researched extensively (Greenberg and Klotz 2000, Suckling et al. 2008, Tanaka et al. 2009, Nishisue et al. 2010, Suckling et al. 2010, Suckling et al. 2011, Sunamura et al. 2011, Choe et al. 2012, Choe et al. 2014). As a stand-alone product, synthetic (*Z*)-9-hexadecenal has shown efficacy at disrupting Argentine ant trails when high concentrations of the pheromone are applied to paper discs and then the treated discs applied to trails (Suckling et al. 2010). However, one limiting factor of the use of synthetic pheromone is the longevity of the pheromone in the field, as it highly volatile in its natural form (Suckling et al. 2008). In light of this issue, Suckling et al. (2010) formulated the synthetic pheromone into a microencapsulated-sprayable product that can be readily dispersed in a field setting. Microencapsulation allows the pheromone to more slowly volatilize and, with this formulation, Suckling et al. (2010) observed an increase in efficacy from around 24 h to around 14 d post-treatment.

In addition to work on essential oils, there are publications espousing the benefits of products that have not been scientifically investigated. In particular, Bader (2006) has claimed numerous plant-based products to be effective as ant repellents or toxicants. Included are claims about cucumber peels, a soybean tea, and tansy leaves. Although there is no scientific evidence to support Bader's claims, given the appeal of utilizing alternatives to conventional insecticides, this presented an opportunity for further investigation. The objectives, therefore, of this study

were to assess the claims made by Bader about the use of several nonconventional products as repellents and/or deterrents to Argentine ants and to ascertain the efficacy of utilizing microencapsulated, synthetic Argentine ant trailing pheromone as a stand-alone product to impact Argentine ant harborage choice in the laboratory and to assess its impact on the foraging intensity of Argentine ants around the perimeter of inhabited buildings in the field.

Materials and Methods

Natural product laboratory trials.

Ants. Argentine ants used in this bioassay were collected from Towaliga Village in Barnesville, Georgia (N 33° 3'17.11", W 84° 9'59.16"). Ants, including brood and queens, were collected along with accompanying soil, leaf litter, and other debris. The collected ants and material were placed in a plastic tub (≈57 x 45 x 13 cm) (Model 400-5N, Del-Tec/Panel Controls Corporation, Greenville, SC). The tub was prepared in advance of ant collection by coating the inside walls with Fluon™ (Northern Products Inc., Woonsocket, RI) to prevent ant escape. In the lab, ants were provided harborage to prevent desiccation but were not provided food.

Ant harborage. Harborage for the collected ants was prepared using polystyrene culture dishes (100 x 25 mm; NalgeNunc International, Rochester, NY) half-filled with Castone™ (Model 99044, Dentsply International Inc., York, PA), a high-strength, water absorbent dental molding material. Castone powder was mixed with water at a ratio of 120 g to 40 ml, after which all of the mixture was placed in a dish. Before the Castone hardened, dishes were gently and repeatedly tapped on a horizontal surface to ensure even distribution of the material and to remove air bubbles. After air drying for ≈24 h, two holes (1.6 mm diam and 180° apart) were drilled through the side of the dish, just above the surface of the dried Castone, to provide entrance and exit holes for the ants. A third hole was drilled in the center of the accompanying

lid of each dish. All dishes and lids were rinsed under running tap water to remove plastic debris and Castone dust prior to use. After rinsing, dishes and lids were placed in an oven (60°C) for 1-3 d to ensure complete drying of the Castone. After drying, and just prior to being used, dishes were filled with water to ensure complete saturation of the Castone. Excess water was decanted and the Castone surface was wiped dry with a paper towel, ensuring that no standing water remained in the dish.

To separate ants from the leaf litter debris, four moistened dishes were placed in the corners of a tub that contained ants and debris, and 1 ml of water applied to each dish daily to maintain a moist harborage. After ≈72 h, as the leaf litter dried out, the ants (workers, brood, and queens) moved into the moistened dishes. Argentine ants are susceptible to desiccation, and readily move from dry/drying habitats to moist habitats. Therefore, with this technique the ants moved from their dirt and leaf litter debris into clean, debris-free harborages that allowed for ease of use in future bioassays. The ants were held at ambient humidity and temperature (20-23 °C) and starved.

Treatments. Laboratory trials were conducted to evaluate the repellency/deterrency of five treatments to Argentine ants: tansy leaf, cucumber peel, a soybean tea, spearmint leaf, rosemary leaf, and control treatments of peppermint oil (positive) and water (negative) (Table 4.1). Peppermint oil was acquired from Polarome International (Jersey City, NJ) and formulated at 1% according to the method of Scocco et al. (2012). Tansy plants, *Tanacetum vulgare* L., were purchased from a commercial nursery (Winterville, GA) and maintained in a greenhouse on the University of Georgia Griffin Campus. Several soybean (Maturity Group 7, Roundup Ready, Georgia Crop Improvement Association, Inc., Athens, Georgia), *Glycine max* L., plants were harvested from the Bledsoe Farm in Williamson, Georgia in June and July. The soybean seeds

were planted in May 2011 and had no insecticides applied after planting. Following removal from the ground, the plants were placed in small plastic storage bags (Ziploc, S.C. Johnson & Son, Inc., Racine, WI) containing ≈250 ml water. The plants remained in the open bag at ambient laboratory conditions until use (no more than 18 h, with temperatures ranging from 23-26°C). Common cucumbers were purchased from a supermarket in Griffin, Georgia, and stored in a refrigerator (temperature ranged from 5-10° C) until being used (no more than 6 h). Fresh spearmint, *Mentha spicata*, and fresh rosemary, *Rosmarinus officinalis*, leaves were obtained from a residence in Griffin, Georgia. The spearmint leaves were placed in a small plastic storage bag with water covering the leaves. The rosemary leaves were placed in a small plastic storage bag without water. The spearmint and rosemary leaves remained in the bags until use (approximately 1-2 h).

To prepare the soybean tea, soybean plants were rinsed under tap water to remove debris. Leaves were removed from the plant and 2.5 cm of the main stem removed (the cut line was approximately 6.5 mm above the first root). Two and one-half cm of the remaining stem was then removed, cut into 6.5 mm sections, and the sections then placed into a plastic vial (57 x 16.5 mm; Sarstedt Inc., Newton, NC) containing 1 ml of tap water. The stems were then allowed to soak for 24 h at ambient laboratory conditions. This procedure is similar to the instructions outlined by Bader (2006).

A cucumber was washed thoroughly under tap water and then peeled using a Farberware Euro Peeler (Lifetime Brands, Inc., Garden City, NY). Tansy and spearmint leaves were removed by hand from live plants. Cucumber peel, tansy leaf, and spearmint leaf were hole-punched using a single, round (≈3.5 mm diam) hole punch (At the office™, Wal-Mart Stores, Inc., Bentonville, AR).

Trial 1. For the dry treatments (tansy and cucumber), 12 pieces of hole-punched material were placed in a Pyrex® glass dish (150 x 75mm; Corning Inc., Lowell, MA) and the dish then placed in an oven (approximately 40°C) for 24 h. Twenty-four h later, and the day of the bioassay, 12 pieces of hole-punched material were prepared and used as the fresh treatments (tansy, cucumber, and spearmint) (Table 4.1). For the soybean tea, 0.25 ml of the tea was used (6.5 mm root equivalent). For the 1% peppermint oil solution control, 0.25 ml of the solution was used. For the water control, 0.25 ml of water was used.

Trial 2. Treatments consisted of 40 pieces of fresh hole-punched material (tansy, cucumber, and spearmint); four leaves of rosemary (approximately 2.5 cm long) that had been removed by hand from a live plant at a local residence and cut in half; 0.25 ml of 1% peppermint oil solution; and 1.0 ml of water for the water control (Table 4.1).

Bioassay. Similar to Scocco et al. (2012), treatment material or substance was applied directly to the surface of small (≈ 35 x 5 mm), Castone-filled dishes, prepared as per Scocco et al. (2012). For trial 1, before each treatment material was added to a dish, with the exception of the soybean tea, 0.25 ml of water was applied to the stone to create an attractive, moist harborage for the ants. For the soybean tea, 0.25 ml of the tea was used in place of additional water. For the peppermint oil treatment (positive control), 0.25 ml of the 1% peppermint oil solution was added to the stone dish and allowed to air-dry for 2 h, then 0.25 ml of water was added to each dish. For the water treatment (negative control), 0.25 of water was added to each dish. Following the application of the treatment material and water, each dish was covered with its lid and placed in a plastic box (19 x 14 x 9.5 cm; Tri-State Plastics, Dixon, KY) with Fluon-lined walls (Figure 4.1).

Trial 2 was executed identically to trial 1, but before each treatment material was applied, with the exception of the soybean tea, 1.0 ml water was applied to the stone to moisten it for the

ants and to create an attractive harborage. For the soybean tea, 1.0 ml of the tea was used in place of additional water. For the peppermint oil treatment (positive control), 0.25 ml of the 1% peppermint oil solution was added to the stone dish and allowed to air-dry for 2 h, then 1 ml of water was added to each dish. For the water treatment (negative control), 1 ml of water was added to each dish. Examples of treated harborages from trial 2 can be found in Figures 4.2 and 4.3.

For both trials 1 and 2, worker ants used in bioassays were collected from laboratory colonies by placing a clean dish (void of collection debris) containing ants (from the laboratory colony) into a small, Fluon-lined, plastic box (31 x 23 x 10 cm; Pioneer Plastics, Inc., Dixon, KY) and allowing several ants to climb onto a small paintbrush. Twenty ants were then gently tapped into a clear, 30 ml plastic cup (Jetware, Jet Plastica Industries, Inc., Hatfield, PA) with the walls and floor coated with Fluon, and the ants then transferred to test arenas containing the freshly-treated dishes for either trial 1 or 2.

Ants had the choice of entering the covered, moistened dish containing the treatment or not. This no-choice design was identical to that described in Scocco et al. (2012). The number of ants that were inside the dish (alive + dead) was recorded after 2 and 4 h. There was no mortality in trial one and negligible mortality (< 1%) in trial two. All bioassays were conducted at room temperature, and each treatment was replicated 12 times. At the

Statistical analysis. For both trials, treatment, time, and the treatment*time interaction were analyzed by mixed model, two-way analysis of variance (PROC GLIMMIX [SAS Institute, Inc. 2010]). For each combination of trial and time, the mean number of live ants inside each treated harborage was analyzed by mixed model, one-way analysis of variance (PROC GLIMMIX [SAS Institute, Inc. 2010]). Following each one-way ANOVA, differences between

least square means were determined using pairwise t-tests (Table 4.2). Residual plots for both trials at both time periods can be found in Figures 4.4 to 4.7.

Pheromone laboratory choice tests.

Ants. Argentine ants used in this bioassay were collected from property adjacent to the UGA Griffin campus in Griffin, Georgia, U.S.A. (N 33°15'58.41", W 84°17'19.98"). On the same day, ants, including brood and queens, were collected in four plastic tubs (≈57 x 45 x 13 cm) along with accompanying soil, leaf litter, and other debris, transferred to the lab, and then visually and equally distributed among four large washing machine overflow pans (71 x 76 x 2.5 cm; Model 34067, Oatey SCS, Cleveland, OH).

Treatments. A choice-test laboratory bioassay was used to evaluate Argentine ant response to three concentrations (0.002%, 0.005%, and 0.02% serially diluted in water) of microencapsulated, synthetic Argentine ant trail pheromone, (*Z*)-9-hexadecenal, (Suterra LLC, Bend, OR); the untreated control treatments consisted of a blank microcap (containing only dodecanol) (Suterra LLC, Bend, OR) and water only. All treatments were applied at a rate of 3.79 liters / 92.9 m^2 (2.9 ml per harborage [below]).

Ant harborages. After separation into pans, the ants were provided a cotton ball soaked in 25% sugar water placed on a soufflé cup lid (Model PL1, Dixie Consumer Products LLC, Atlanta, GA) and a frozen cricket. The ants were undisturbed for 24 h to acclimate before the experiment began. After 24 h, two pheromone- or blank microcap-treated and two water-only Castone dishes were placed in the corners of each pan (Figure 4.8). Castone dishes were prepared as before, with the exception that dishes were larger (140 x 20 mm; Thermo Fisher Scientific, Pittsburgh, PA) and 150 g of Castone was mixed with 50 ml water. After mixing, 190 g of the Castone mixture was placed in each polystyrene dish. Following drying (48 h at 60°C),

17 ml of water was added to each dish followed by the addition of 2.9 ml of either treatment material (pheromone or blank microcap) or additional water to bring the total to 20 mls (Figure 4.9). Dishes were then placed in the pans with the ants.

Bioassay. Each pan contained two water-only harborages and two pheromone-treated harborages. After 14 d, visual counts of ants were made for dishes containing < 20 ants. Harborages containing > 20 ants were removed and immediately placed in 3.79 liter plastic storage bags and then placed in a freezer (0°C). After one week, the harborages were removed from the freezer, the dead ants transferred to a 30 ml plastic cup, with lid (Jetware, Jet Plastica Industries, Inc., Hatfield, PA), and cups placed in an oven (60°C) for 48 h. After 48 h, the dried ants were weighed to obtain total weight for each harborage. Tests were conducted at room temperature and each treatment combination (two treated and two untreated dishes) was replicated 12 times.

Response variable. The weight of ants entering each dish was expressed as the percentage of ants, within a replicate, that were inside a harborage at the completion of the trial (after 14 d). The weights were then combined by treatment and replicate.

Pheromone field trial.

Research site and treatments. A field trial evaluating the effect of three concentrations (0.002%, 0.005%, and 0.02% serially diluted) of microencapsulated, synthetic Argentine ant trail pheromone (Suterra LLC, Bend, OR) and an untreated blank microcap (Suterra LLC, Bend, OR) was conducted on 16 residential buildings (Towaliga Village, Barnesville, Georgia, N 33°3'20.10", W 84°10'0.96"). Buildings consisted of two different architectural styles and there were either 63.1 or 72.5 linear meters around each structure (Figure 4.10). All treatments were applied at a rate of 3.79 liters / 92.9 m^2, in a 60.96 cm-wide band (30.48 cm up on the wall from

ground and 30.48 cm out from the wall on the grass and soil) around the exterior perimeter of the buildings. Each treatment was applied to four buildings using a recently purchased, unused compressed air sprayer (11.4 liter, Model 430-3G, Solo, Newport News, VA).

Response variable. Prior to treatment, a visual inspection was performed on each building wherein an ant trailing intensity rating was assigned to each of the buildings. The ratings for each wall section ranged from 0 to 3, where 0 was no visible ant foraging activity and 3 was high ant foraging activity. Each building consisted of 12 wall sections and each wall section was independently rated. The individual wall section ratings were summed to provide a total rating for each building, which ranged from 0 to 36.

Ant foraging activity was qualitatively rated on an intensity scale of zero, low, medium, and high. Low ratings consisted of at least more than two ants moving in a manner consistent and in the same direction as the others. One ant moving in a direction opposite of another ant did not constitute a rating of one because this was not a consistent trail. Medium ratings consisted of trails at least 2 ants wide, and typically involved movement in two directions (i.e. left and right on the foundations). High ratings consisted of trails >4 ants wide, and typically involved movement in two directions.

Application schedule. The 0.02% rate of microencapsulated Argentine ant trail pheromone and the blank control were applied only once. The 0.005% rate was applied twice, with 28 d between treatments. The 0.002% rate was applied three times, with 14 d between treatments. Following the first treatment, visual inspections of the buildings were made at 1, 10, 14, 15, 24, 28, and 29-d post-treatment. Treatment days and post-treatment reading days are listed in Table 4.3. Application frequencies differed to provide the maximum amount of applied material allowable under U.S. E.P.A. regulations (2006) (Table 4.4). Percent reductions in ant

activity, for each post-treatment sampling date, was calculated for each building with the following formula: $\frac{\text{pretreatment building rating} - \text{post treatment builing rating}}{\text{pretreatment building rating}}$ x 100. The mean percent reduction was then statistically analyzed and graphed.

Statistical analysis. For the pheromone lab trial, the weight of ants in harborages, expressed as a percentage of the total weight of ants inside harborages within each replicate, was analyzed by mixed model, one-way analysis of variance (PROC GLIMMIX [SAS Institute, Inc. 2010]). Following analysis, differences between least square means were determined using pairwise t-tests (Figure 4.11). A residual plot of these data can be found in Figure 4.12. Visual observation of the residual plot suggests that these data are normalized, and thus justify the use of ANOVA.

For the field trial, treatment, day post-treatment, and the treatment*day post-treatment interaction were analyzed by two-way analysis of variance (PROC GLM [SAS Institute, Inc. 2010]). Following analysis, differences between least square means among treatments, for the entire study and for each period post-treatment, were determined using pairwise t-tests (Table 4.5). Then, at each day post-treatment, treatment was analyzed by one-way analysis of variance (PROC GLM [SAS Institute, Inc. 2010), and again differences between least square means determined using pairwise t-tests (Table 4.6). A residual plot of all data can be found in Figure 4.13. Visual observation of residual plots suggests that data are normalized, and thus justify the use of ANOVA.

Results

Natural product laboratory trial 1. For trial 1, treatment (F = 18.43; df = 7, 176; P <0.0001) and time (F = 4.97; df = 1, 176; P = 0.0271) were significantly different among treatments, but their interaction was not (F = 0.64; df = 7, 176; P = 0.7205).

For trial 1, only the 1% peppermint oil (positive control) deterred ants from entering dishes (Table 4.2); no other treatment deterred ants from entering dishes at either 2 or 4 hrs. Excluding 1% peppermint oil, after 2 hrs the mean number of ants inside treated dishes was not significantly different (F = 8.73; df = 7, 88; P <0.0001); results were similar after 4 hrs (F = 14.56; df = 7, 88; P <0.0001). After 2 h, all treatments, had ≥88% of ants inside the treated harborages (Table 4.2); after 4 h, the response was ≥92.5% (Table 4.2). There was little change in response from 2 to 4 h for a majority of the treatments.

Natural product laboratory trial 2. . For trial 2, treatment (F = 32.29; df = 6, 123.3; P <0.0001) and time (F = 5.61; df = 1, 154; P = 0.0191) were significant, but their interaction was not (F = 0.88; df = 6, 123.3; P = 0.5085).After 2 hours there were significantly fewer ants in dishes containing freshly-collected spearmint and rosemary than in dishes containing any other treatment, including water but excluding 1% peppermint oil (F = 10.98; df = 6, 77; P <0.0001). Moreover, there were significantly fewer ants in dishes containing fresh rosemary in comparison to dishes containing fresh spearmint The results had not changed appreciably after 4 h ((F = 20.64; df = 6, 77; P <0.0001). Increasing the concentration of fresh cucumber and fresh tansy 10-fold, and soybean tea 4-fold from trial 1 to trial 2 did not change the effectiveness of these natural products, which were each non-deterrent in both trials. The response of ants to fresh spearmint, however, appeared to be concentration dependent; in trial 1 ants were not deterred, but when the concentration was increased 10-fold (trial 2), fresh spearmint deterred ants from entering treated dishes.

For trial 2, after 2 h, the water (negative control) and soybean tea had similar responses of >83% of ants inside the treated harborages (Table 4.2). Tansy and cucumber were similar with >65% of ants inside the treated harborages. Spearmint, rosemary, and the peppermint oil solution

were significantly different, but all demonstrated repellency with <50% of ants inside treated harborages, with the peppermint oil solution demonstrating a similar effect as in trial 1. After 4 h, ant response was greater and more ants were inside the treated harborages (Table 4.2). The water control, soybean tea, and cucumber had similar responses of >90% of ants inside the treated harborages after 4 hours. Tansy had around 75% and spearmint had around 50% of ants inside treated harborages. Rosemary had around 25% of ants inside treated harborages. All treatments, with the exception of fresh spearmint, fresh rosemary, and the peppermint oil solution, had >70% of ants inside the dishes at the conclusion of the trial (after 4 h).

Pheromone laboratory choice tests. Although significantly more ants were attracted to and harbored in pheromone-treated harborages than harborages treated with water only, the difference did not appear to be concentration dependent as the response of ants across all pheromone concentrations was not significantly different ($F = 10.69$; df = 7, 86; $P < 0.0001$) (Figure 4.11). Interestingly, the percentage of ants inside harborages treated with blank controls was significantly greater than the percentage of ants in water-treated harborage (Figure 4.11), suggesting that the microcap, in the absence of pheromone, is a deterrent to Argentine ants. Of the ants that chose to enter a harborage, 78 to 81% entered and harbored in a harborage treated with the pheromone (Figure 4.11).

Pheromone Field Trial. Treatment was highly significant ($F = 8.95$; df = 3, 84; $P < 0.0001$), while neither day post-treatment ($F = 1.90$; df = 6, 84; $P = 0.0895$) or their interaction ($F = 0.89$; df = 18, 84; $P = 0.5865$) were. Percentage reduction in Argentine an activity was significant for days 1, 28, and 29 post-treatment (Table 4.6). For day 1, the pheromone treatments did not reduce activity as effectively as the control ($F = 4.70$; df = 3, 12; $P = 0.0215$.

For days 28 (F = 3.47; df = 3, 12; P = 0.0507) and 29 (F = 6.09; df = 3, 12; P = 0.0092), the pheromone treatments did significantly reduce activity versus the control.

Overall, the pheromone treatments were significantly different from the control (F = 2.01; df = 27, 84; P = 0.0082), with the low pheromone treatment having a mean percentage reduction from pretreatment scores of ≈30% (±17.04%); the high treatment having no noticeable change in foraging activity, with changes from pretreatment and post-treatment scores remaining nearly the same with an average increase of 1% (±17.04%); the max treatment having a mean change of 10.7% (±17.04%); and the control treatment having a mean increase of 85% (±17.04%).

Discussion

Plant volatiles have been used in agriculture for pest management purposes for over two millennia (Isman 2006). Essential oils represent one of four major types of botanical products in use for pest control, with pyrethrum, rotenone, and neem being the other three (Isman 2006). Recent, increased attention given to plant essential oils for alternatives to synthetic chemical insecticides is partly due to their being exempted from registration by the Environmental Protection Agency and classified as so-called "25(b)" products, owing to the name of the federal act that granted exemption (Drees 2005, Geiger and Tootelian 2005, Isman 2006). According to Isman (2006), this has caused an increase in the development and production of essential oil-based insecticides, fungicides, and herbicides for both commercial and residential use. Adding to the appeal of essential oil use and product development is that they possess low mammalian toxicity, which is in contrast with many of the conventional insecticides and other synthetically-derived insecticides (Table 4.7) (Rajendran and Sriranjini 2008, Scocco et al. 2012). Oils of particular interest are rosemary, cedar, clove, mint, and thyme oils (Appel et al. 2001, Meissner

and Silverman 2003, Isman 2006, Phillips et al. 2010). Rosemary, for example, has fumigant, repellent, and contact toxic properties against several insects, including stored product pests such as the bean weevil, *Acanthoscelides obtectus* (Papachristos and Stamopoulos 2002, Isman et al. 2008). Given consistent interest in alternatives to conventional insecticides by the public, it is not surprising a number of products have become available and more "natural" remedies have been suggested to meet this demand (Potter and Bessin 1995, Bader 2006).

Rosemary oil is toxic to adult turnip aphids, *Lipaphis pseudobrassicae*, the human head louse, *Pediculus humanus capitis*, the two-spotted spider mite, *Tetranychus urticae*, the armyworm, *Pseudaletia unipuncta*, and the cabbage looper, *Trichoplusia ni* (Yang et al. 2004, Sampson et al. 2005, Miresmailli et al. 2006, Isman et al. 2008). In addition to toxic characteristics, rosemary oil has shown promise as a viable repellent, demonstrating repellency against four mosquito species, *Anopheles stephensi, Aedes aegypti, Culex quinquefasciatus*, and *Culex pipiens pallens* (Choi et al. 2002, Prajapati et al. 2005). Plant essential oils have been tested as repellents and insecticides against *L. humile*, however, there have only been a limited number of laboratory tests and no field studies to date (Wiltz et al. 2007, Scocco et al. 2012). The results from our natural product trials indicate that in addition to being repellent to numerous other pest insects, rosemary oil and spearmint might also have repellent properties against Argentine ants. Although the exact mechanism responsible for any potential repellency was beyond the scope of this study, if the oil proves to be repellent, it might provide further opportunity for research in field settings against Argentine ants. Additionally, spearmint oil should also be further researched, especially in field settings, given the results from the natural product trials. Scocco et al. (2012) demonstrated that spearmint oil (0.1%, 1%, and 10%) was repellent to Argentine ants in laboratory trials.

While evaluating different products, a complete appreciation for the insect's biology and physiology should be considered. Often, this is lacking with the general public with, for example, Bader (2006) recommending the use of common plant material, including tansy and cucumber, to repel ants—plant material that is known to serve as host to many species of honeydew-excreting hempiterans, many of which are tended by ants, including *L. humile* (Stadler 2004, Powell and Silverman 2010). Also included are recommendations to use soybean oil or a soybean tea and corn grit. Although *L. humile* do not prefer to feed on baits made of defatted corn grit and soybean oil, these are typically baits manufactured for control of the red imported fire ant, *Solenopsis invicta* Buren (Kabashima et al. 2007). Although there is no need to evaluate Bader's claim that the ants will "eat" the grit and die due to it being physiologically impossible, there was an opportunity to test some of his other claims in hopes of putting to rest some of these highly improbable, yet often highly appealing to the general public, claims.

We decided to evaluate repellency/deterrency of these products, with repellency being indicated by an avoidance of the treated harborage by the Argentine worker ant and deterrence being indicated by an avoidance of the material inside the harborage by the ants. As such, we found all of the products, with the exception of peppermint oil and rosemary, not to be repellent. However, in trial 2, we found fresh rosemary and spearmint to be significantly different and have fewer ants inside at 2 and 4 hours than the water control and thus be considered deterrent. The 1% peppermint oil solution (positive control) was highly repellent with <3% (less than 1 ant on average) of worker ants inside the dishes for the entire study. Likewise, the water only treatments (negative control) were not repellent.

The purpose of documenting and comparing the number of worker ants inside the dishes at both 2 and 4 h after beginning the study was to quickly ascertain whether the products were

repellent or not and to control for the potential volatility of the active ingredients of each product. It is assumed that if worker ants enter a treated dish within 2 h at high numbers then the product is not repellent. If, however, high numbers of worker ants do not enter after 2 h but do enter after 4 h, then we can assume the product is repellent but that the chemical or chemicals responsible for the repellency volatilized and were no longer as effective at 4 h. The disparities between each time reading are not large and do not indicate that any of the treatments, with the exception of the 1% peppermint oil in trial 1 are repellent at 2 or 4 h and only rosemary, spearmint, and 1% peppermint oil were repellent at 2 and 4 h in trial 2.

We dried some of the treatments before using them to determine if the structure of the plant, separate from the potentially volatile oils, would be repellent. The purpose of utilizing a no-choice experimental design is that it is an efficient method to preliminarily determine whether any of the products were repellent (Scocco et al. 2012). Further study would be warranted if any of the products had been significantly repellent (≤ 10% of worker ants inside the dishes at either 2 or 4 h). Although fresh rosemary and spearmint should be more fully researched—especially in field settings, it was beyond the scope of this study to do so. Finally, given that none of the experimental treatments were significantly repellent, the need to do more elaborate choice studies and possibly field evaluations did not arise.

According to Shorey (1973), three factors are necessary for effective communication to occur between two organisms: one of the organisms must emit a message; a medium must be available through which the message is transmitted; and the other organism must respond in some way when exposed to the message. Pheromones are chemicals used for communication between two or more animals of the same species (Karlson and Butenandt 1959). The use of pheromones, particularly sex pheromones, as both attractants and deterrents is particularly

appealing because of three factors: they are species specific; they are active in very small amounts; and the majority are not known to be toxic to animals (Witzgall et al. 2010). What is interesting about pheromones is that most stimuli that attract at certain, often low concentrations will repel at higher concentrations (Baker 1989). Following the moth mating disruption paradigm, the use of trail pheromone to reduce Argentine ant foraging has been well documented in the literature recently, and poses the potential to be as at least an aid if not a replacement for conventional insecticidal treatments in treating for Argentine ants (Witzgall et al. 2010, Suckling et al. 2011). Given recent interest in utilizing the Argentine ant trail pheromone, (*Z*)-9-hexadecenal, as a standalone product and in conjunction with conventional treatments, we chose to evaluate a relatively new formulation of the pheromone in microencapsulated form (Suckling et al. 2008, Suckling et al. 2010, Ward et al. 2010, Suckling et al. 2011, Choe et al. 2014).

In the pheromone laboratory trial, the purpose of choosing a 2x2 choice assay was to ensure adequate choice between treated and non-treated dishes and because initial research demonstrating trail disruption by this product has already been done by Suckling et al. (2010).The high rate that was tested in the pheromone repellency laboratory and field trials is the rate used by Suckling et al. (2010). In addition to using the same rate as Suckling et al. (2010), a rate higher and lower than the rate previously used were also evaluated. In this application, the pheromone product seems to be attractive to the worker ants as a larger proportion of the ants were in dishes treated with the pheromone after 14 d. In addition to more worker ants being located in pheromone-treated dishes, more brood were observed in the pheromone-treated dishes as well (J.B.H., personal observation).

In the pheromone field trial, the Suckling et al. (2010) rate was also evaluated, as well as a rate higher and lower than this rate. Our results indicate the pheromone-treatments and the

control treatment are significantly different, with the low rate having an overall decrease in foraging intensity of ≈30%. The max rate also significantly reduced foraging intensity overall of around 10%. However, the Suckling et al. (2010) rate (high rate evaluated) did not result in a net reduction from pretreatment intensity ratings, but did differ significantly from the control. Whether this difference is due to the pheromone product being a deterrent is not clear. Additionally, despite each treatment being applied a different number of times (except the control and max treatments which were each applied only once), application timing and frequency did not significantly affect foraging ratings. Given these results, application rate does, but frequency does not, seem to affect the efficacy of the pheromone product.

In conclusion, the natural products evaluated in these trials, with the exceptions of peppermint oil, fresh rosemary and spearmint, were not repellent or deterrent. Our results indicate further investigation of rosemary and spearmint is warranted and might provide valuable insight into effective plant-based management for *L. humile*. Likewise, despite our research not reinforcing the results obtained from Suckling et al. (2010) in their studies, there remains much to be done regarding the development of a more comprehensive understanding of the different concentrations and delivery methods of pheromones as they pertain to management strategies for Argentine ants. In particular, the mechanism(s) responsible for the synthetic trail pheromone remains unknown. Additionally, the potential use of synthetic trail pheromone as a part of a "push-pull" pest management strategy also poses potential given the attractant and deterrent properties of the pheromone at different concentrations. In particular, more research should be done given the results from the pheromone lab trial demonstrating the pheromone product to be attractant, and results from Suckling et al. (2011) showing high concentrations of synthetic trail pheromone near active trails leading to a buildup of ants in the area. As Suckling et al. (2011)

noted, for synthetic trail pheromone to be a viable management option, disruption must reduce foraging efficiency and suppress ant populations as well. Our results support other studies demonstrating foraging reductions, but suppression of ant populations has yet to be demonstrated (Suckling et al. 2008, Tanaka et al. 2009, Nishisue et al. 2010, Suckling et al. 2010, Ward et al. 2010, Suckling et al. 2011).

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Table 4.1. Amount and surface area of treatment material applied to harborage for natural product repellency trials.

	Trial 1		Trial 2	
Treatment	**Amount Applied***	**Surface Area***	**Amount Applied****	**Surface Area****
Fresh Cucumber	4 hole punched sections	11.3 cm^2	40 hole punched sections	113.04 cm^2
Dry Cucumber	4 hole punched sections	11.3 cm^2	-	-
Fresh Tansy	4 hole punched sections	11.3 cm^2	40 hole punched sections	113.04 cm^2
Dry Tansy	4 hole punched sections	11.3 cm^2	-	-
Soybean Tea	0.25 ml	NA	1.0 ml	113.04 cm^2
Fresh Spearmint	4 hole punched sections	11.3 cm^2	40 hole punched sections	113.04 cm^2
Fresh Rosemary	-	-	40 hole punched sections	113.04 cm^2
Water Only Control	0.25 ml	NA	1.0 ml	NA
1% Peppermint Oil Solution	0.25 ml	NA	1.0 ml	NA

*For trial 1, 0.25 ml of water was added to all dishes with the exception of the soybean tea, where the 0.25 ml of the tea was used in place of water. Water only control received 0.25 ml total water. **For trial 2, 1.0 ml of water was added to all dishes with the exception of the soybean tea, where the 1.0 ml of the tea was used in place of water. Water only control received 1.0 ml total water.

Table 4.2. Response of Argentine ants to various treatments applied to harborages for natural product repellency trials.

	Number (mean ± S.E.) of live ants inside dish at hour			
	Trial 1		**Trial 2**	
Treatment	**2**	**4**	**2**	**4**
Dry Cucumber	19.4 ± 1.3 A	19.8 ± 1.3 A	-	-
Fresh Cucumber	17.6 ± 1.2 A	18.5 ± 1.2 A	13.6 ± 2.5 A,B	18.5 ± 2.2 A
Dry Tansy	18.9 ± 1.3 A	18.7 ± 1.2 A	-	-
Fresh Tansy	18.4 ± 1.2 A	19.6 ± 1.3 A	14.8 ± 2.7 A,B	14.8 ± 1.9 A,B
Soybean Tea	19.3 ± 1.3 A	19.4 ± 1.3 A	16.8 ± 3.1 A	19.2 ± 2.3 A
Fresh Spearmint	17.6 ± 1.2 A	19.2 ± 1.3 A	9.33 ± 1.8 B	10.7 ± 1.4 B
Fresh Rosemary	-	-	3.42 ± 0.8 C	5.17 ± 0.8 C
Water Only Control	18.4 ± 1.2 A	19.1 ± 1.3 A	18.3 ± 3.3 A	19.1 ± 2.3 A
1% Peppermint Oil	0.28 ± 0.2 B	0.92 ± 0.3 B	0.08 ± 0.09 D	0.50 ± 0.2 D
	$F = 8.73$ df = 7, 88 $P < 0.0001$	$F = 14.56$ df = 7, 88 $P < 0.0001$	$F = 10.98$ df = 6, 77 $P < 0.0001$	$F = 20.64$ df = 6, 77 $P < 0.0001$

Following mixed model, 1-way ANOVA (PROC GLIMMIX), differences between least square means, for each combination of Trial and Hour, were determined using pairwise t-tests; means within a column followed by the same letter are not significantly different.

Table 4.3. Pheromone field trial treatment days and post-treatment reading days.

Treatment	Treatment application dates*	Post-treatment reading dates*
0.002% Pheromone (Low)	July 1, 15, and 29	All treated buildings were read on
0.005% Pheromone (High)	July 1 and 29	July 2, 11, 15, 16, 25, 29, and 30.
0.02% Pheromone (Max)	July 1	
Blank Control	July 1	

*All dates are from 2013

Table 4.4. Applications based on maximum application rate of a pheromone of 150 grams active ingredient/acre/year (3.5 grams active ingredient/92.9 m^2/year) (United States Environmental Protection Agency 2006).

Application Rate:	**Maximum Application Per Year**
Low Rate (0.002%)	Up to 8 applications per year at this rate
High Rate (0.005%	Up to 4 applications per year at this rate
Maximum Rate (0.02%)	Up to 1 application per year at this rate

Table 4.5. Percentage reduction (mean ± S.E.) in activity of ants around structures after being treated with various concentrations and numbers of applications of a microencapsulated-sprayable product containing the Argentine ant trail pheromone, (Z)-9-hexadecenal. Negative means denote an increase in activity

Treatment	**Percentage Reduction (mean ± S.E.)**
0.002% Pheromone (Low)	31.3 ± 17.0 A
0.005% Pheromone (High)	-1.0 ± 17.0 A
0.02% Pheromone (Max)	10.7 ± 17.0 A
Blank Control	-84.7 ± 17.0 B

Following ANOVA (PROC GLM), differences between least square means, for the entire study, were determined using pairwise t-tests; means followed by the same letter are not significantly different.

Table 4.6. Percentage reduction (mean ± S.E.) in foraging activity, given by changes from pretreatment rating, of Argentine ants around structures after being treated with various concentrations and numbers of applications of a microencapsulated-sprayable product containing the Argentine ant trail pheromone, (*Z*)-9-hexadecenal. Negative means denote an increase in activity.

	Percent Reduction (mean ± S.E.) in Argentine Ant Activity Rating at Day Post-Treatment						
Treatment	**1**	**10**	**14**	**15**	**24**	**28**	**29**
0.002% Pheromone (Low)	11.9 ± 12.9 B	-46.7 ± 59.0 A	44.5 ± 52.6 A	67.4 ± 54.6 A	26.9 ± 48.7 A	44.5 ± 34.2 A	70.5 ± 35.8 A
0.005% Pheromone (High)	33.5 ± 12.9 B	-77.0 ± 59.0 A	0.49 ± 52.6 A	-2.56 ± 54.6 A, B	-16.8 ± 48.7 A. B	20.8 ± 34.2 A	34.7 ± 35.8 A
0.02% Pheromone (Max)	35.1 ± 12.9 B	-8.1 ± 59.0 A	-19.4 ± 52.6 A	5.94 ± 54.6 A, B	-6.92 ± 48.7 A, B	40.6 ± 34.2 A	27.5 ± 35.8 A
Control	78.6 ± 12.9 A	-95.2 ± 59.0 A	-102.4 ± 52.6 A	-102.4 ± 54.6 B	-152.4 ± 48.7 B	-90.5 ± 34.2 B	-128.6 ± 35.8 B
	$F = 4.70$	$F = 0.42$	$F = 1.37$	$F = 1.65$	$F = 2.63$	$F = 3.47$	$F = 6.09$
	df = 3, 12	df = 3, 12	df = 3, 12	df = 3, 12	df = 3, 12	df = 3, 12	df = 3, 12
	$P = 0.0215$	$P = 0.7435$	$P = 0.2989$	$P = 0.2292$	$P = 0.0981$	$P = 0.0507$	$P = 0.0092$

Percent reduction in ant activity was analyzed, for each day post-treatment, by one-way analysis of variance (PROC GLM [SAS Institute, Inc. 2010]). Following each analysis, differences between least square means were determined using pairwise t-tests. Means within a column followed by the same letter are not significantly different.

Table 4.7. LD_{50} (acute oral toxicity—combined male and female rat) for various active ingredients in pure form.

Product	**LD_{50} (mg/kg)**	**Source**
Rosemary Oil	5000	Sigma-Aldrich (2014)
Bifenthrin	53.4 to 210.4	National Pesticide Information Center (2014a)
Indoxacarb	< 1,000	United States Environmental Protection Agency (2000)
Fipronil	97	National Pesticide Information Center (2014b)

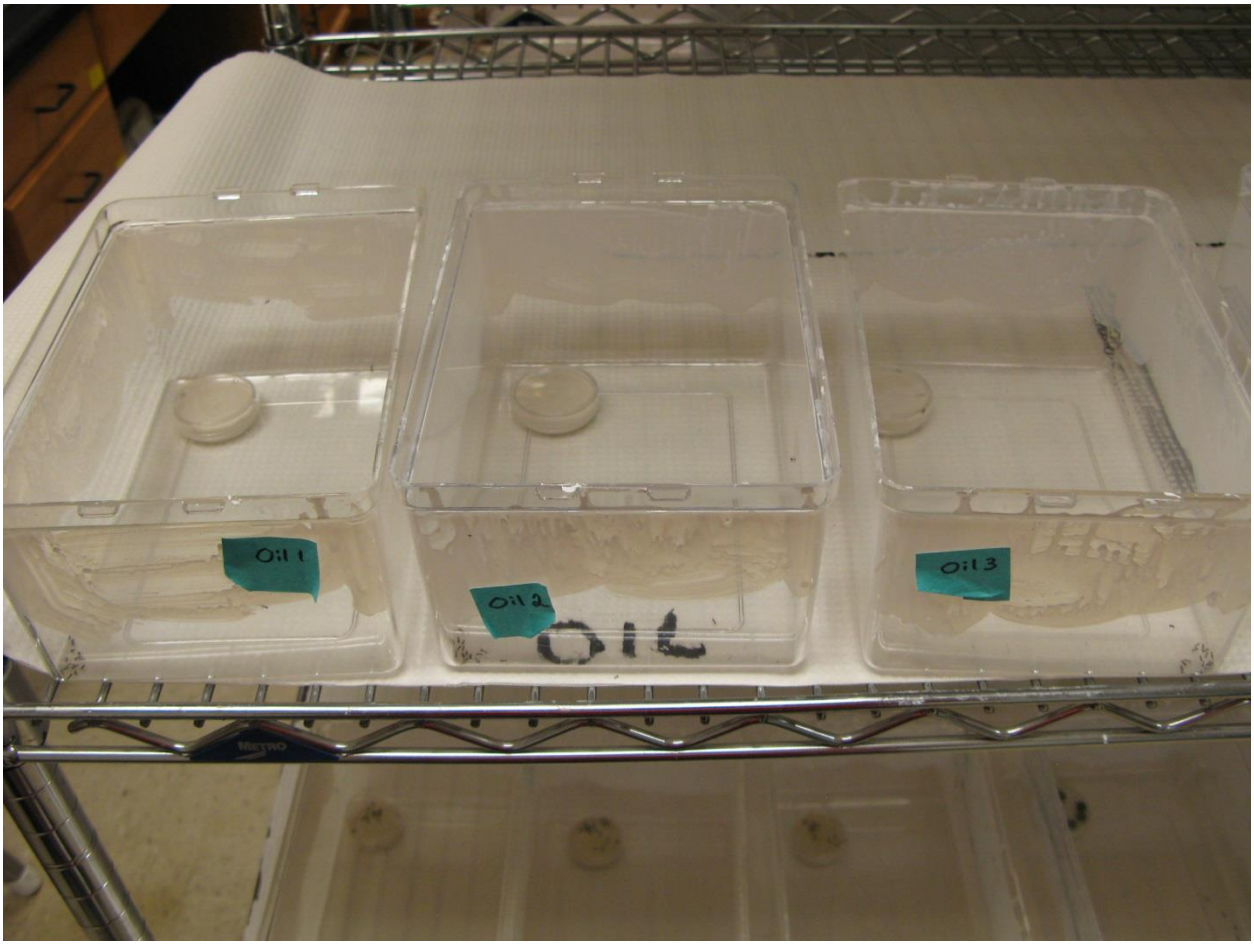

Figure 4.1. Plastic boxes containing Castone-filled dishes (harborages) for the laboratory trials.

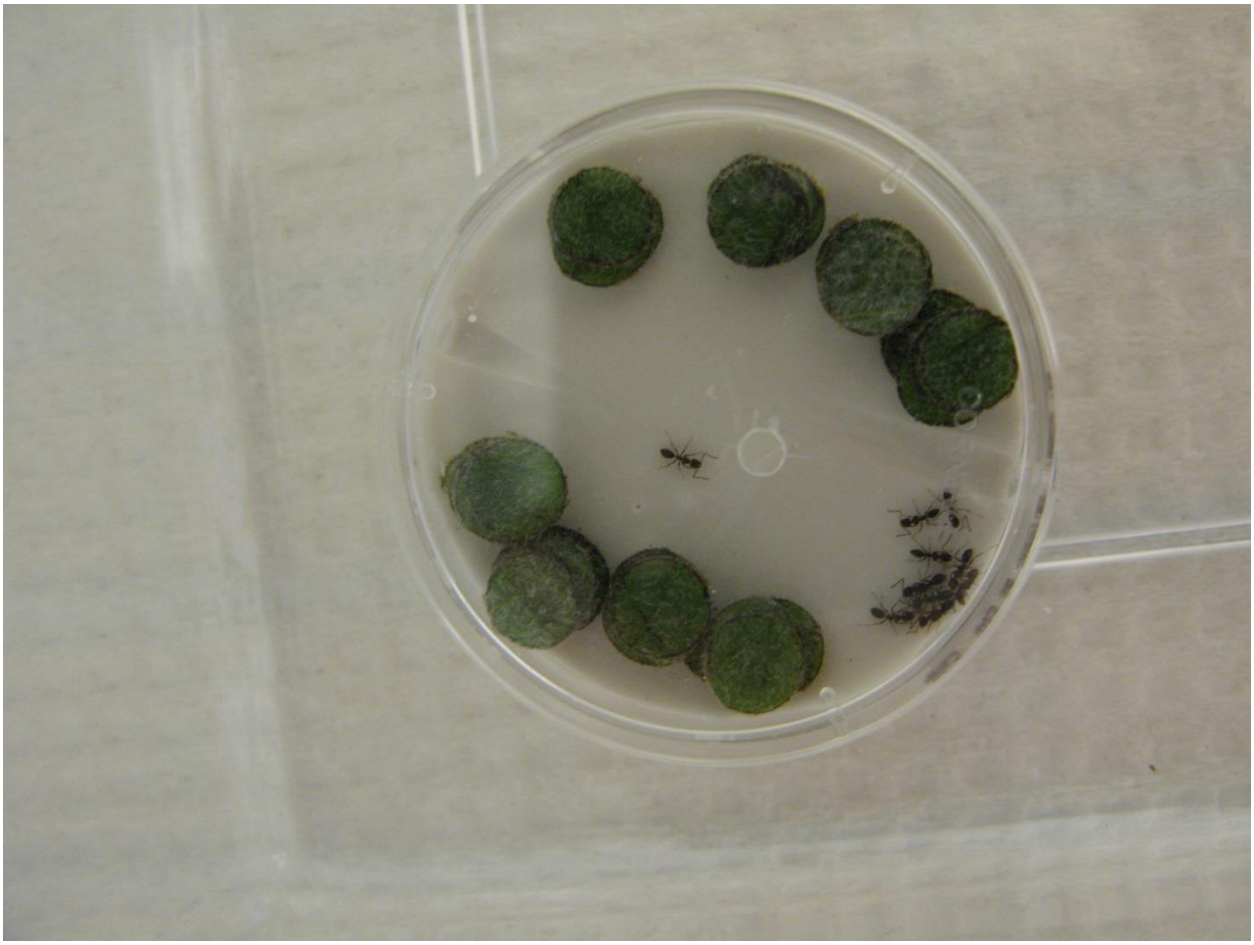

Figure 4.2. Close-up of Castone-filled culture dish (harborage) containing spearmint treatment and worker ants from natural product repellency trial 2.

Figure 4.3. Close-up of Castone-filled culture dish (harborage) containing rosemary treatment and worker ants from natural product repellency trial 2.

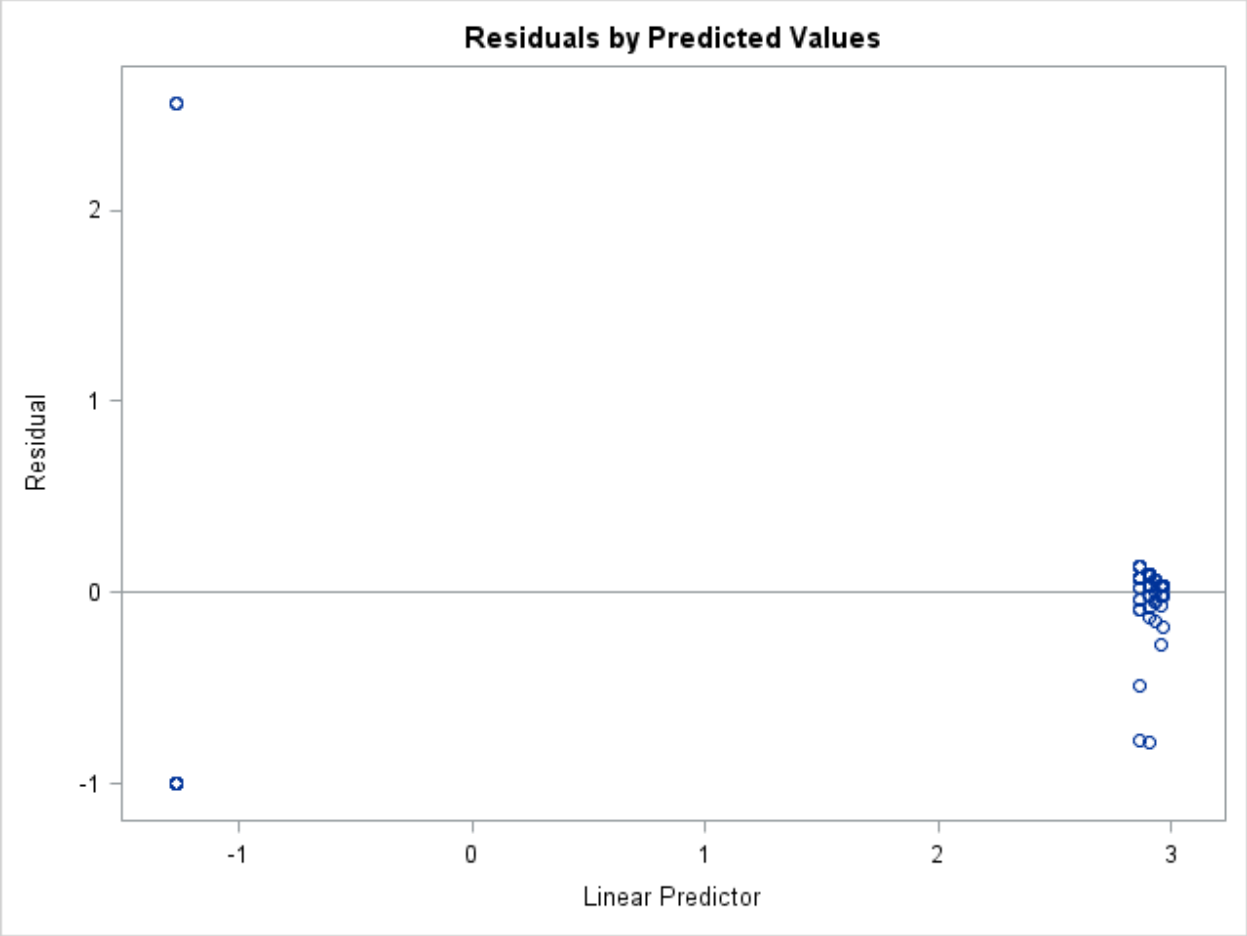

Figure 4.4. Residual plot of percentage of ants inside treated dishes after 2 h for the natural product repellency trial 1.

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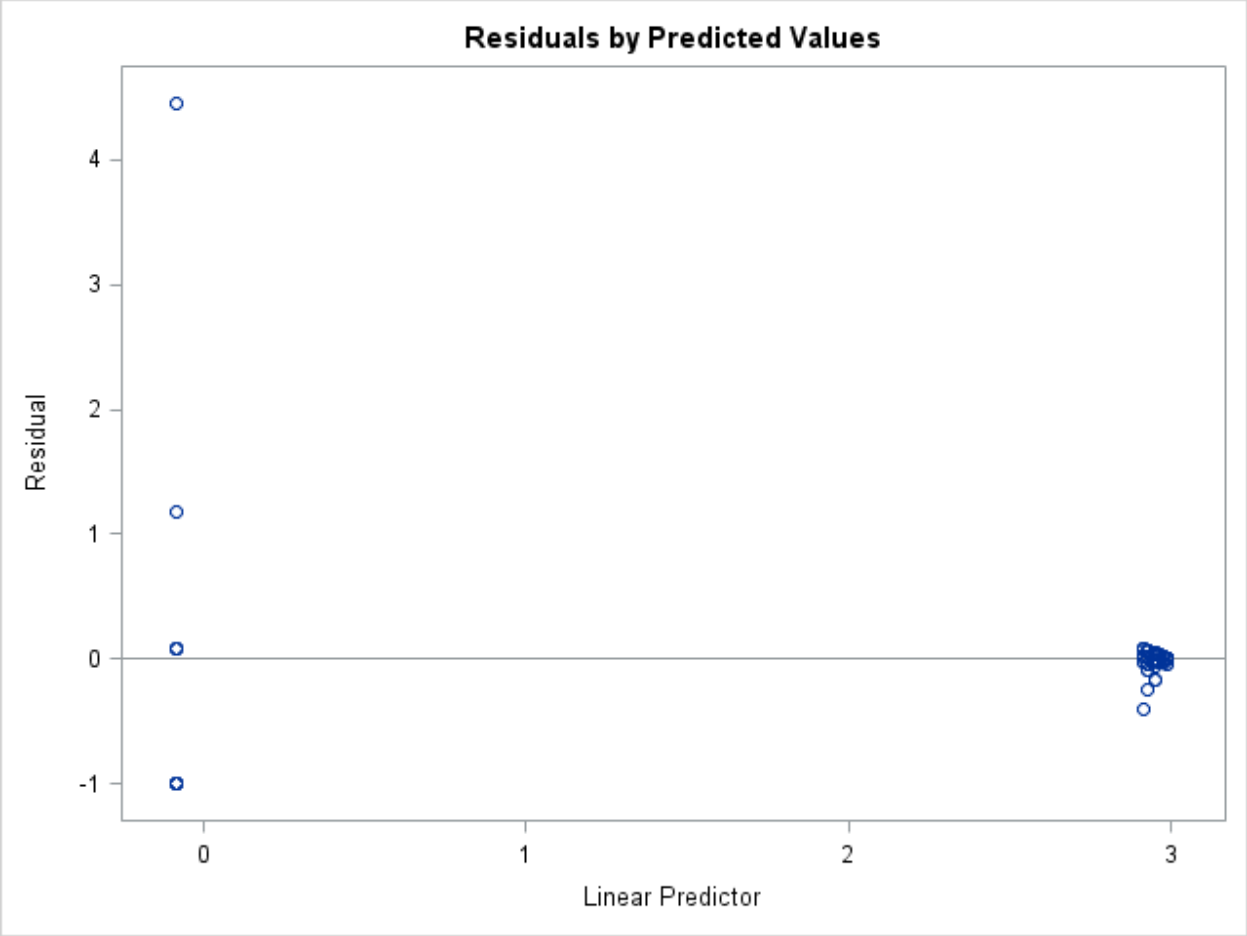

Figure 4.5. Residual plot of percentage of ants inside treated dishes after 4 h for the natural product repellency trial 1.

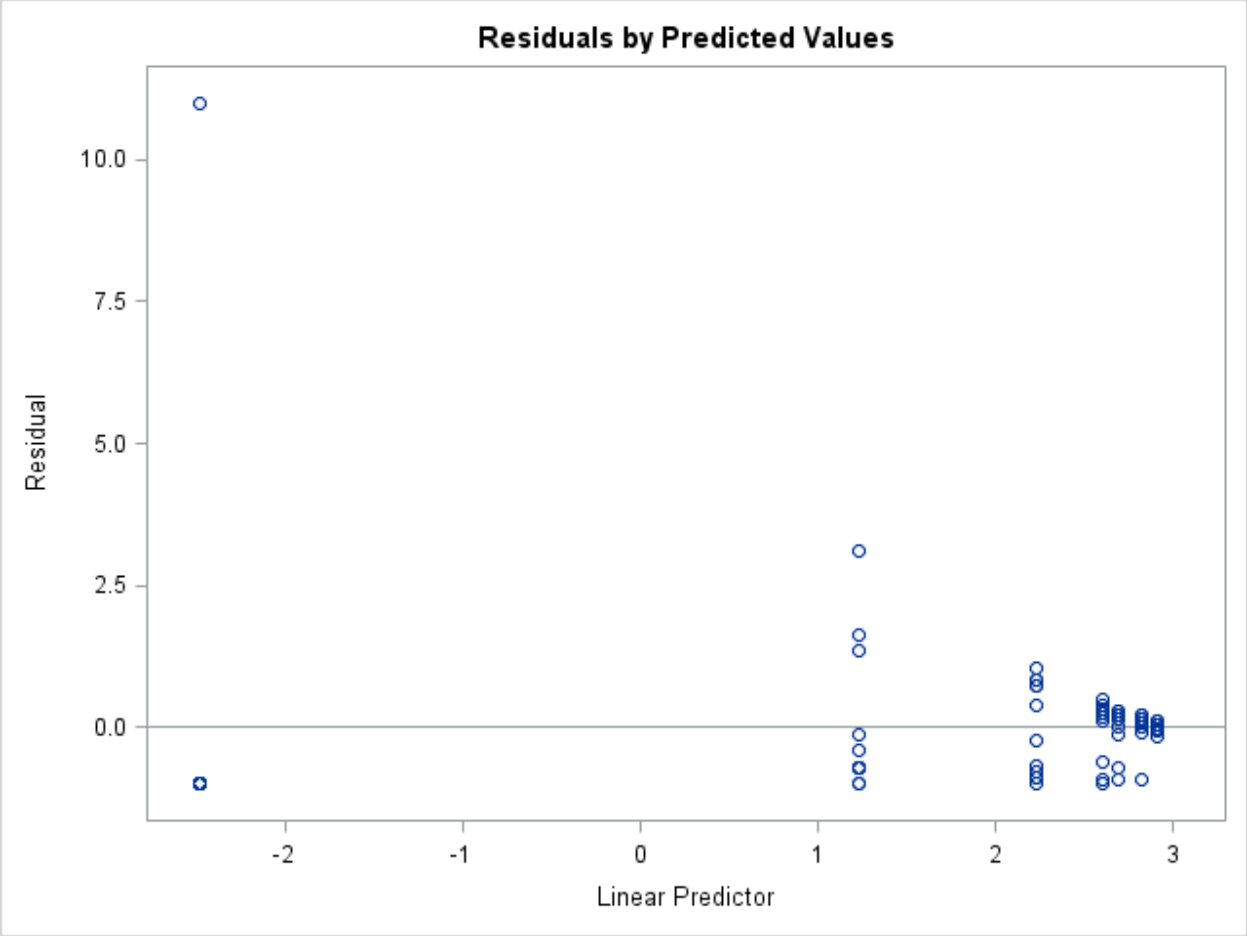

Figure 4.6. Residual plot of percentage of ants inside treated dishes after 2 h for the natural product repellency trial 2.

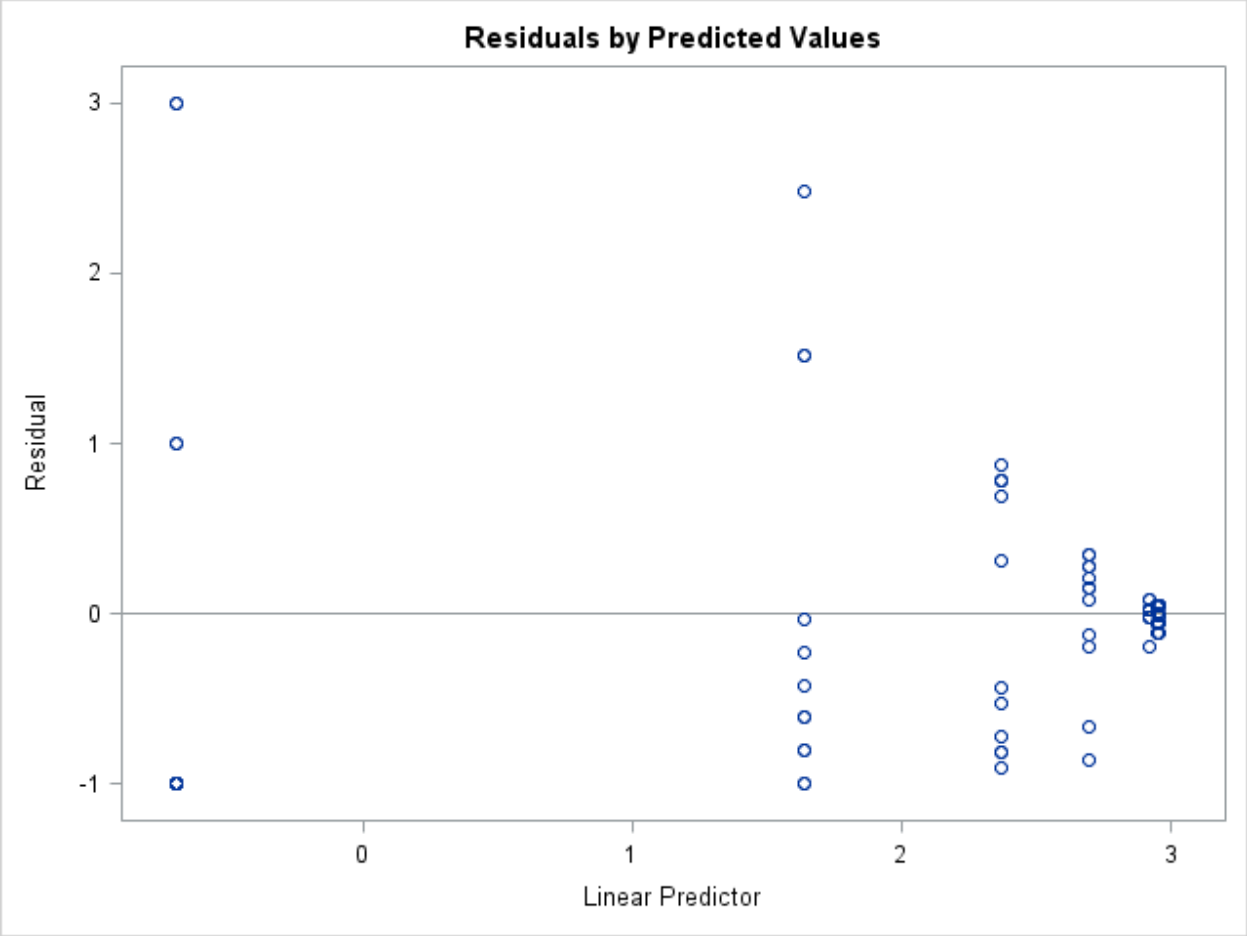

Figure 4.7. Residual plot of percentage of ants inside treated dishes after 4 h for the natural product repellency trial 2.

Figure 4.8. Washing machine pan with ants, accompanying leaf litter, and the two pheromone-treated (or blank microcap-treated) and two untreated (water only) dishes in the corners.

Figure 4.9. Close-up of blank microcap-treated harborage with ants.

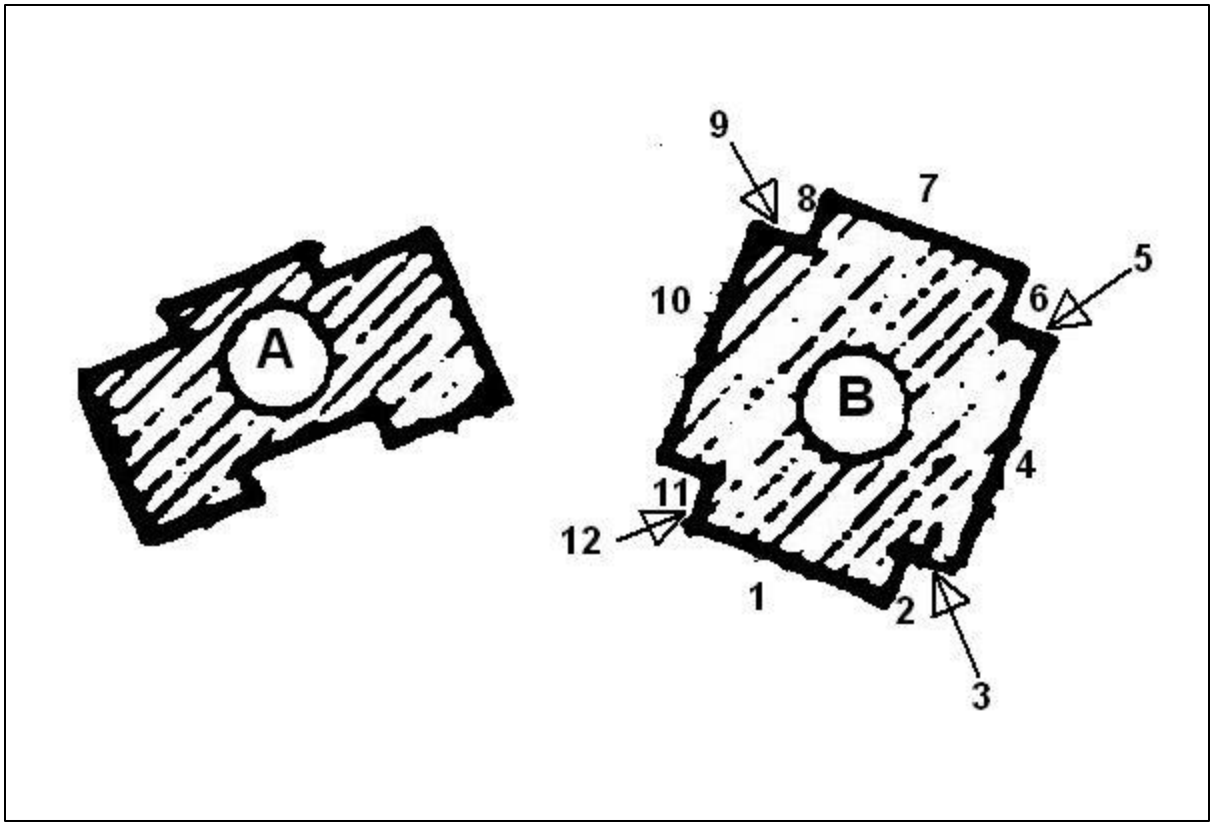

Figure 4.10. A sketch representing the two different architectural building styles (A and B) included in the pheromone field trial and the division of walls into numerical sections on building style B.

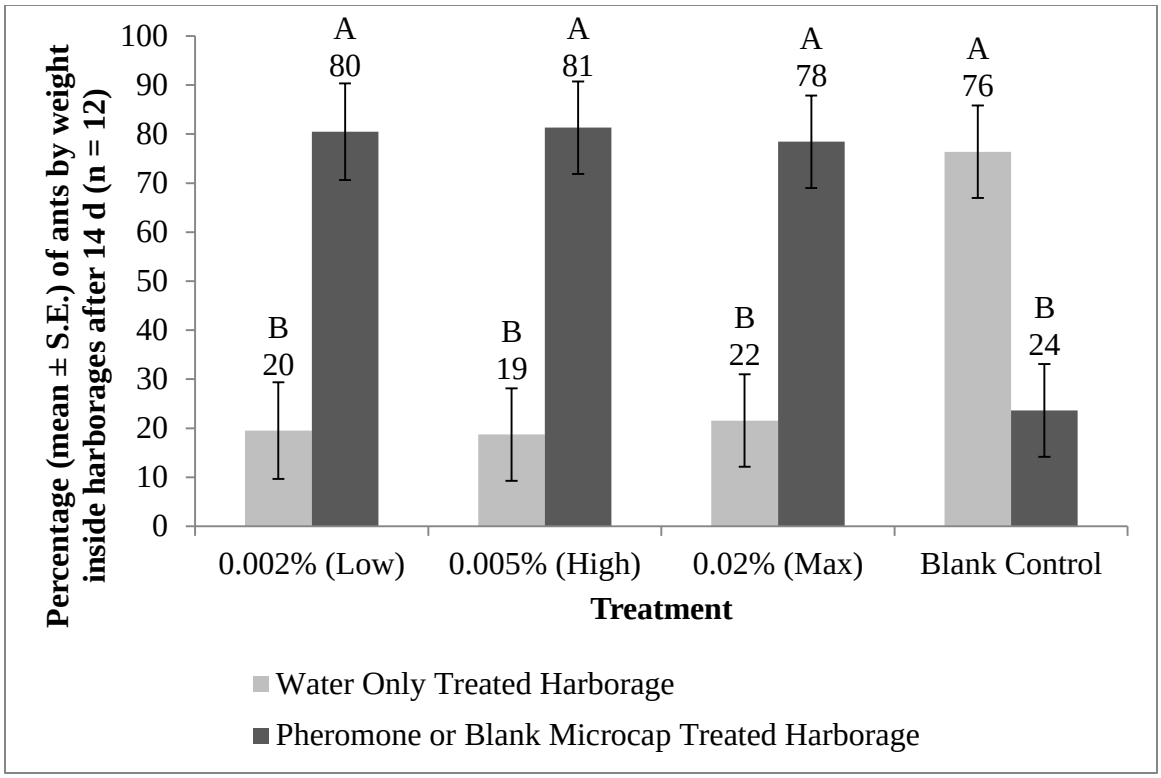

Figure 4.11 Percentage of ants, by weight (mean ± S.E.), inside harborages for the pheromone laboratory trial. Means followed by the same letter are not significantly different. Data were analyzed by one-way ANOVA (PROC GLIMMIX [SAS Institute, Inc. 2010]; $F = 10.69$; df = 7, 86; $P < 0.0001$).

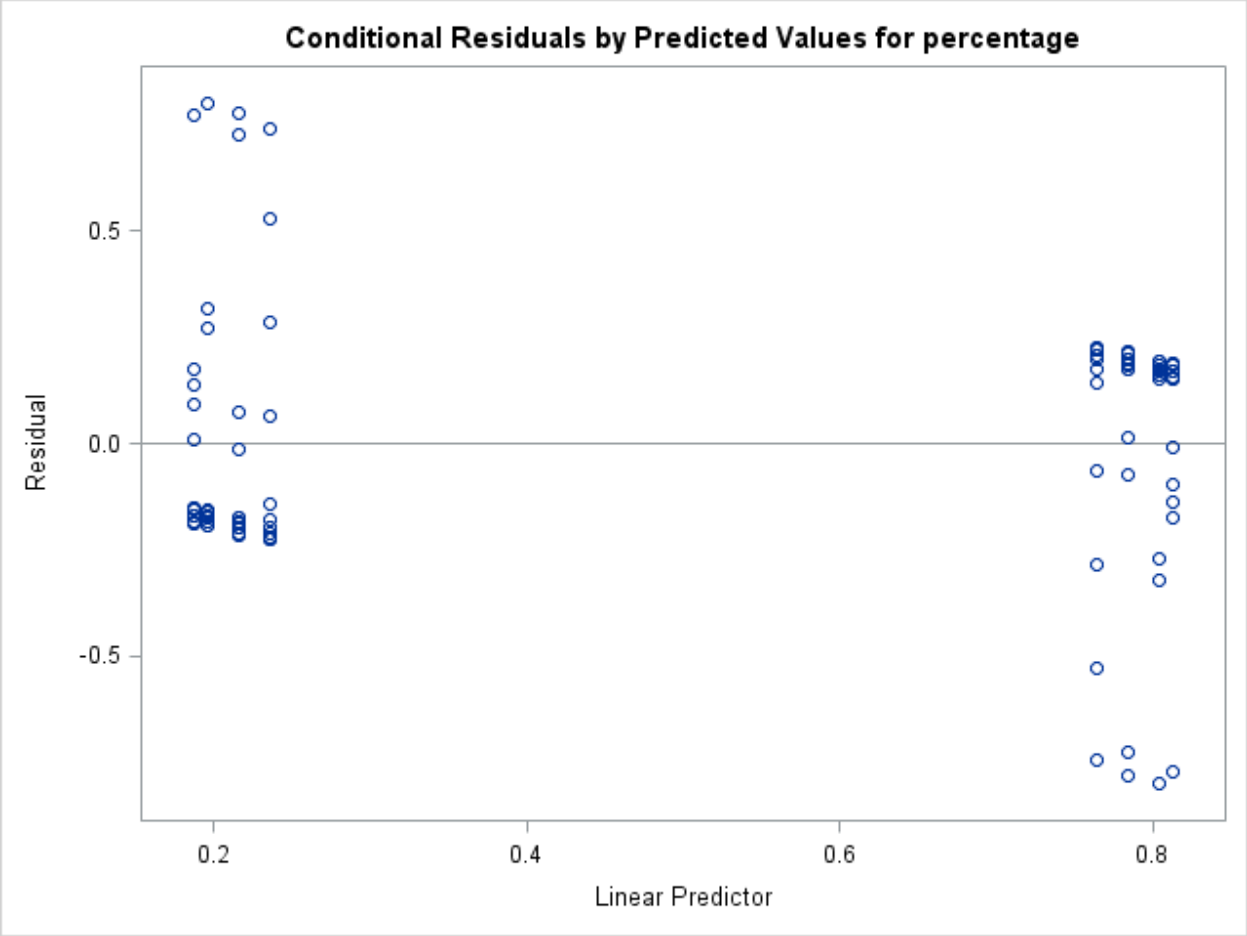

Figure 4.12. Residual plot for percentage of ants by weight inside dishes after 14 d in pheromone laboratory trial.

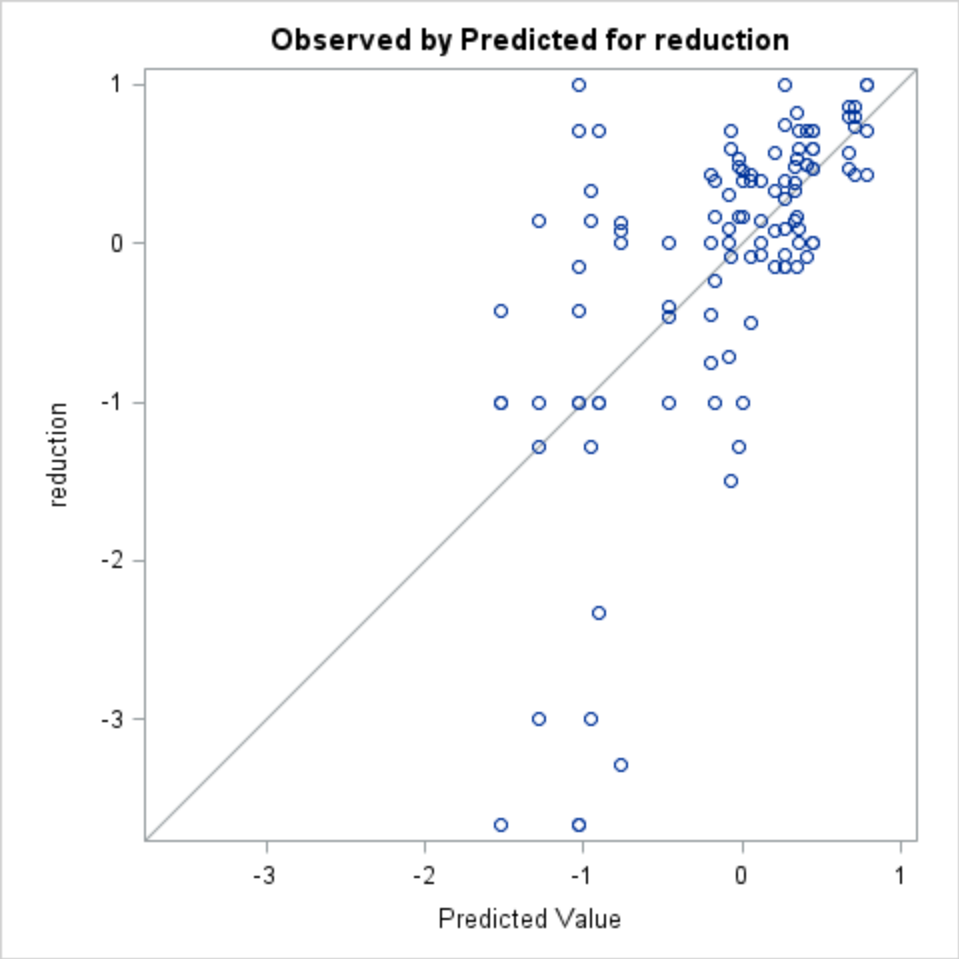

Figure 4.13. Residual plot for mean percentage reduction of foraging activity post-treatment for pheromone field trial.

CHAPTER 5

CONCLUSION

These studies have shown that continued research needs to be done in order to fully evaluate and discover viable alternatives to conventional chemical pest management strategies against the Argentine ant. Implementing the tenets of an integrated pest management program is one step in diminishing the negative environmental effects of a reliance on chemical inputs. Despite the allure of alternative products to conventional insecticides, the fact remains that conventional insecticides provide the most effective reductions in Argentine ant activity in both laboratory and field settings. One problem with resorting to unconventional and often untested means is the potential health and environmental harm that may be done. Over the course of the seasonal foraging study, numerous instances of treatments were encountered that incorporated products and chemicals, including bleach, detergents, grease, and excessive amounts of Borax and other insecticides, applied on the ground or on the structure. It is hoped that by evaluating some of these unconventional products, the claims made from various sources about them that their impact on Argentine ants can be elucidated.

While these studies did not discover a panacea to managing the Argentine ant, our results do indicate that further research should be done on rosemary, and possibly spearmint, as viable repellents/deterrents to Argentine foraging activity. As far as conventional insecticides fare, our results corroborate existing research on the efficacy of fipronil and bifenthrin as effective active ingredients against the Argentine ant and provide the first evidence of indoxacarb's efficacy as a treatment against Argentine ants—specifically its efficacy at reducing ant foraging intensity

around urban structures, and its secondary kill in a laboratory setting. The results from the seasonal foraging study showed ant foraging activity in Barnesville, Georgia to be highest in the warmer months (June through October), with activity peaking in July; highest in air temperatures ranging from 20-30°C without regard to photoperiod; and relative humidity having no significant impact on foraging activity. Future research regarding foraging activity might focus on surface temperature and its impact on foraging intensity and behavior. Additionally, utilizing video tracking software with continuous video over 24 h of foraging ants, along with surface and air temperature, might provide greater insight into foraging behavior.