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EVALUATION OF FORAGING PATTERNS OF LINEPITHEMA HUMILE (MAYR), THE ARGENTINE ANT, TO IMPROVE BAIT PLACEMENT IN NATURAL PARK HABITATS

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EVALUATION OF FORAGING PATTERNS OF *LINEPITHEMA HUMILE* (MAYR), THE ARGENTINE ANT, TO IMPROVE BAIT PLACEMENT IN NATURAL PARK HABITATS

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ABSTRACT

Linepithema humile (Mayr), the Argentine ant, is an invasive ant species and a significant pest in natural and managed habitats of the southeastern United States. In the natural sites of Lake Greenwood State Park (LGSP) in the Piedmont region of South Carolina, *L. humile* has invaded. Although park personnel treat problem areas with liquid insecticides, control of *L. humile* through the park areas is minimal. The primary objectives of this study were to determine the optimal foraging distance of *L. humile*, evaluate over-wintering nest temperatures and foraging activity of *L. humile*, and evaluate liquid bait placement to control *L. humile.*

A Double Antibody-Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA) procedure was used to detect individual ants that had consumed rabbit Immunoglobin (IgG) protein (Sigma Chemical Co., St. Louis, MO) for marking and tracking. In this study, the optimal concentration of IgG in an individual ant necessary for detection was determined to be 0.01mg/ml. In both lab and field conditions, there was a significant difference in the detection of IgG in ants fed the protein marker mixed with sugar water compared to ants only fed sugar water. Additional field studies found that an individual ant could retain detectable levels of the protein marker for 3 d and that ants feeding IgG containing bait could be significantly detected up to15 m from the original bait source.

In a field at LGSP, Greenwood, SC, bait stations containing 300ml of 30% sugar-water with 0.01 mg/ml of IgG protein were placed in a grid pattern with nine stations placed at 10 m apart and were compared to nine stations in a grid pattern place at 20 m apart. This study was replicated three times. When the distance between the two bait station placements was compared, the amount of IgG detected in *L. humile* was significantly higher in ants foraging at stations 10 m apart compared to ants foraging at stations at 20 m apart. However, IgG could be detected in ants foraging to stations 20 m. To be cost effective for the amount of bait needed, stations needed and time for labor, 20 m was selected for a later field trial to control *L. humile.*

The over-wintering habitat study showed that mean *L. humile* nest temperatures were less variable than mean ambient temperatures. From January to March, 2012, the range from lowest to highest temperature was 12.4°C in the nests and 21.7°C in the ambient environment. During this period, the lowest mean temperature recorded in the ambient environment was 8.27°C and 10.01°C in the nests. The highest mean temperature was 30.0°C in the ambient environment and 22.5°C in the nests. Even though ambient and nest temperature fluctuated, the mean foraging activity of *L. humile* increased from 12 February to 29 February. After 16 March, both the mean ambient temperatures and the nest temperatures continued to be over 15°C. At this temperature, *L. humile* began to actively forage. This result suggested the optimal bait placement date for control of *L. humile* was after 16 March due to temperature (ambient and nest) and ant foraging activity.

A bait study was conducted in natural areas of LGSP to determine early season control of *L. humile* using the 20 m bait placement discovered in the earlier study. When temperatures were continuously above 15°C in 2012, three treatment areas were established. These areas included a natural control area with no bait placement, a bait control area with stations containing 200 ml of 25% sugar water, and a bait treatment area with stations filled with 180 ml of 25% sugar water mixed with 20 ml Maxforce Quantum Ant Bait (0.03% imidacloprid). In each area, 10 trees with active *L. humile* trails were selected to assess foraging activity. The ant trail with the greatest number of individuals on each tree was counted weekly. Liquid bait stations were placed in three rows at 20 m apart. Bait was replaced weekly for three months. The mean number of *L. humile* was recorded from spring to fall in 2012 and from spring to early summer in 2013. No bait was used after June 2012. Liquid ant bait decreased the *L. humile* population in the treatment area after one season of baiting as compared with the control areas. It was conclude that early season liquid baiting (mid-March), with a specific placement distance (20 m), was an effective method for controlling *L. humile* in a natural park habitat in South Carolina.

ACKNOWLEDGMENTS

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CHAPTER ONE

Introduction and Literature Review

Linepithema humile (Mayr), the Argentine ant, is an invasive ant species and a significant pest in natural and managed habitats (Holway et al. 2002a). It has become established on six continents and many oceanic islands where it is a serious ecological, agricultural and urban pest. In the United States, *L. humile* is steadily spreading in all directions throughout the southern and western states (Buczkowski et al. 2004; Holway et al. 2002a; Barber 1916). Its invasive success can be attributed to change in social behavior and colony structure (Giraud et al. 2002). Even though *L. humile* are incapable of penetrating closed-canopy forests, they have established in native open-canopy woodland habitats (Rowles et al. 2007). *L. humile* has similar traits to other invasive ant species including polygyny, polydomy, unicoloniality, and monomorphic worker (Holldobler and Wilson, 1990 and Passera 1993). They also have super colonies that dominate native ant communities (Brightwell and Siverman 2007).

The most serious result of *L. humile* invading natural habitats is the reduction in native ant diversity and the possible negative effect on other trophic levels (Heller et al. 2006). Recent behavioral studies indicate that in its native region, *L. humile* is multicolonial, with territorial boundaries between colonies beging well defined and nests are aggressively defended against conspecifics (Suarez et al. 1999). However in regions into which it has been introduced, including California and Southern Europe, *L. humile* is unicolonial with weak territorial boundaries between colonies and multiple interconnected nests (Suarez et al. 1999 and Giraud et al. 2002). Often *L. humile* lack

intraspecific colony aggression. Instead of agonistic aggression, workers invest in rearing brood from different colonies and queens (Holway et al. 2002a).

Linepithema humile are aggressive foragers often dominating other ant species in the areas where they are established (Heller et al. 2006). The foraging range of *L. humile* colonies in urban areas of southern California extended at least 61 m from feeding stations (Vega and Rust 2001). Although *L. humile* do not damage crops directly, they might protect phloem feeding Hemiptera and adversely impact beehives and irrigation systems (Vega and Rust 2001). *L. humile* is not a public health threat but it is a considerable nuisance for homes and buildings (Barber 1916). The control of *L. humile* is difficult because of rapid reinfestation. Their mass recruitment and trail pheromone allow them to rapidly find food sources at a greater rate than many other ant species (Aron et al. 1990).

Taxonomy

Ants are members of family Formicidae and belong to the order Hymenoptera, which also includes bees, sawflies, and wasps (Grimaldi and Agosti 2001). In 1900, there were 11 subfamilies and 297 genera of Formicidae (Holldobler and Wilson 1990). By 2013, 15,835 species were classified (AntWeb 2015), with estimates of a possible 20,000 species. Species were classified by a pentanomial system which was used for naming subgenera, species, subspecies, and variety (Creighton 1938). Gustav Mayr first described Argentine ants in 1866 and named them *Hyoclinea humilis* (Newell 1908). Emery transferred the species to the genus *Iridomyrmex* in 1888 (Bolton 1995). However,

according to Shattuck, the species was poorly placed in this genus and in 1992 Shattuck transferred the species to the genus *Linepithema* (Bolton 1995).

Linepithema humile are in the subfamily Dolichoderinae. According to Shattuck (1992), Forel established the subfamily Dolichoderinae by separating a portion from the subfamily Formicinae in 1878. There were two different characters on which Shattuck based the two groups. The Dolichoderinae have the gaster terminating in a slit-like opening, and the Formicinae have the gaster terminating in a circular orifice. The queens and workers in the subfamily Dolichoderinae are characterized with a single segmented petiole, no gaster constriction between the first and second segments, and a slit-like opening in the posterior of the gaster. Males are distinguished from other subfamilies by having a single-segmented petiole with a short anterior peduncle, no gaster constriction between the first and second segments, and no teeth in a subgenital plate (Shattuck 1992).

Workers of *L. humile* are monomorphic and range in size from 2.2 to 2.6 mm with a single abdominal pedicel, the petiole, a promesonotal suture, no sting or acidopore, and a single pointed node between the propodeum and the gaster. They are a uniformly light brown to brown with a slender body and oval gaster. Workers have mandibles with two apical teeth followed by denticles and lack of hair on the thorax (Smith 1965).

Life cycle

Eggs: The egg is typically elliptical and white. Time from oviposition to hatch depends on temperature and humidity. The incubation period during the summer averages 15 days (Barber 1916). Queens are reported to lay 3 to 60 eggs per day in a laboratory environment (Barber 1916, Thompson 1990).

Larvae: After hatching, larvae are white in appearance and scarabaeiform (Barber 1916) with anterior and posterior ends together. Larval morphology makes it difficult to distinguish first larvae from eggs (Newell 1908). Larvae straighten as they grow. During this period workers groom and feed the larvae in the colony. Workers also move larvae to optimal areas within the colony in response to changing weather conditions. The larval stage averages 13 days, but can vary depending on ambient conditions (Barber 1916).

Pupae: Pupae are initially white except for one black compound eye on each side of the head. Pupae turn light brown to medium brown as they mature (Newell 1908). During the pupal stage the immature morphs into an adult form which allows gender to be determined (Barber 1916). Worker pupae are typically 2 mm long. A male pupa's abdomen is smaller than its thorax. This characteristic of male pupae can be distinguished from worker pupae. The queen's pupal body size is on average twice the size of the female worker pupae. The pedicel of the queen pupae is more constricted than that of male pupae (Barber 1916).

Adults: Workers are 2.2-2.9 mm in length and brown with a single node pedicel anterior to the gaster (Thompson 1990). The winged adults or alates comprise up to 15% of the colony when present. Alate males are around 3mm in length (Barber 1916). Males often are most active between May and June and are observed flying near lights in the evening (Markin 1967). Queens are approximately 5 mm in length and are two times longer than workers. Queens have the same coloration as other colony members (Newell and Barber 1913). Adult queens emerge from the nests between April-June. Queens retain

their wings until copulation is completed (Markin 1967). Egg laying begins after removal of the wings (Passera and Aron 1993).

Habitat

Linepithema humile is well-established in the southeastern United States. Queens produce a large number of individuals in a colony. Large multiple colonies often have the ability to nest in many diverse habitats (Smith 1965). In general, *L. humile* prefer to nest in areas with suitable amounts of moisture and in close proximity to food (Mallis 2011). They also tend to remain hidden in concealed areas of a nest, except for entrances (Vega and Rust 2001). During fall, winter and early spring, nests are usually found in soil or under organic debris adjacent to structures, or near the bases of trees, logs, stumps, or other natural features. During warmer months, *L. humile* foragers become more active and increase the number of nesting sites over a greater area (Mallis 2011).

Food preference

Linepithema humile have two main preferences foods: 1) solid animal prey and 2) liquids such as honeydew produced by plant-feeding aphids and related insects (Markin 1967). Solid food items often provide protein and liquid foods often provide carbohydrates. Many *L. humile* nests are in close proximity to vegetation harboring honeydew-producing insects (Markin 1970; Holway et al 2002b; Smith 1965). According to Markin (1967), protein is the predominant nutrient for larval and queen diets and carbohydrates are the predominant nutrients for workers (Vega 2001). During warm seasons workers often forage for carbohydrates (Mallis 2011) from honeydew-secreted

by mealy bugs and aphids (Smith 1965). During the winter season the workers often forage for other nutrients due to lack of plant-feeding and honeydew-secreting insects.

Economic Impact

Initially the wide distribution of *L. humile* in the United States resulted from commercial shipments of plants, plant products and household goods (Barber 1916). The ecological success of *L. humile* results from the ant's ability to tolerate a wide range of habitats, establishing polygynic and polydomic nests, and performing massive and rapid recruitment by using pheromone odor trails (Aron 1990). They can be a pest in urban and agricultural areas. In urban areas, *L. humile* is often a nuisance pest, but one that also can result in economic loss. They invade houses and can penetrate containers and foul food (Davis and Van Schagen 1993). When nests are disturbed or disrupted, *L. humile* will crawl up legs and arms of people. While bites from this species are not common, some people are highly sensitive to *L. humile*'s bite. *Linepithema humile* also has the potential to carry and spread disease around hospitals (Flower et al. 1993). *Linepithma humile* can be a serious pest in agricultural areas. When found in corn, cotton, and sugar cane fields, aphids and mealy bugs are consistently present. Because *L. humile* defend aphids and mealy bugs from potential predators, these pests may cause serious damage to crops (Barber 1916). *Linepithema humile* can also destroy honey bee colonies by entering the hives to rob honey and may cause honey bees to abandon their nests. Consequently, a decrease in honey bees reduces pollination and can decrease crop production (Vega and Rust 2001).

Control

Linepithema humile can also adversely impact natural areas in the southeastern United States. In the Piedmont region of South Carolina, there is a documented problem with *L. humile* invading campsites of recreational campers in state parks, often in close proximity to lakes and streams (Ellis 2009). They have been reported to infest personal recreational vehicles, tents, public facilities, and a variety of locations accessed by campers (Stan Hutto, pers. com. 2006). In a survey conducted by Ellis (2009) 30% of campers surveyed at two state parks during summer months were undecided about visiting the campground again due to infestations of *L. humile* in their sites. Ellis (2009) extrapolated that if 30% of campers did not return because of *L. humile* infestations, the South Carolina State Park Service could lose up to $137,900 in funds per year at just two selected campgrounds.

Linepithema humile continues to be a pest in the Piedmont region of South Carolina State Parks, invading the campsites of recreational campers (Ellis 2009). While some state parks have tried to implement a control program, the most widely used "program" is spraying insecticides when *L. humile* populations become unbearable in campsites or park facilities. Seasoned campers frequently come with their own "control" products often using cleaning powders, lime, insecticide powders and a wide variety of insecticide sprays placed around campsites near streams, lakes, sleeping and eating areas. In many state parks, it is not uncommon to see rings of powder in multiple sites around campgrounds.

In 2007, three SC state parks were selected (Greenwood, Baker and Calhoun Falls) to study *L. humile* distribution and prevalence. Insecticidal sprays alone compared

to insecticidal sprays combined with granular ant baits were evaluated for control (Ellis 2009). In these studies, granular bait combined with sprays did not significantly reduce ant numbers when compared to untreated control sites (Ellis 2009). Some insecticidal sprays did significantly reduce ant numbers for one month compared to untreated control sites, but sprays can be costly and may adversely impact natural areas of state park campgrounds (Ellis 2009). The major dietary component of the Argentine ant consists of carbohydrates such as honey dew (Rust et al, 2000). Thus, Argentine ants prefer to forage on insecticide mixed with sugar water rather than granular protein bait. Additionally Silverman and Roulston (2001) stated that even though ant populations on the gel bait were higher, liquid bait composition could be more effective control for Argentine ants than gel bait due to more time spread on bait and mortality (Silverman and Roulston 2001).

During February of 2008, Ellis did a preliminary, short-term study to locate over-wintering nests of *L. humile* in a few South Carolina state park campgrounds. Ellis found many ants aggregated at the base of pine trees in shallow nests, but they were surprisingly active on 53% of the trees surveyed. This may indicate *L. humile* will forage for food as early as February in the Piedmont Region of South Carolina in natural areas if temperatures are high enough. Ellis (2009) stated that future research is needed to find more effective and environmentally sustainable methods to control *L. humile* infestations in South Carolina state park campgrounds. For example early season baiting, perhaps at the base of trees where *L. humile* over-winter, may be an effective control method and more environmentally friendly than warm-season area sprays.

However, the dispersal pattern and foraging distance of *L. humile* are not studied well in natural area. Previous research findings suggest that the rabbit IgG has a greater retention time than chicken IgG solution. Furthermore rabbit IgG solution has a higher mean absorbance value than chicken IgG solution (Hagler 1997). Using Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS- ELISA; Hagler 1997) Buczkowski and Bennet (2006) tracked foragers and reported that *Tapinoma sessile* (Say) exhibited high foraging site fidelity, traveled along well-established trails and foraged on a scale insect (Buczkowski and Bennett 2006). According to Hagler (1997), the direct ELISA test was less sensitive for detecting Rabbit IgG on the marked insects than the Sandwich ELISA test. In general *Tapinoma sessile* did not forage far for food. In contrast, Cooper et al. (2008) reported that *L. humile* foragers fed IgG rabbit protein could be detected as far away as 72 m, but the majority of ants marked stayed within 20 m of the marking site. Cooper et al. (2008) reported that bait station placement for *L. humile* control in citrus orchards in Southern California could be optimized by understanding the foraging distances *L. humile* individuals would travel. It was reported that baiting *L. humile* in Southern California was most effective for colony control during the spring (April) when new reproductive and worker brood were being produced at the highest level.

The goals of this research are to 1) evaluate of the use of a sandwich ELISA test to determine optimal bait station placement for Argentine ant control in South Carolina state park natural areas 2) evaluate over-wintering *L. humile* nest locations and early season foraging of *L. humile* in South Carolina state park natural areas and 3) evaluate

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early season bait strategies for *L. humile* control in South Carolina state park natural

areas.

References

AntWeb. 2015. Bolton World Catalog Ants: www.antweb.org. 9 April 2015.

Aron, S., S. G. Pasteels, and J. L. Deneubourg. 1990. Self-organizing spatial patterns in the Argentine ant *Iridomyrmex* humilis (Mayr). Applied Myrmecology: a world perspective. Westview press, Boulder, Colorado. 438-451 pp.

Barber, E. R. 1916. The Argentine ant: Distribution and control in the United States. U.S.D.A. Bulletin 377: 1-23.

Bolton B. 1995. A new general catalog of the ants of the world. Harvard University Press. Cambridge, Massachusetts. 504 pp.

Brightwell R. J. and J. Siverman. 2007. Argentine ant foraging activity and interspecific competition in complete vs. queenless and broodless colonies. Insectes Sociaux. 54: 329-333.

Creighton, W. S. 1938. On formicid nomenclature. J. New York Entomol. Soc. 46: 1-9.

Davis, P., J. Van Schagen. 1993. Effective control of pest ants. Journal of Agriculture, Western Australia 34:92-95.

Ellis, B. R., E. P. Benson, P. A. Zungoli, and W. C. Bridges. 2009. Evaluation of chemical control of *Linepithema humile* (Mayr) (Hymenoptera: Formicidae) in South Carolina state park campground. J. Agr Urban Entomol.

Fowler, H. G., O. C. Bueno, T. Sadatsune, A. C. Montelli. 1993. Ants as potential vectors of pathogens in hospitals in the state of Sao Paulo, Brazil. Insect Sci Appl. 14:367-370.

Giraud T, J. S. Pedersen., and J. Keller. 2002. Evolution of supercolonies: the Argentine ants of southern Europe. Proceedings of the National Academy of Sciences, 99, 6075–6079.

Grimaldi D, D. Agosti. 2001. A formicine in New Jersey Cretaceous amber (Hymenoptera: Formicidae) and early evolution of the ants Proc Natl Acad Sci USA. 97:13678–13683.

Hagler, J. R., and S. E. Naranjo. 1997. Measuring the sensitivity of an indirect predator gut content ELISA: Detectability of prey remains in relation to predator species, temperature, time, and meal size. Biol control. 9, 112-119.

Heller, E. Nicole, and D.M. Gordon. 2006. Seasonal spatial dynamics and causes of nest movement in colonies of the invasive Argentine ant *(Linepithema humile)* Ecol Entomol. 31: 499-510.

Holldobler, B. and E.O. Wilson. 1990. The ants. Belknap Press of Harvard University Press. Cambridge, Mass. 732pp.

Holway, D. A., A. V. Suarez, and Case, T.J. 2002a. The role of abiotic factors in governing susceptibility to invasion: a test with a widespread invasive social insect. *Ecology*, 83, 1610 – 1619.

Holway, D. A., L. Lach, A. V. Suarez, N. D. Tsutsui, and T. J. Case. 2002b. The causes and consequence of ant invasions. Annu. Rev. Ecol. Syst. 33: 181-233.

Mallis, A. 2011. 10th Edition. Handbook of Pest Control [ed.] Stoey Hedges. Franzak and Foster Company. Cleveland, OH.

Markin, G. P. 1967. Food distribution within colonies of the Argentine ant, *Iridomyrmex humilis* (Mayr). Ph.D. Dissertation. University of California, Riverside.

Meissner, H. E. and J. Silverman. 2003. Effect of aromatic cedar mulch on argentine ant (Hymenoptera: Formicidae) foraging activity and nest establishment. J Econ Entomol. 96: 850-855.

Newell, W. and T. C. Barber. 1913. The Argentine ant. U.S.D.A. Bur. Entomol. Bull. 122: 98.

Newell, W. 1908. Notes on the habits of the Argentine or "New Orleans" ant, *Iridomyrmex humils* Mayr. J. of Econ Entomol. 21-34 pp.

Passera, L. and S. Aron. 1993. Factors controlling dealation and egg laying in virgin queens of the Argentine ant *Linepithema humile* (Mayr)(= *Iridomyrmex humilis*). Ethology 93:225-235.

Shattuck, S. O. 1992. Generic revision of the ant subfamily Dolichoderinae (Hymenoptera: Formicidae). Sociobiology. 21: 1-176.

Roulston, T. H. and J. Silverman. 2002. The effect of food size and dispersion pattern on retrieval rate by the Argentine ant, *Linepithema humile* (Hymenoptera: Formicidae). J. Insect Behav. 15: 633-648.

Rowles, A. and D. O'Dowd. 2007. Interference competition by Argentine ants displaces native ants: implications for biotic resistance to invasion. Biol. invasions 9: 73-85.

Rust, M. K., D. A. Reierson, E. Paine, L. J. Blum. 2000. Seasonal activity and bait preferences of the Argentine ant (Hymenoptera: Formicidae). J Agric Urban Entomol. 17:12.

Shattuck, S. O. 1992. Generic revision of the ant subfamily Dolichoderinae (Hymenoptera: Formicidae). Sociobiology. 21: 1-177.

Silverman J. and T. H. Roulston. 2001. Acceptance and intake of gel and liquid sucrose compositions by the Argentine ant (Hymenoptera: Formicidae). J. Econ. Entomol. 94: 544-515.

Smith, M. R. 1965. House-infesting ants of the Eastern United States. U.S.D.A. Tech. Bull. No. 1326: 17-105.

Suarez A. V., N. D. Tsutsui, D. A. Holway. 1999. Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. Biol Invasions, **1**, 43–53.

Thompson, C. R. 1990. Ants that have pest status in the United States. In R.K. Vander Meer, K. Jaffe, and A. Cedeno [eds.], Applied myrmecology: a world perspective, Westview Press, Boulder, CO.

Vega, S. J. and M. K. Rust. 2001. The Argentine ant – a significant invasive species in agricultural, urban, and natural environments. Sociobiology 37: 3-25.

CHAPTER TWO

Using the DAS-ELISA Test to Determine the Optimal Placement of Bait Stations for

Control of *Linepithema humile*

Introduction

Linepithema humile (Mayr), the Argentine ant, is an invasive pest introduced to

the United States in 1891 (Newell and Barber 1913). It has spread throughout the

Southern and Western United States. Even though *L. humile* is an urban pest, it causes

ecological problems in natural habitats by displacing native ants and other arthropods

(Markin 1970 and Holway et al. 2002). Many ants, including *L. humile,* take part in

central foraging. The foragers collect food around the nest and bring it back to the colony

(Holldobler and Wilson 1990). *Linepithema humile* does not show intraspecific

aggression (Suarez et al. 1999, Holway et al. 2002), thus Argentine ants form large,

overlapping, colonies housing multiple queens (Aron et al 1990). Additionally *L. humile*

takes part in dispersed central-placed foraging to reduce foraging time and energy

expenditure (Holway and Case 2000). The ants transport workers and brood to resources

rather than bringing food back to the nest (Holway and Case 2000). *L. humile* foraging

patterns and distances have been studied in urban areas (Cooper et al. 2008), but not in

natural habitats. In order to make the use of baiting most effective for the control of

Argentine ants in natural habitats, an understanding of the foraging range of *L. humile* is

essential.

The enzyme-linked Immunosorbent assay (ELISA) is a valuable test method for detecting and quantifying a specific protein in a complex mixture. Engval and Perlmann (1971) demonstrated quantitative measurement of IgG in rabbit serum when linked to the enzyme alkaline phosphatase. Buczkowski and Bennett (2006) used rabbit immunoglobin (IgG) protein (Sigma Chemical Co., St. Louis, MO) in 30% sucrose-water solution to mark the odorous house ants, *Tapinoma sessile* (Say)*, to evaluate central-place foraging. Previous research findings (Hagler 1997) suggest that because rabbit IgG degrade less quickly than chicken IgG, rabbit IgG is retained by ants for a longer period of time post feeding than chicken IgG. Furthermore a hornworm caterpillar, *Manduca sexta* (Lepidoptera: Sphingidae), fed with rabbit IgG solution has a greater mark retention time than hornworm caterpillar fed with chicken IgG (Kelly et al. 2012). Using Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay (DAS-ELISA; Hagler 1997), Buczkowski and Bennet (2006) tracked foragers and reported that *T. sessile* exhibited high foraging site fidelity, traveled along well-established trails, "foraged on a local scale" and in general *T. sessile* did not forage far for food. (Buczkowski and Bennett 2006). In contrast, Cooper et al. (2008) reported that *L. humile* foragers fed IgG rabbit protein could be detected as far away as 72 m from the marking site but the majority of ants marked stayed within 20 m of the marking site. According to Hagler and Naranjo (1997), the direct ELISA test was less sensitive for detecting rabbit IgG on the marked insects than the Sandwich ELISA test.

The purpose of this study was to detect ants carrying technical-grade rabbit immunoglobin (Ig G) protein marker by using the double antibody-sandwich ELISA test (DAS-ELISA) and thereby determine the foraging distance of *L. humile* and calculate the

optimal placement of bait stations that would be needed for control of this species. The following specific objectives were: 1) to determine the concentration of protein marker that provided the optimal retention time of protein marker in *L. humile*, 2) determine the distance from the nest at which protein marker in foragers could be significantly differentiated from a non-marked control, and 3) to use the results of objectives 1 and 2 to estimate the optimal placement of bait stations based on foraging distances determine by earlier experiments..

Materials and Methods

Much of the preparatory work for these experiments were completed using either, *L. humile* individuals collected, or colonies located on the Clemson University campus.

ELISA Test

A DAS-ELISA procedure was used for detecting the presence of technical grade rabbit IgG protein marker in ants in all experiments. Each well of a 96-microtiter plate was coated with 100µl goat anti-rabbit IgG (Sigma Chemical Co., St. Louis, MO) diluted 1:500 with carbonate coating buffer (0.05 M sodium carbonate + 0.02% sodium azide, pH 9.6) and incubated at room temperature for one hour. The goat anti-rabbit IgG was discarded and the microtiter plate washed three times with phosphate buffered saline (PBS), pH 7.4 + 0.5ml/liter Tween-20 (PBS-Tween) for three minutes per wash. Individual frozen ants were ground in 150µl PBS buffer and added into a single well. The plates were incubated overnight at 4°C. After incubation overnight, the samples were discarded and washed two times with PBS-Tween and one time with tris-buffered saline (TBS) 10 mM Tris, 150 mM NaCl, pH 7.5) + 0.5ml/liter Tween-20 (TBS-Tween) for

three minutes per wash. Blocking solution (1% dried milk [Carnation Brand] + 0.5% bovine serum albumen in TBS was added to each well for one hour at room temperature to block non-specific binding sites. Each well was washed three times for three minutes per wash with TBS-Tween. Then, 100µl of anti-rabbit IgG alkaline phosphatase conjugate (Sigma Chemical Co., St. Louis, MO) diluted 1:5000 in blocking solution (diluted 1:10 with TBS-Tween) was added into each well and incubated for four hours at room temperature. Each well was washed three times for three minutes per wash with TBS- Tween. 100µl of p-nitrophenol phosphate substrate (1mg/ml) in 10% diethanolamine (pH 9.8) was added to each well. After one hour of incubation, the absorbance at 405 nm was determined by using an E^{max} microplate reader (Molecular devices, Sunnyvale CA) and readings were subjected to Analysis of Variance and comparison with a control were analyzed by using a Dunnett's test unless otherwise stated.

Optimal IgG protein marker concentration

To determine optimal concentration of technical grade rabbit IgG protein required to mark individual ants, specimens of *L. humile* were collected and fed rabbit IgG (I8140, Sigma Chemical Co., St. Louis, MO). According to Buczkowski and Bennett (2006), 0.5mg/ml in 30% sugar water was a minimal concentration to mark odorous house ants. However, a preliminary test showed that 1mg/ml of IgG protein marker would be easily detected in *L. humile*. To reduce potential costs we selected several concentrations of IgG protein marker (0.1, 0.01, and 0.001mg/ml) below 1mg/ml. The ants were placed into plastic boxes and fed with 30% sugar water. After 1 week, a group of at least 10 ants

was placed into a Petri dish and fed with one of three concentrations of IgG serum protein, 0.1, 0.01, or 0.001mg/ml, diluted into a 30% sugar-water solution. After 3 d the ants were collected at random and frozen at -20°C. A DAS-ELISA test was performed on individual ants and the mean absorbance was recorded.

Optimal retention time of IgG protein marker under laboratory and field conditions

To test retention time of the protein marker in the laboratory, *L. humile* were collected and placed into Petri dishes and fed with the protein marker (0.1 mg/ml) in 30% sugar water. After 3, 5, and 7 d post feeding, ants were recollected and frozen at -20°C. For field tests of the retention time of the IgG protein marker, a 30% sugar water solution was prepared and placed in bait stations. The stations used were KM AntPro liquid bait stations (KM AntPro LLC; P.O. Box 967, Nokomis, FL 34275). KM AntPro bait stations are designed to slowly release bait, hold enough bait to feed many ants, and protect the bait from degradation. In other research this bait station has been successfully used for control of *L. humile* in a citrus orchard (Greenberg et al. 2006). Control stations with only 300 ml sugar water were compared to stations containing 300 ml sugar water and 0.01mg/ml of the IgG protein marker. Control stations were placed over 100 m from stations containing the IgG protein marker to avoid ants in the control area foraging to the stations containing IgG. Ants were collected at 2, 5, and 10 h, and 1, 2, and 3 d post exposure to the bait stations and stored in -20°C. A DAS-ELISA test was performed and the mean absorbance of individual ants and a control was recorded.

Field Foraging Distance for Which the IgG protein Marker is Detectable in *L. humile*

A test was performed in natural areas of the Clemson University campus to determine the distance from bait stations at which the IgG protein marker could be detected in field-collected *L. humile*. KM AntPro bait stations were used in these field trials. Control stations had only a 30% sugar water solution and were compared with stations containing 30% sugar water (300 ml) and 300µl of protein markers (0.1 mg/ml). Bait stations used as controls were placed at least 100 m away from stations containing the IgG protein marker to avoid having ants in the control areas reach and feed on stations containing IgG. Three days post bait station placement in natural areas on the Clemson campus, several foraging trails were identified at the bait stations. Each foraging trail distance was measured from a feeding source to their nest. The longest trail out of several foraging trails was selected. Ten ants were collected at 5, 10, 15, 20, 25, and 30 m away from feeding stations. The ant samples were stored in a -20°C freezer. The DAS-ELISA test was performed and the mean absorbance of individual ants and a control was recorded. The data were used to evaluate the optimal foraging distance range of an individual *L. humile*.

Effective Distance Between Bait Stations in the Field

This test was conducted at Lake Greenwood State Park (LGSR) (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) to determine the optimal distance between KM AntPro bait station placement. LGSR had previously been shown to have large *L. humile* infestations and was ideal for a bait placement study. It was difficult to find any other ant species in the area. We hypothesized that significant differences in bait station placement would occur between 10 and 20 m. Nine bait stations were placed in three rows of three

at 10 and 20 m apart. The distance between each experimental area was at least 100m to avoid collecting ants from neighboring stations. Each station contained 300 ml of sugar water with 0.1 mg/ml protein markers. After three days, *L. humile* specimens were collected at point's mid-way between stations in each experimental area. Detection of the protein marker was determined using DAS-ELISA. This test was replicated three times. Mean absorbance for each distance were compared using a mixed model analysis of variance with distance as a fixed effect and replicate as a random effect.

Results

Optimal IgG protein marker concentration

Preliminary evaluation with a 1mg/ml IgG protein marker produced a significant absorbance (>0.2). In order to determine the lowest concentration of IgG that allowed for reliable detection of individuals and to reduce protein marker expense *L. humile* were fed with 0.1, 0.01, and 0.001 mg/ml IgG protein marker. The mean absorbance recorded for individual ants fed each concentration was 0.218, 0.108, and 0.082, respectively (Fig. 1). The average absorbance for ants fed 0.1mg/ml IgG marker was almost three times greater than the absorbance of the control (buffer solution 0.081). The absorbance recorded for an individual ant fed with either 0.1 or 0.01mg/ml IgG protein marker was significantly different from the control (P<0.05). The mean absorbance of ants fed with 0.001 mg/ml IgG protein marker did not differ from the mean absorbance for the control. Even though the 0.1 mg/ml absorbance value was much higher than the 0.01 mg/ml absorbance, the 0.01mg/ml was still significantly higher than the control. Since 0.01mg/ml was 10 times lower compared to 0.1 mg/ml, it was more cost effective. Based on these results, *L.*

humile foragers were fed 0.01 mg/ml of IgG protein marker in sugar-water baits for the subsequent studies.

Optimal retention time of IgG protein marker under laboratory and field conditions

In a laboratory test completed on ants fed 3 d previously with 0.01mg/ml of IgG in sugar-water, the mean absorbance of an individual ant (0.204) was significantly different from the control (0.09) ($P<0.05$) (Fig. 2). However, there was no significant difference for the mean absorbance recorded for ant samples tested 5 d post feeding (0.0125) compared to the control. At 7 d post feeding, the mean absorbance value was almost the same as the control value, indicating there was almost no protein marker left in a single ant.

After setting up bait stations, ants were collected after 3 d based on the laboratory test results. There was a significantly different mean absorbance for individual ants collected 2 h, 5h, 1 d, 2 d, and 3 d after exposure to a food resource compared with the control ($P<0.05$) (Fig. 3). The mean amount of IgG protein marker detected in ants increased until 2 d. At 2 h, 5 h, 1 d, and 2 d the mean absorbance value were 1.346, 1.885, 2.119, and 2.77, respectively. After 2 d the amount of protein dropped quickly. At 3 d, the mean absorbance was less, but still detectable at 1.467.

Field Foraging Distance for Which the IgG protein Marker is Detectable in *L. humile*

There was a significant difference among the mean absorbance value for individual ants collected 5, 10, and 15 m away from a food resource compared to the control ($P<0.05$) (Fig. 4). The absorbance of the samples was 2.280, 1.955, and 1.064, respectively. The mean background absorbance in the sugar-water control was 0.082. The

mean absorbance of samples collected 20, 25, and 30 m away from a food source was 0.902, 0.638 and 0.238, respectively. However, these values were not significantly different from the control. This result showed the highest absorbance for an ant's foraging trail was detected at 5 m from the food feeding source. In contrast, the lowest absorbance number was detected at 30 m from the feeding source where a nest was located, but IgG levels were still clearly detectable at distances of 10, 15 and 20 m from stations with sugar-water bait containing 0.01 mg/ml of IgG.

Effective Distance Between Bait Stations in the Field

The mean absorbance of ants fed IgG at LGSP was significantly different between bait stations placed at 10 m and 20 m as shown in Fig. 5 (F=4.23, P=0.0451). The mean absorbance of each treatment was 0.363 and 0.192 respectively. The mean absorbance of the ants fed at 10 m bait station placement sample was almost two times higher than the 20 m bait station placement. While this indicated that the closer bait stations had more *L. humile* feeding, marked ants could still be detected at 20 m. Since it was clear that ants were foraging up to 20 m, stations were set at this distance to reduce material costs of the bait, the bait stations and the labor involved in the evaluation of control of *L. humile* in a natural area.

Discussion

Optimal IgG protein Marker Concentration.

According to Buczkowski and Bennett (2006), a 0.5mg IgG/ml concentration was selected for a feeding study of *T. sessile* because it allowed detection of ants fed IgG.

However, our preliminary test detected a significant difference between the absorbance recorded for controls (ants not fed IgG) and those fed only 0.1mg/ml of IgG. This may reflect a difference between the species involved. Three different IgG concentrations (0.1, 0.01, and 0.001mg/ml) were selected based on our preliminary test to determine the optimal IgG concentration for feeding *L. humile* workers. Workers fed 0.01mg IgG/ml had the lowest optimal IgG concentration. Hagler (2004) applied 5mg/ml rabbit IgG protein to *Hipodamia convergens* for a mark-recapture test. In this work we were able to reduce protein marker costs based on our results. Our selected IgG protein marker concentration was 50 times less than the selected protein concentration of Buczkowski and Bennett (2006).

Optimal Retention Time of IgG Protein Marker Under Laboratory and Field Conditions

Optimal retention time of 0.1mg/ml IgG under our laboratory condition as indicated by mean absorbance was significantly different from the control after day 3 (P<0.05) at 0.2039 and 0.0895, respectively. Data showed that at day 3 the mean absorbance was highest but dropped quickly by day 5. These results were similar to the pattern reported by Buczkowski and Bennett (2006) where the average absorbance dropped sharply by day 4 (Buczkowski and Bennett 2006). In addition, the protein marker concentration was higher in their study than the concentrations we used. In our laboratory study, the optimal retention time was day 3, after which the absorbance dropped.

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In field studies, the level of IgG protein detected in the ants increased over a day 2 period and then dropped sharply. The absorbance at day 3 was 0.3739. However, this value was high enough to detect IgG protein and was significantly different from the control. Buczkowski and Bennett (2006) also mentioned that at 72 h the retention time of protein marker in a field study was shorter than in a lab study. These results also showed that retention time of protein marker in a field test could be relatively shorter than under laboratory conditions. The results in both lab and field studies showed no significant difference in absorbance at 5 d post feeding. This suggests that the optimal sample collection time of *L. humile* fed with IgG protein was 3 d.

Field Foraging Distance for Which IgG Protein Marker is Detectable in *L. humile*

The mean absorbance of samples collected at 5 m from a feeding station was highest; and the mean absorbance at 30 m was lowest. In general, the absorbance of IgG protein marker was reduced in ants heading back to the nest. We assumed that the foragers digested some IgG protein marker while travelling. We also assumed that *L. humile* shared food with nestmates on the trail by trophallaxis (Flanagan et al. 2013). Results of these data also indicated there was a significant difference in mean absorbance at distances up to 15 m from the feeding source (P<0.05). The absorbance at 5, 10, 15 m were 2.28, 1.956, and 1.064, respectively. The mean absorbance at 20, 25, and 30 m were 0.902, 0.638 and 0.238, and were not significantly different than the sugar-water control, suggesting that the majority of *L. humile* populations stayed within 15 m. However, the mean absorbance at 20 m was almost ten times higher than control. Furthermore, Cooper et al. (2008) speculated that most ants stayed within 20 m of a food source and that

carrying bait beyond 20 m from a food source occurred in less than 10% of the ants. Even though absorbance value at 20, 25, and 30 m were not a significantly different from the control, IgG could be detected a percentage of the ants sampled. Cooper et al (2008) focused on the positive percentage of IgG detection, but this study determined the significant difference of mean absorbance between an individual ant sample and control. If our results were applied to the same data analysis method as the Cooper et al. (2008) study, the percentage of *L. humile* carrying IgG protein marker was 100% and 70% at 25 m and 30 m from a food resource, respectively. In spite of the high percentage of *L. humile* carrying IgG protein marker at 20, 25, and 30 m, there was no significant difference of the absorbance between each individual samples and control, indicating that 15 m is the optimal foraging distance. However, up to 20 m appeared to be a suitable foraging distance due to higher mean absorbance (0.902) compared with control (0.082). Buczkowski and Bennett (2006) also suggested that foraging range and pattern studies were required for obtaining better results when using bait for control. Therefore, the foraging distance studies of *L. humile* were a very useful tool to determine an effective distance between bait stations placed in natural areas in South Carolina.

Effective Distance Between Bait Stations in the Field.

To determine the optimal distance for bait placement within the selected natural areas at LGSP, three rows of bait stations were placed at either 10 m or 20 m apart. The results of these DAS-ELISA data indicated that there was a significant difference in mean absorbance for individual ants from bait stations placed at 10 and 20 m (P=0.0451) apart. The mean absorbance number was 0.363 and 0.192 at 10 m and 20 m sample. Samples of

90 ants were collected from each plot. After conducting the ELISA test, we determined

there were only a few *L. humile* samples that did not have IgG protein. The absorbance

number was almost the same as the control value in 17 of 90 ants at 10 m bait stations

placement. In addition, 20 of the 90 ants at 20 m were close to the control number. This

suggested that *L. humile* feeding at the 10 m bait station were more likely to obtain IgG

protein marker than *L. humile* at 20 m. Because workers share food with colony members

via trophallaxis (Knight and Rust 1991), *L. humile* could distribute the liquid bait faster

with a 10 m bait station placement than at 20 m. Therefore, this study suggested that bait

station placement at around 10 m apart would be an optimal distance. However, the

previous study showed that the mean absorbance (0.902) at 20 m apart was ten times

greater than control (0.082). The mean absorbance (0.902) at 20 m was almost equal to

the mean number (1.064) at 15 m. It suggested that the majority of foraging workers of *L.

humile* could look for food up to 20 m. For this reason, 20 m was selected to minimize

the cost of labor and equipment, as well as insecticide use in the natural area at LGSP.

References

Aron, S., S. G. Pasteels, and J. L. Deneubourg. 1990. Self-organizing spatial patterns in the Argentine ant *Iridomyrmex humilis* (Mayr). Applied Myrmecology: a world perspective. Westview press, Boulder, Colorado. pp. 438-451.

Buczkowski G. and G. W. Bennett. 2006. Dispersed central-place foraging in the polydomous odorous house ant, *Tapinoma sessil* as revealed by a protein marker. Insects Sociaux, 53: 282-290

Cooper, M. L., K. M Daane, E. H. Nelson, L. G. Varela, M. C. Battany, N. D. Tsutsuj, and M. K. Rust. 2008. Liquid baits control Argentine ants sustainably in coastal vineyards. Calif. Agric. Vol. 62, Issue 4, 177-183.

Daane, K. M., K. R. Sime, B. N. Hogg, M. L. Bianchi, M. L. Cooper, M. K. Rust, and J. H. Ellis, B. R., E. P. Benson, P. A. Zungoli and W. C. Bridges. 2010. Evaluation of

chemical control strategies for *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), in South Carolina state park campgrounds. J. Agric. Urban Entomol. 25(4), pp. 223-232.

Engvall, E., and P. Perlmann. 1971. Enzyme-linked Immunosorbent assay (ELISA) quantitative assay of immunoglobulin G. Immunochemistry, Volume 8. Issue 9. 871-874.

Flanagan, T. P., N. M. Pinter-Wollman, M. E. Moses, and D. M. Gordon. 2013. Fast and Flexible: Argentine ants recruit from nearby trails. PLoS ONE, Volume 8. Issue 8.

Greenberg, L., J. H. Klotz, and M. K. Rust. 2006. Liquid borate bait for control of the Argentine ant, *Linepithema humile*, in organic citrus (Hymenoptera: Formicidae). Florida Entomologist. Volume 89. Issue 4. 469- 474.

Hagler, R., and S. E. Naranjo. 1997. Measuring the sensitivity of an indirect predator gut content ELISA: Detectability of prey remains in relation to predator species, temperature, time, and meal size. Biol. Control 9. 112–119.

Hagler, J. R. 2004. Optimizing a protein-specific ELISA for the detection of protein-marked insects. Int. J. Pest Manage. 50(3), 209-214.

Holldobler, B. and E. O. Wilson. 1990. The ants. Belknap Press of Harvard University Press. Cambridge, Mass. 732 pp.

Holway, D. A. and T. J. Case. 2000. Mechanisms of dispersed central-place foraging in polydomous colonies of the Argentine ant. Anim. Behav., 59 (2), 433-441.

Holway, D. A., A. V. Suarez, and T. J. Case. 2002. The role of abiotic factors in governing susceptibility to invasion: a test with a widespread invasive social insect. *Ecology*, 83, 1610 – 1619.

Kelly, J. L., J. R. Hagler, and I. Kaplan. 2012. Employing immunomarkers to track dispersal and trophic relationships of a piercing-sucking predator, *Podisus maculiventris*. Environ. Entomol. 41 (6): 1527-1533.

Klotz. 2006. Effects of liquid insecticide baits on Argentine ants in California's costal vineyards. Crop. Prot. 25: 592-603.

Knight, R. L., and M. K. Rust. 1991. Efficacy of formulated baits for control of Argentine ant (Hymenoptera: Formicidae). J. Econ. Entomol. 84: 510-514.

Markin, G. P. 1970. The seasonal life cycle of the Argentine ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae), in southern California. Ann. Entomol. Soc. Am. 63: 1238–1242.

Moreno, D. S., P. B. Haney, and R. F. Luck. 1987. Chlorpyrifos and diazinon as barriers to Argentine ant (Hymenoptera: Formicidae) foraging on citrus trees. J. Econ. Entomol. 80: 208-214.

Nelson, E. H. and K. M. Daane. 2007. Improving liquid bait programs for Argentine ant control: Bait station density. Environ. Entomol. 36 (6): 1475-1484 (2007).

Newell, W. and T. C. Barber. 1913. The Argentine ant. U.S.D.A. Bur. Entomol. Bull. 122: 98.

SAS Institue Inc. **2011**. *SAS/STAT® 9.3 User's Guide*. Cary, NC: SAS Institute, Inc.

Suarez A. V., N. D. Tsutsui, and D. A. Holway. 1999. Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. *Biol. Invasions*, 1, 43–53.
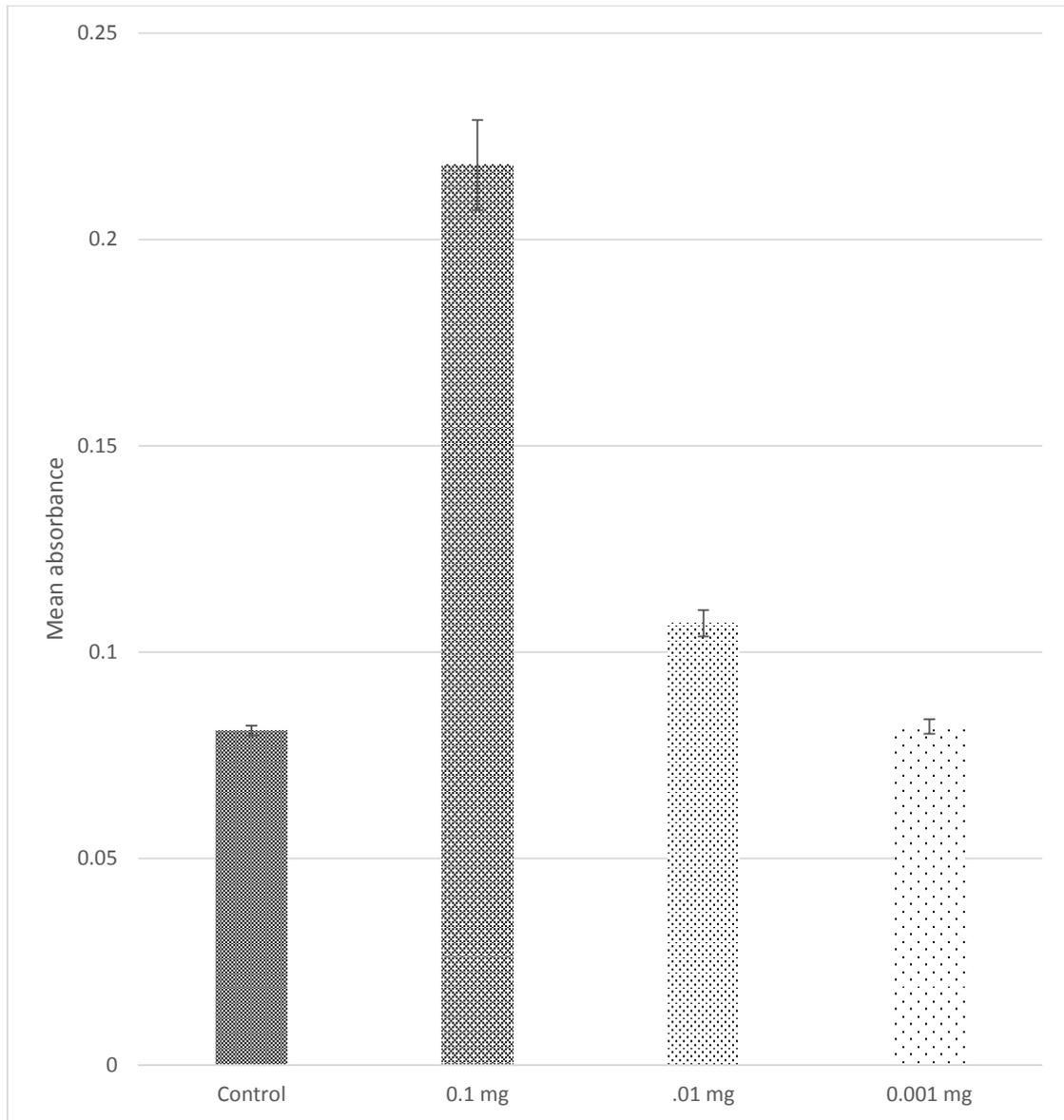
Fig. 2.1. Optimal IgG protein marker concentration. Mean absorbance detected for individual *Linepithema humile* (Mayr) (10 ants per treatment) fed with rabbit IgG protein marker (Sigma Chemical Co., St. Louis, MO) at concentrations of 0.1, 0.01, 0.001 mg/ml ± standard deviation (SD) in 2011. Readings were subjected to Analysis of Variance and comparison with a control were analyzed by using a Dunnett's test.
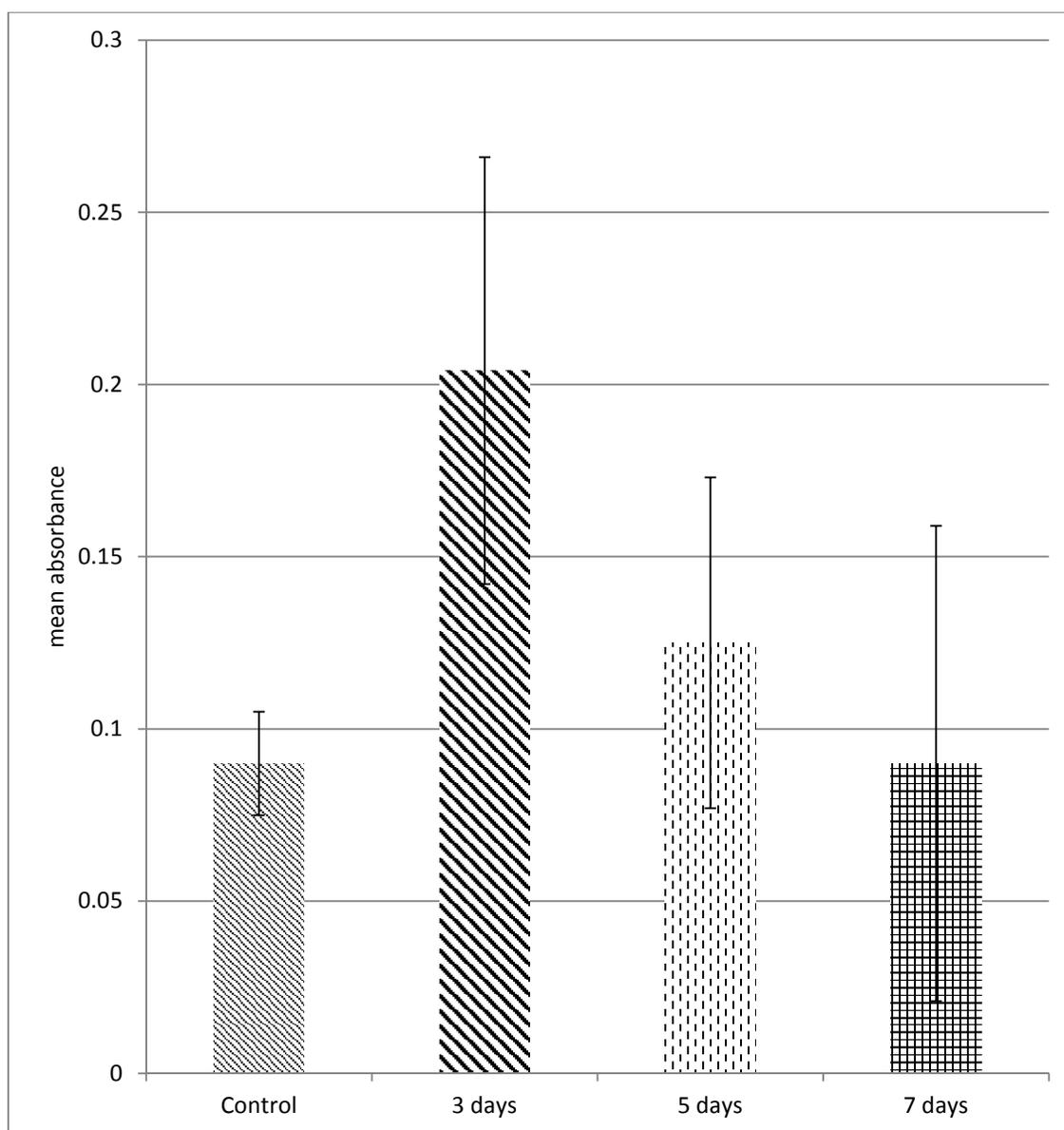
Fig. 2.2. Optimal retention time of rabbit IgG protein marker (Sigma Chemical Co., St. Louis, MO) under laboratory conditions. Mean absorbance number of *Linepithema humile* (Mayr) (10 ants per treatment) under laboratory condition in 2011. Mean absorbance ± standard deviation (SD) are given (n=10). Readings were subjected to Analysis of Variance and comparison with a control were analyzed by using a Dunnett's test.
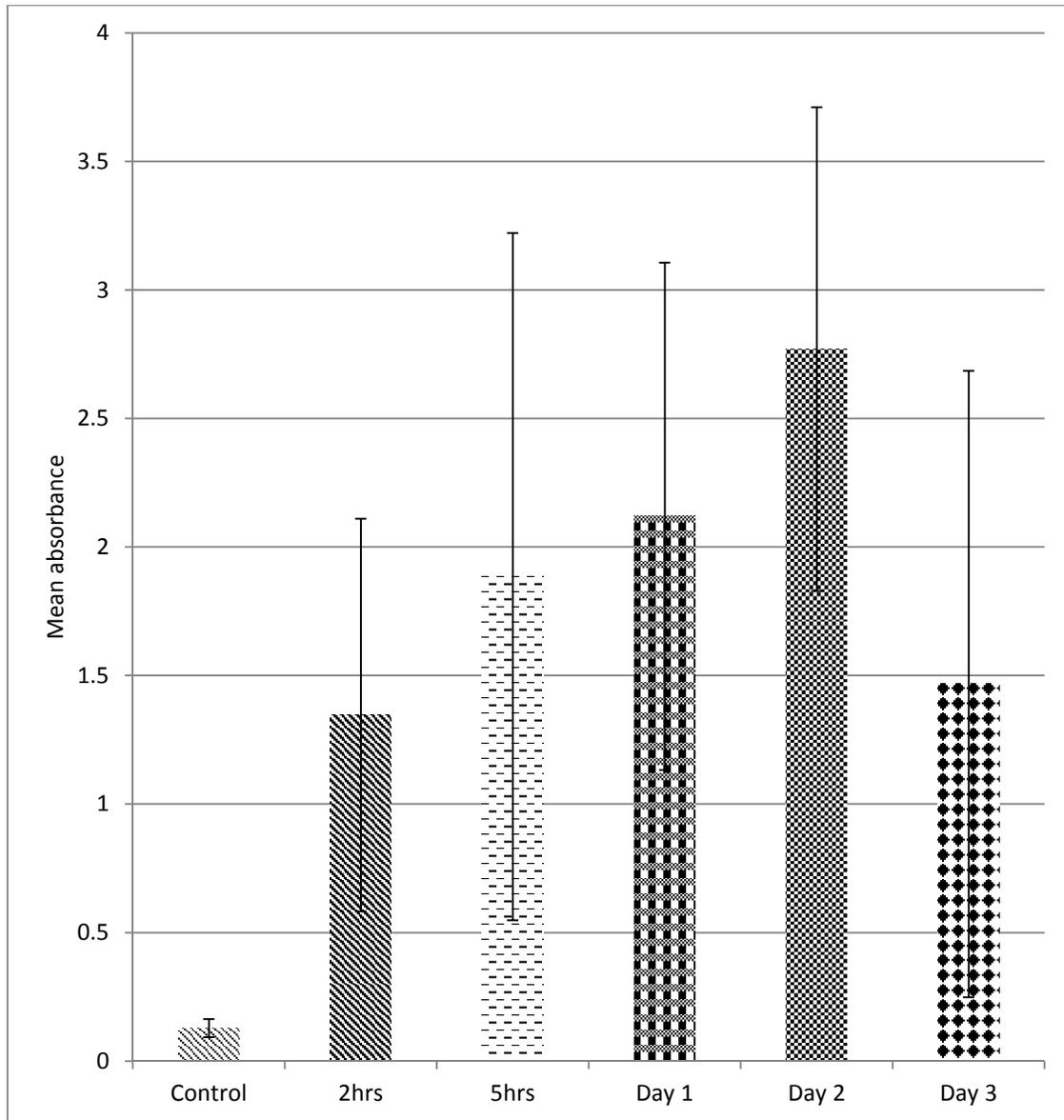
Fig. 2.3. Optimal retention time of protein marker under field conditions. Mean absorbance number of *Linepithema humile* (Mayr) (10 ants per treatment) fed with rabbit IgG protein marker (Sigma Chemical Co., St. Louis, MO) collected from under field condition in 2011. Mean absorbance ± SD are given (n=10). Readings were subjected to Analysis of Variance and comparison with a control were analyzed by using a Dunnett's test.
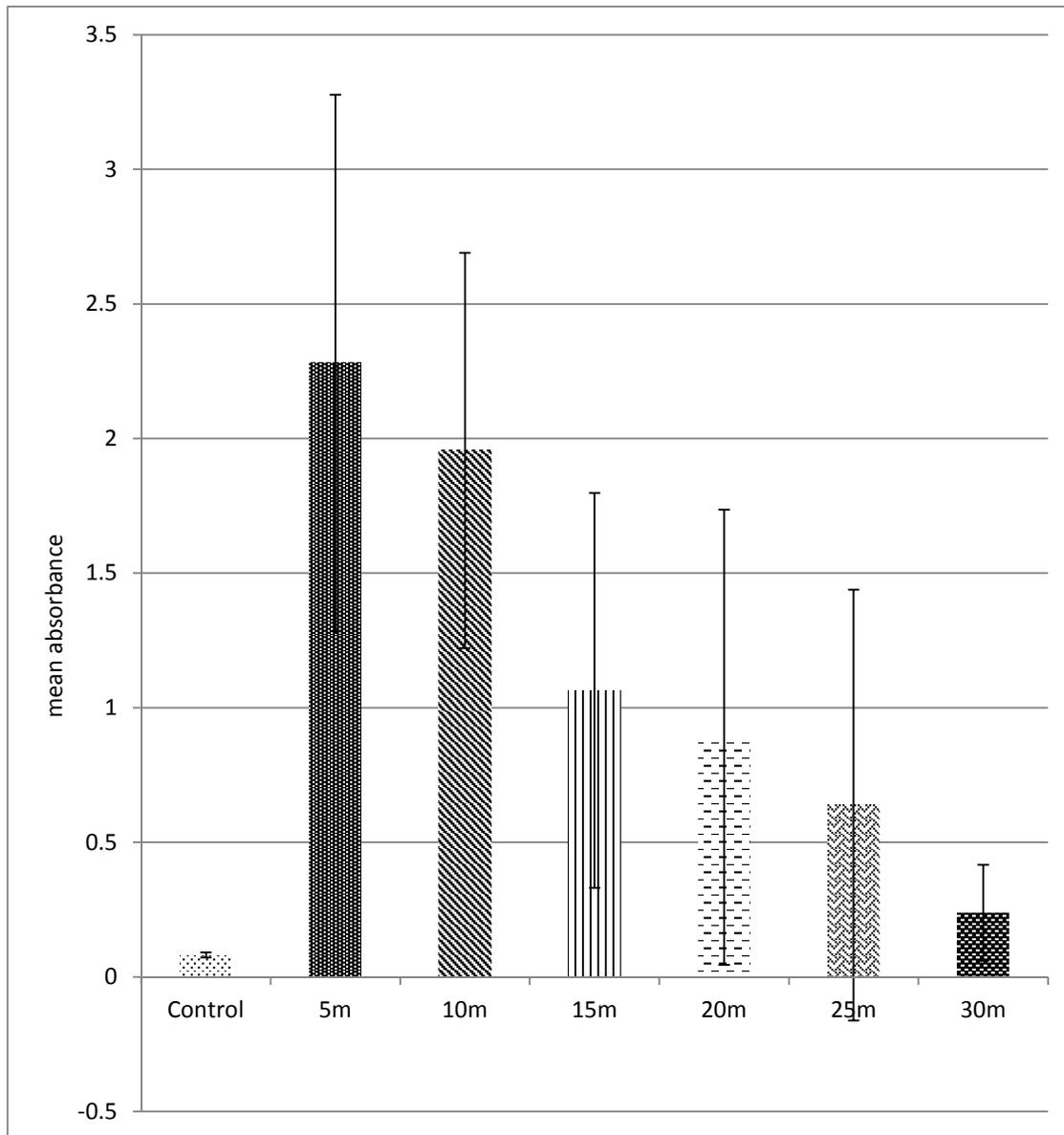
Fig. 2.4. Field foraging distance for which the IgG protein marker is detectable in *Linepithema humile* (Mayr). Mean absorbance number of *L. humile* (10 ants per treatment) fed with rabbit IgG protein marker (Sigma Chemical Co., St. Louis, MO) collected 5, 10, 15, and 20 m away from a food resource in 2011. Mean absorbance ± SD are given (n=10). Readings were subjected to Analysis of Variance and comparison with a control were analyzed by using a Dunnett's test.
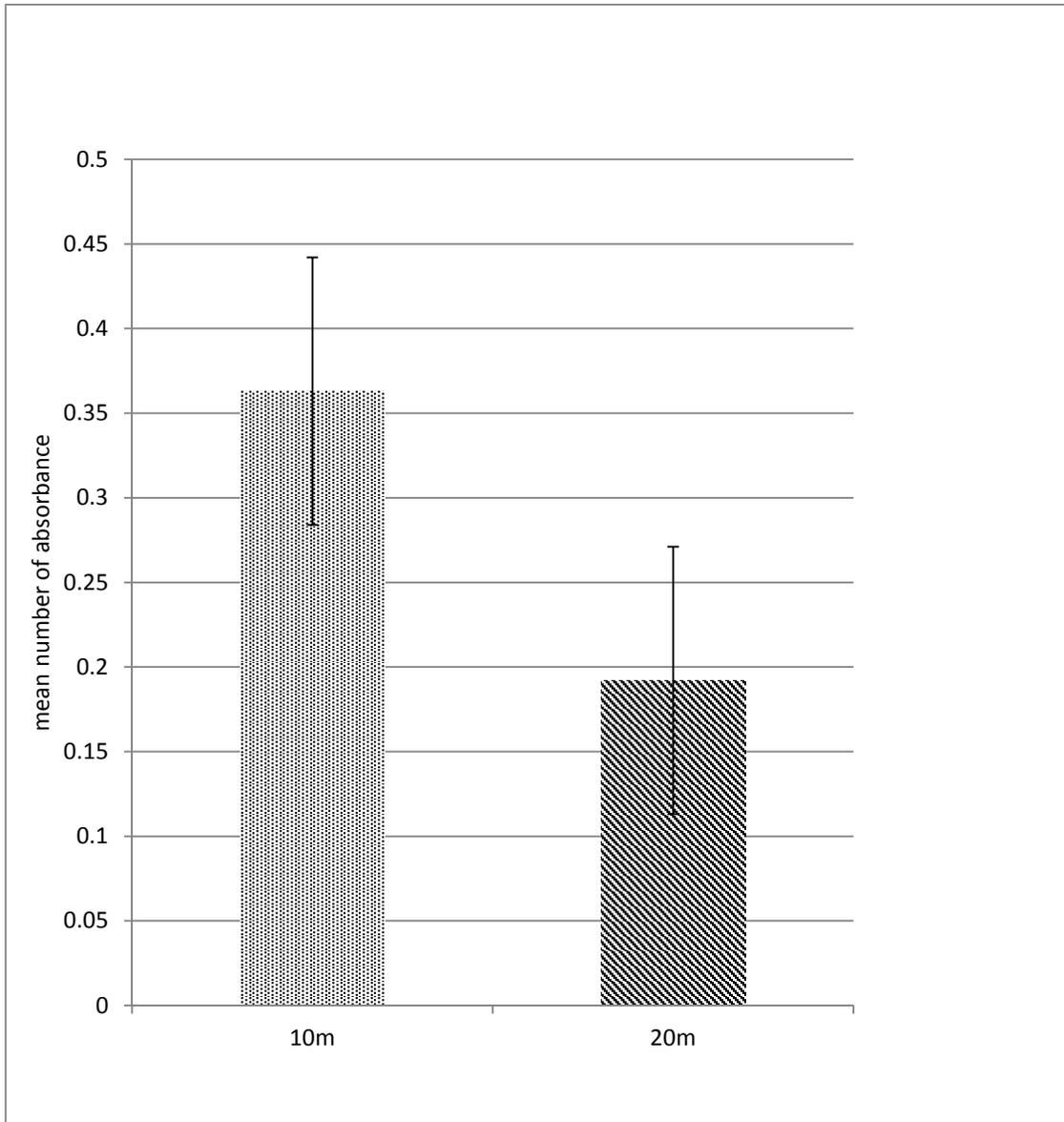
Fig. 2.5. Effective distance between bait stations in the field. Mean absorbance number of *Linepithema humile* (Mayr) (10 ants per treatment) fed with rabbit IgG protein marker (Sigma Chemical Co., St. Louis, MO) collected 10 and 20 m away from a food resource at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) 2011. Mean absorbance ± standard error (SE) are given (n=9). Mean absorbance for each distance were compared using a mixed model analysis of variance with distance as a fixed effect and replicate as a random effect.

CHAPTER THREE

Evaluation of Over-wintering Nests and Early Season Foraging of *Linepithema humile*
in South Carolina State Park Natural Areas

Introduction

Linepithema humile (Mayr) is a foreign, invasive pest and is well established in

the United States throughout the South, California, and Hawaii. They are also found in

Arizona, Missouri, Illinois, Indiana, Maryland, Oregon, and Washington (Mallis 2011).

Linepithema humile can be found from subtropical to warm temperate regions (Roura-

Pascual et al. 2006). In general, *L. humile* prefer to nest in areas with suitable amounts of

moisture and in close proximity of foods (Mallis 2011).

During the fall, winter and early spring, nests are usually found in soil or under

organic debris adjacent to structures or near the base trees, logs, stumps or other natural

features (Human et al. 1998). During colder weather, foraging activity of *L. humile* is

generally reduced especially from January to February (Human et al. 1998 and Vega et

al. 2001). Nests have been found to join and form larger colonies to maintain warm

conditions more efficiently (Barber 1916), thus reducing their colony spatial range (Sudd

1969). This joining creates their winter nests, which are placed under rocks and near

plant structures often with a southern exposure (Enriquez et al. 2013). The south-facing

base of trees is often a suitable winter nesting location. According to Brightwell and

Silverman (2011), *Pinus taeda L.*, loblolly pine, provides an appropriate nest site for *L.*

humile that consolidate around the base of these trees. Nest depths are approximately 20 cm, but during dry seasons *L. humile* can make an underground nest up to 60 cm deep by excavation (Mallis 2011). To survive in the unfavorable winter season *L. humile* need a foraging opportunity and food sources. *Pinus taeda* is not only a food source but also a suitable nest (Brightwell and Silverman 2011). Ellis (2009) found that *Pinus spp.* was the only tree for foraging activity of *L. humile* within the South Carolina campgrounds during winter months. According to Markin (1970b), when the daily temperature is between 15°C and 30°C, *L. humile* is highly active. The egg development rate was zero below 18ºC and upper 32ºC (Abril et al. 2010). Even though ambient temperature was below 5°C, *L. humile* workers would forage up and down *P. taeda* (Markin 1970a). *Linepithema humile* reduce carbohydrate foraging from December through February (Mallis 2011).

The purpose of this study was to evaluate 1) over-wintering nest temperatures and 2) foraging activity of *L. humile* in Lake Greenwood State Park (LGSP) (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) in relation to winter temperatures. Monitoring ambient and nest temperatures and *L. humile* foraging activity was performed to determine a suitable date for early season bait placement It was hypothesized that there was no significant difference between ambient temperature and Argentine ant nest temperature, and that there was no significant difference of foraging activity from ambient temperature.

Materials and Methods

Nest selection and foraging activity

Nine *L. humile* L. nests were identified near loblolly pine, *Pinus taeda* or shortleaf pine*, Pinus echinata* Mill.*,* at a natural area in LGSP. These two pine tree species are some of the most common tree species at LGSP in natural areas. These *Pinus spp.* provides not only access to food resources such as honeydew but also nest locations for *L. humile*. The pine trees were also in close proximity to water areas, where *L. humile* could forage for moisture. Because bark on the south side of pine trees was exposed to sun, temperatures on this south side remained above the threshold during daylight, *Linepithema humile* in this natural area could forage during winter. Plastic vials (7 dram) were placed in the selected experimental area and a cotton ball soaked with 25% sugar water was placed into each vial. After *L. humile* had located the sugar vials and actively foraged, trails were visually followed from vial to nest entrance. Once nine *L. humile* nests had been visually identified, sugar-baited vials were placed near the nest. *Linepithema humile* foraging activity was recorded once a week from January through March. All ants counted in the foraging trail were summed weekly and then averaged to provide a mean number of ants for each week.

Station design

To record ambient and internal nest temperatures, HOBO H8 Temp/ RH/2x external channel data loggers (Onset Computer Corp. Bourne, Massachusetts) were used. Thermocouples (TMC6-HA Onset Computer Corp. Bourne, Massachusetts) were attached to the data logger. One of two external thermocouples was placed 25 cm into an underground nest and the other thermocouple was placed 10 cm above the station in the open air. The thermocouples recorded temperature between -40°C and 100°C.

To protect the data loggers from extreme weather, wild animals, and tamper-resistant waterproof stations were used (Figure 1). The main housing of a station was a 100 mm diameter polyvinyl chloride (PVC) pipe cap (Charlotte Pipe and Foundry, Charlotte, NC), with a 100 mm drainage grate placed to fit inside the pipe cap. Velcro tape (Velcro USA Inc., Manchester, NH) was used to attach data logger to the drainage grate. A 2.5 cm bolt on the top center of the station was installed to connect with a wooden stake that allowed for placement into the ground. Stations were taped with camouflage tape (Realtree® Hardwoods HD Duct Tape, Henkel consumer Adhesives, Dusseldorf, Germany) to make the stations less obvious. To connect the HOBO data loggers with the thermocouples, two holes were drilled through the PVC cap. The station was attached to the wooden stake and placed near each *L. humile* nest. HOBO data loggers were placed onto sun-warmed, natural areas during daylight where nine *L. humile* nests were located around *Pinus spp.* areas at LGSP. Each HOBO data logger recorded ambient and nest temperature every two hour for three months.

Raw data of temperature and foraging activity was used to characterize the winter habitat of *L. humile*. Data was used to determine the relationship between temperature and foraging activity. A graph was generated by SGPLOT procedure of SAS program. The data was also used to determine a significant difference between ambient and nest temperature. Differences between ambient and nest temperatures at each location were analyzed by using a mixed model for significant differences accounting for location, date and location by data interaction as random effects. This test was conducted for each week of the study. (Proc Mixed, SAS Institute 2011).

Results

Overall, the mean nest temperatures were less variable than mean ambient temperatures. From January to March, the range from lowest to highest temperature was 12.4°C in the nests and 21.7°C in the ambient environment. During this period, the lowest mean temperature recorded in the ambient environment was 8.27°C and 10.01°C in the nests. The highest mean temperature was 30.0°C in the ambient environment and 22.5°C in the nests.

The mean foraging number of *L. humile* gradually increased from January to March, except for 9 February, when the minimum ambient temperature dropped below 10°C (Figure 3.11). After 9 February, nest temperatures generally increased in a similar pattern compared to ambient temperatures though there were differences.

At the start of the study, there was a significant difference between mean ambient temperature and mean nest temperature from 18 January to 24 January (P= 0.048). The ambient temperature was lower than nest temperature (t= -2.47). The mean ambient and nest temperature were 8.27±2.99°C and 10.01±1.47°C, respectively (Figure 3.2). The mean foraging ant number was 9.4 (Figure 3.11).

Mean ambient and nest temperature gradually increased during week 2 and 3. There was no significant difference between the weekly mean ambient temperature and the nest temperature during week 2 (P= 0.397). The mean ambient temperature was lower than the mean nest temperature (t= -0.91). The mean ambient and nest temperatures were 10.74±2.56 and 11.02±1.2, respectively (Figure 3.3). The mean foraging ant number during this time was 10.8 (Figure 3.11).

There was high foraging activity in early February. When the mean ambient temperature was 12.14±3.61°C during week 3 from 1 February to 7 February, the mean nest temperature was 12.5±1.49 °C (Figure 3.4). The mean foraging number of *L. humile* was 26.3 (Fig. 3.11). However, there was no significant difference in mean weekly temperature between the ambient environment and the nests (P=0.444). The ambient temperature was lower than the nest temperature (t=-0.82).

There was a quick drop in foraging activity from 8 February to 14 February (week 4). There was a significantly difference in mean weekly temperature between ambient and nest (P=0.005). The ambient temperature was lower than nest temperature (t= -4.39). The mean foraging ant number also decreased from approximately from 26 to 7 (Figure 3.11). The mean weekly ambient and nest temperature was 6.08±2.29 °C and 9.27±0.95°C, respectively (Figure 3.5). The lowest ambient temperature during this study was 2.31±1.37°C on 12 February. The mean foraging ant number was 7.2 (Figure 3.11).

After 12 February the mean ambient and nest temperature gradually increased. The mean foraging ant number also gradually increased. There was no significant difference in mean temperature between ambient and nest during week 5 from 15 February to 21 February (P=0.091). The ambient temperature was lower than nest temperature (-2.01). The mean ambient and nest temperature was 9.81±2.24 and 10.46±1.3, respectively (Figure 3.6). The mean foraging ant number was 15.7 (Figure 3.11).

During week 6 from 22 February to 29 February the mean temperature was not significantly different between the ambient environment and the nests (P= 0.71), though

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the ambient temperature average was higher than the nest temperature average (t=0.38). The mean weekly ambient and nest temperature were 12.85±4.29 and 12.19±1.98, respectively (Figure 3.7). The mean foraging ant number was 20 (Figure 3.11). After 22 February the mean nest temperature continued to be over 13°C through 20 March.

There was no significant difference in mean weekly temperature between the ambient environment and the nests during week 7, from 1 March to 7 March (P= 0.5). The ambient temperature was lower than nest temperature (t=0.71). The mean weekly ambient and nest temperature were 14.49±5.15 and 13.47±1.98, respectively (Figure 3.8). The mean foraging ant number was 20.2 (Figure 3.11).

There was no significant difference in mean weekly temperature between ambient and nest during week 8, from 8 March to 15 March (P=0.815). The ambient temperature was lower than nest temperature (t= -0.24). The mean weekly ambient and nest temperature were 14.57±2.37 and 14.54±1.65 (Figure 3.9). The mean foraging ant number was 28.2 (Figure 3.11).

There was a significant difference in mean weekly temperature between the ambient environment and the nests during week 9, from 15 March to 21 March (P=0.007). The mean weekly ambient and nest temperatures were 21.82±1.01 and 18.52±1.05 respectively (Figure 3.10). The ambient temperature was higher than nest temperature (t= 4.09). The mean foraging ant number was 26.1 (Figure 3.11).

Even though the ambient and nest temperature fluctuated, the mean foraging activity of *L. humile* increased from 12 February to 29 February. After 16 March, both the mean ambient temperatures and the nest temperatures continued to be over 15°C. This

result suggested the optimal bait placement date for this study was after 16 March due to temperature (ambient and nest) and foraging activity (Figure 3.11).

Discussion

A season of foraging activity is correlated with the mean seasonal temperature (Markin 1970c). *Linepithema humile* is active in a wider range of abiotic conditions than most native ant species (Human et al. 1998). During warmer months, *L. humile* foragers become more active and increase the number of nesting sites over a greater area (Mallis 2011). A broad environmental tolerance allows *L. humile* to overcome local abiotic conditions and successfully survive (Human et al. 1998). According to Markin (1970a), *L. humile* are active up to 30°C. When the temperature was over 30°C, the foraging activity of *L. humile* decreased. The physical structure of the nest allows them to manage heat effectively (Brian 1973). The ambient temperature of a *P. taeda* trunk was higher on the sunny side, as compared to the shaded side. When these ants find their nest, abiotic factors are important in selecting nest sites. Abiotic factors such as temperature and humidity inside the nest could be modulated by the physical structure of nests (Brian 1973). Soil temperature and humidity are known to be important abiotic factors for successful nest establishment (Menke and Holway 2006; Hartley et al. 2010). A stable thermal condition can reduce the mortality of *L. humile*. Furthermore, an abiotic factor like water may affect the distribution of *L. humile*. Argentine ant workers may be more vulnerable to desiccation because of their small size. They prefer to select nest sites based on closeness to water (Human et al. 1998).

Mean temperature and foraging activity were monitored to determine the optimal time for bait placement. Even though temperature was below 5°C, *L. humile* were be found in the state park natural areas. Although the lowest mean ambient temperature (0.88°C) and mean nest temperature (7.5°C) was on 12 February, the mean *L. humile* foraging activity was not collected on this date. After 12 February, both mean nest and ambient temperature gradually increased. When the mean ambient temperature was about 7°C on 9 February, the foraging mean worker of *L. humile* numbered 7.2. Brightwell et al. (2011) mention that when the ambient temperature exceeds 12°C, *L. humile* may forage more effectively along the length of the branches. Because the ambient temperature was over 12°C from 1:00 to 3:00pm during the monitored day, *L. humile* could forage for food sources year round at LGSP. While temperature data were collected and analyzed, minimum ambient temperature was just under 0°C for a few days in 2012. When the minimum ambient temperature was around 0°C on 12 February, it was early morning. While the ambient and nest temperature fluctuated from 12 February to 11 March, foraging activity gradually increased. However, if foraging activity was recorded every day, the temperature data would be different as compared to current temperature data. The foraging activity was affected by the ambient temperature. In other studies when surface temperatures are over 32°C or below 15°C, *L. humile* stop foraging (Hedges 1998; Hartley and Lester 2003). *Linepithema humile* workers cannot develop completely below 15°C (Hartley and Lester 2003). The egg hatching rate is less than 2% below 18°C (Abril et al. 2008). As ambient temperature is below 10°C, *L. humile* would be limited for foraging (Markin 1970a). When ambient temperature is below 5°C, *L. humile* would cease foraging and starve (Brightwell et al. 2010).

This research suggested that the suitable date for 2012 bait placement was mid-March. When the ambient temperature continued to be over 15°C, *L. humile* began to actively forage. Markin (1970b) mentioned that the temperature range between 15 and 30°C was highly active foraging. The mean ambient temperature of week 7 and 8 was around 15°C. The mean ambient temperature of week 8 was almost equal to the mean nest temperature. It is assumed that workers could forage actively in the field. These data showed that after 12 March 2012 the nest temperature was continually over 15°C, and that *L. humile* workers could potentially develop completely. There was a significant difference in the mean weekly temperature between ambient and nest during week 9 from 15 March to 21 March 2012. The mean ambient temperature (21.82°C) are higher than the mean nest temperatures (18.52) (t= 4.07). After 16 March the nest temperature continued to be over 18°C, and *L. humile* workers could potentially develop completely and produce eggs. The data suggested that *L. humile* could forage actively and was ready to increase their population. The data also suggest that the middle of March in 2012 was a suitable time to set up bait to prevent population growth. Pest management professionals could monitor known nest areas for *L. humile* activity during the winter. As temperatures warm, typically in February and March for this study location, the ant number could be assessed. When *L. humile* activity increases to (25) ants passing a point in 30 seconds, this is an indication that the active foraging season is starting and it is a good time to bait. Liquid, sugar-based baits would be a good choice because they kill colonies rather than only foraging workers. Sprays and barrier insecticides kill only foraging workers rather than workers, larvae, and queens in a nest. Major food sources for *L. humile* include carbohydrate the rich sources such as honey dew (Rust et al; 2000). About 99% of food

of *L. humile* brought to nest from citrus tree was in liquid form (Markin 1970b).

Therefore, the use of liquid, sugar-based baits is a good choice for effective control of *L. humile* in natural areas.

References

Abril, S., Oliveras J., Gómez, C. 2008*. Effect of temperature on the oviposition rate of Argentine ant queens *(Linepithema humile* Mayr*) under monogynous and polygynous experimental conditions. *J. Insect Physiol.* 54*: 265–272.

Abril, S., Oliveras, J., Gómez, C. 2010. Effect of temperautre on the development and survival of the Argentine ant, *Linepithema humile*. J. Insect. Sci. 10: 97.

Barber, E. R. 1916. The Argentine ant: distribution and control in the United States. U.S. Dept. Agric. Bull. 377: 1-23.

Brian, M. V. 1973. Temperature choice and its relevance to brood survival and caste determination in the ant *Myrmica rubra*. Physiol. Zool. 46: 254-262.

Brightwell R. J. and J. Siverman. 2007. Argentine ant foraging activity and interspecific competition in complete vs. queenless and broodless colonies. Insectes Sociaux. 54: 329-333.

Brightwell, R. J., P. L. Labadie and J. Silverman. 2010. Northward expansion of the invasive Argentine ant, *Linepithema humile*, (Hymenoptera: Formicidae) in the eastern U.S. is constrained by winter soil temperatures. Environ. Entomol. 39: 1659-1665.

Brightwell, R. J. and J. Silverman. 2011. The Argentine ant persists through unfavorable winters via a mutualism facilitated by a native tree. Environ. Entomol. 40: 1019-1026.

Ellis, B. R. 2009. Managing *Linepithema humile* (Mayr), the Argentine ant, in South Carolina state park campgrounds. M.S. Thesis, Clemson University. 93 pp.

Enriquez, M. L., S. Abril, M. Diaz, and C. Gomez. 2013. Nest site selection by the Argentine ant and suitability of artificial nests as a control tool. Insectes Sociaux., volume 60, Issue 4, pp 507-516.

Hartley, S., and P. J. Lester. 2003. Temperature-dependent development of the Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae): a degree-day model with implications for range limits in New Zealand. N. Z. Entomol. 26: 91-100.

Hartley, S., Krushelnycky, P. D., and Lester, P. J. 2010. Integrating physiology, population dynamics and climate to make multi-scale predictions for the spread of an invasive insect: the Argentine ant at Haleakala National Park, Hawaii. Ecography 33: 83–94.

Hedges. S. A. 1998. Field guide for the management of structure-invading ants. Franzak and Foster, Cleveland, OH.

Human, K. G., S. Weiss, A. Weiss, B. Sandler, and D. M. Gordon. 1998. Effects of Abiotic factors on the distribution and activity of the invasive Argentine ant (Hymenoptera: Formicidae). Population Ecology.

Mallis, A. 2011. 10th Edition. Handbook of Pest Control [ed.] Stoy Hedges. Franzak and Foster Company. Cleveland, OH.

Markin, G.P. 1970a. Food distribution within laboratory colonies of the Argentine ant, Iridomyrmex humilis (Mayr). Insectes Soc. 17: 127-158.

Markin, G. P. 1970b. Foraging behavior of the Argentine ant in a California citrus grove. J. Econ. Entomol. 63: 740-744.

Markin, G. P. 1970c. The seasonal life cycle of the Argentine ant, *Iridomyrmex humilis* (Hymenoptera: Formicidae), in southern California. Ann. Entomol. Soc. Am. 63: 1238-1242.

Menke, S. B., and D. A. Holway. 2006. Abiotic factors control invasion by argentine ants at the community scale. J. Animal. Ecology. Volume 75, Issue 2, pp 368-376.

Roura-Pascual, N., A.V. Suarez, K. McNyset, C. Gomez, P pons, Y. Touyama, A. L. Wild, F. Gascon, and A. T. Peterson. 2006. Niche differentiation and fine-scale projections for argentine ants based on remotely sensed data. Ecological Application 16:1832-1841 Sudd, J.H. 1969.

Rust, M. K., D. A. Reierson, E. Paine, and L. J. Blum. 2000. Seasonal activity and bait preferences of the Argentine ant (Hymenoptera: Formicidae). J. Agric. Urban Entomol. 17:201–212.

SAS Institute Inc. 2011. *SAS/STAT® 9.3 User's Guide*. Cary, NC: SAS Institute, Inc.

Sudd, J. H. 1969. The excavation of soil by ants. Z. Tierpsychol., 26: 257-276.

Tremper, B. S. 1976. Distribution of the Argentine ant, *Iridomyrmex humilis* Mayr, in relation to certain native ants of California, ecological, physiological, and behavioral aspect. Ph.D. dissertation, University of California, Berkeley.

Vega, S. J. & M. K. Rust 2001. The Argentine ant – a significant invasive species in agriculture, urban and natural environments. Sociobiology 37: 3-25.

Fig. 3.1. A HOBO data logger was placed into the waterproof station at Lake Greenwood State Park. Thermocouples were attached to data loggers and placed 25 cm into an underground.
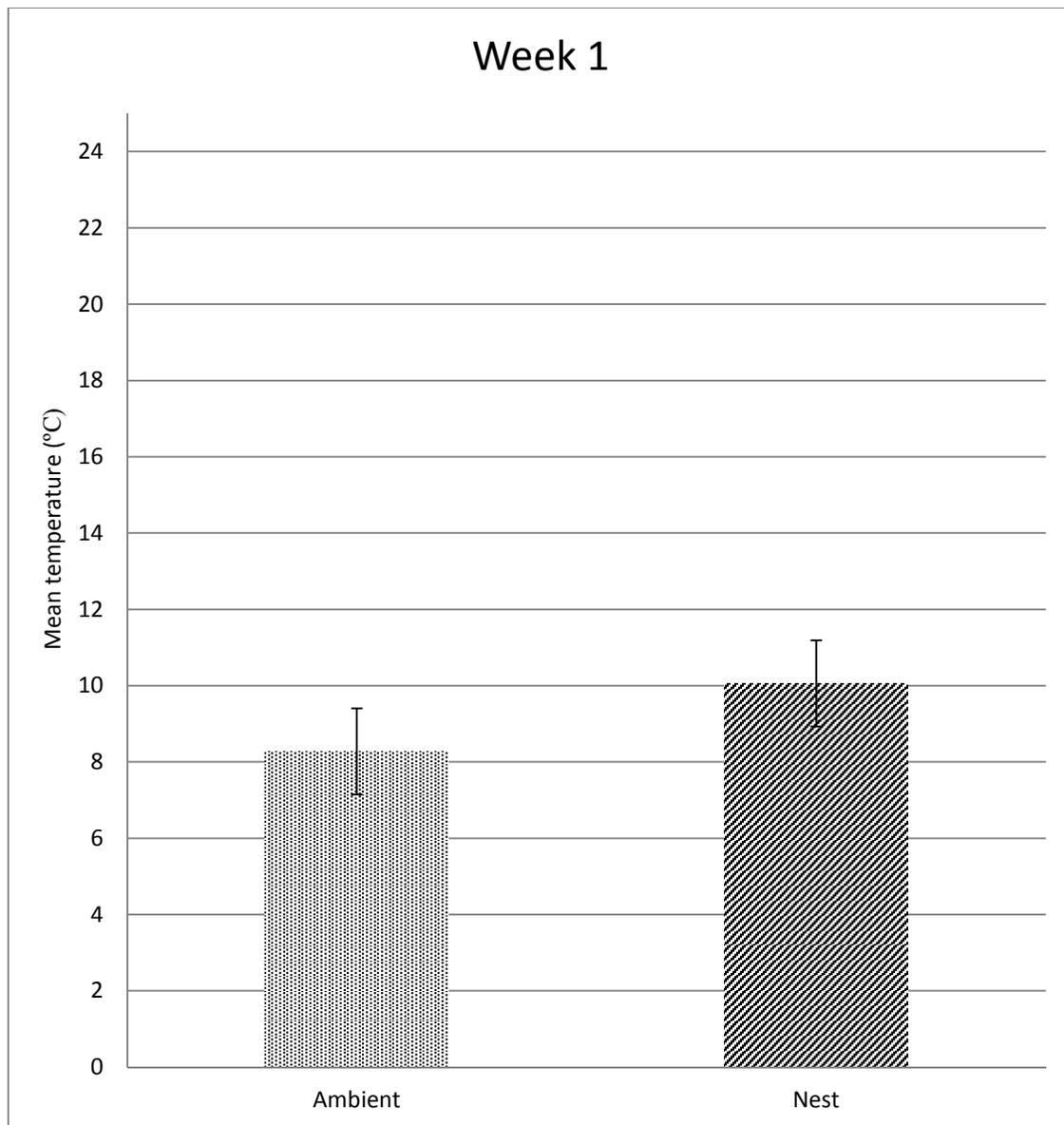
Fig. 3.2. Mean (± SE) weekly ambient and *Linepithema humile* (Mayr) nest temperature at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) for week 1 in 2012. There was a significant difference in the mean number of weekly temperature between ambient and nest at P=0.048. (Means were analyzed by T-test)
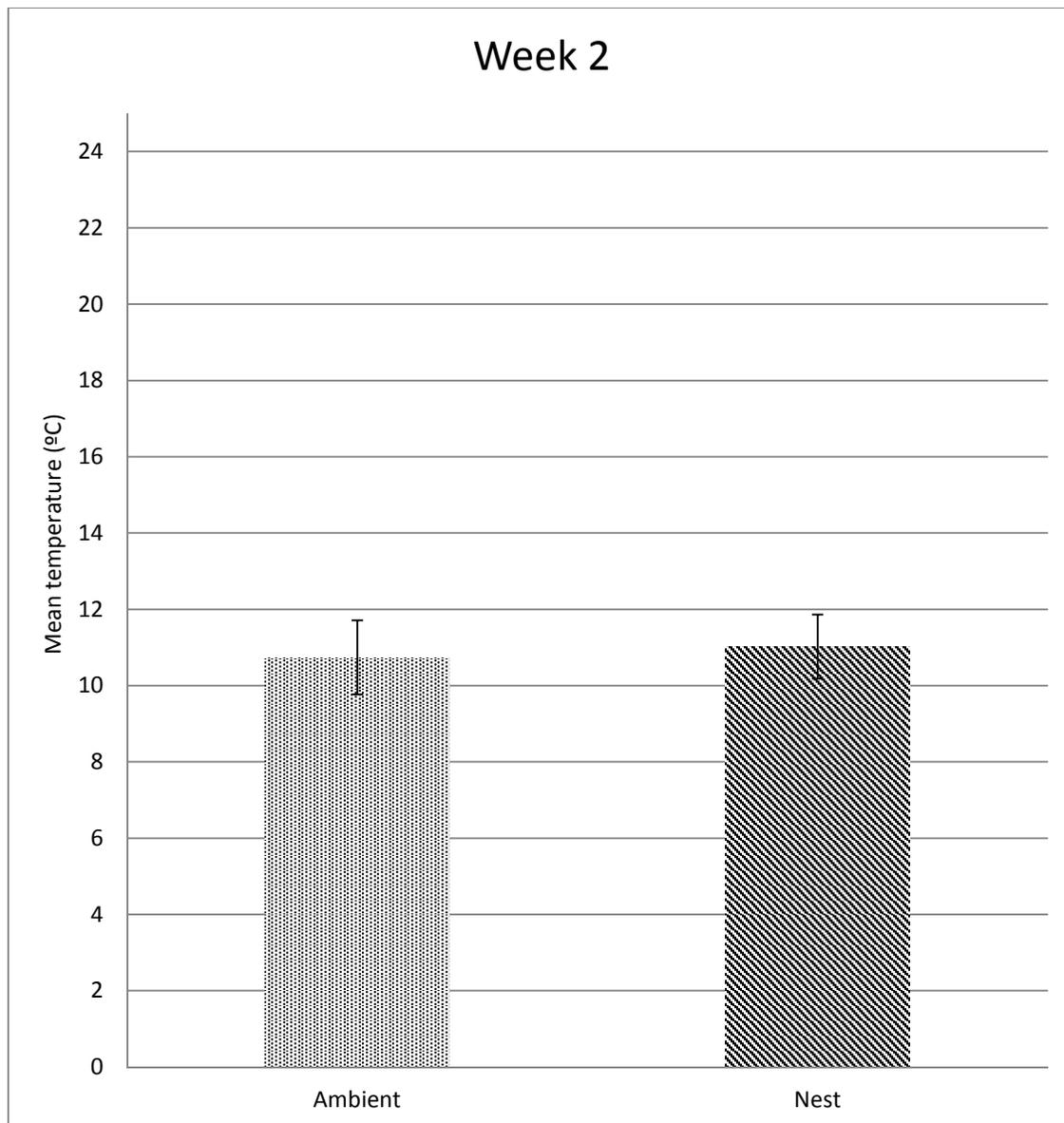
Fig. 3.3 Mean (± SE) weekly ambient and *Linepithema humile* (Mayr) nest temperature at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) for week 2 in 2012. There was not significant different mean number of weekly temperature between ambient and nest at P=0.397. (Means were analyzed by T-test)
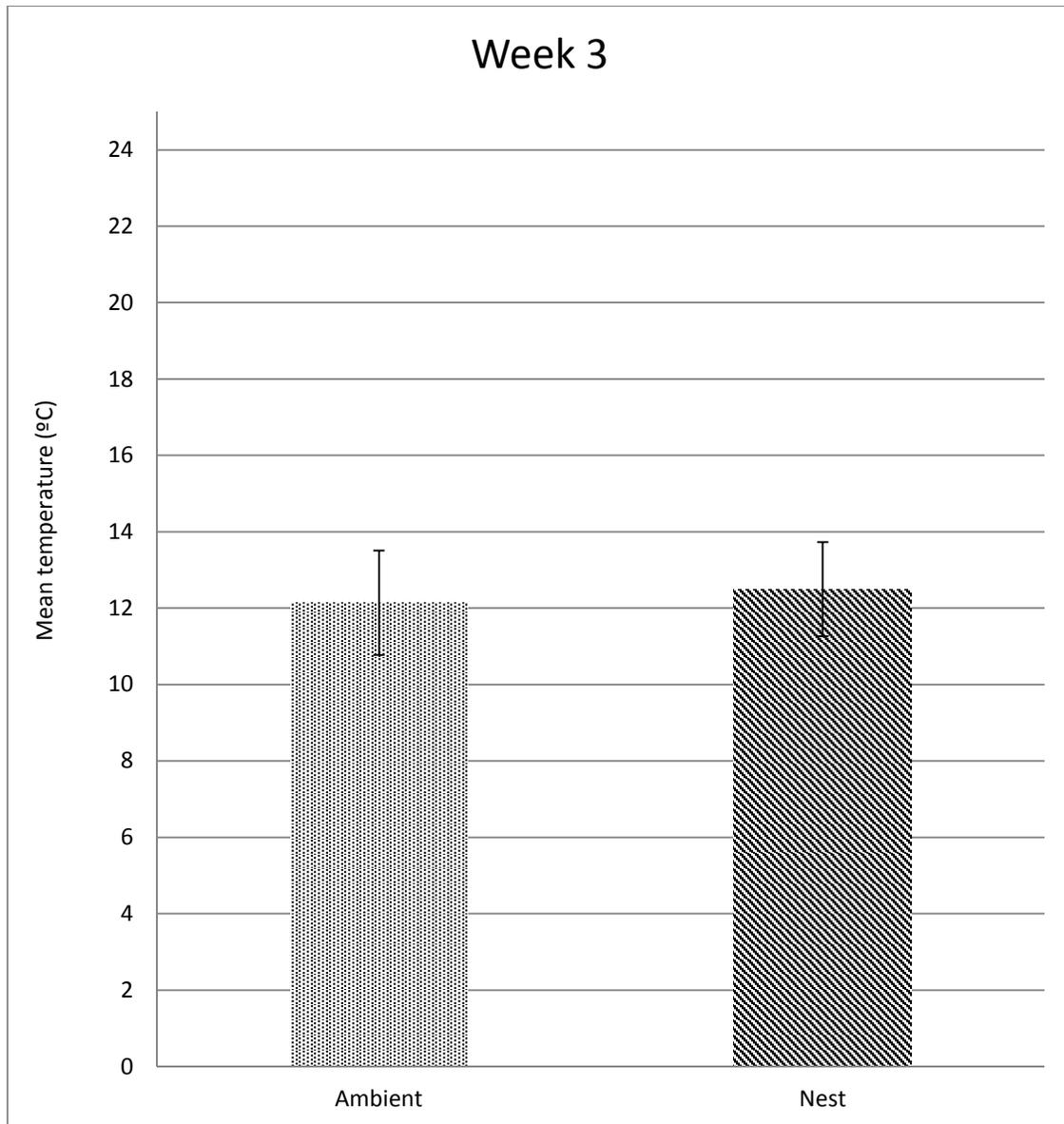
Fig. 3.4. Mean (± SE) weekly ambient and *Linepithema humile* (Mayr) nest temperature at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) for week 3 in 2012. There was no significant difference of mean weekly temperature between ambient and nest at P=0.444. (Means were analyzed by T-test)
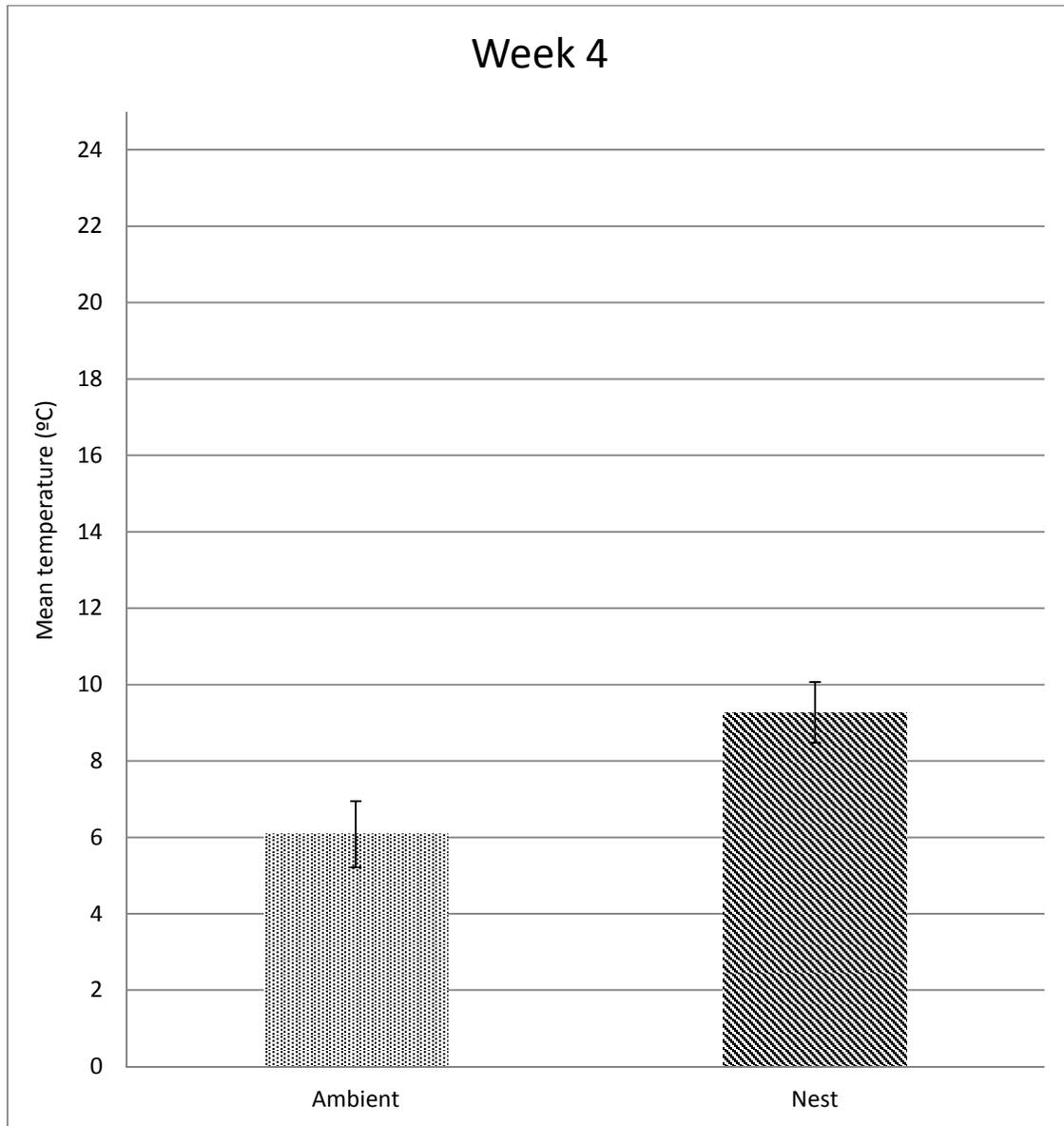
Fig. 3.5. Mean (± SE) weekly ambient and *Linepithema humile* (Mayr) nest temperature at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) for week 4. There was a significant difference of mean weekly temperature between ambient and nest at P=0.005. (Means were analyzed by T-test)
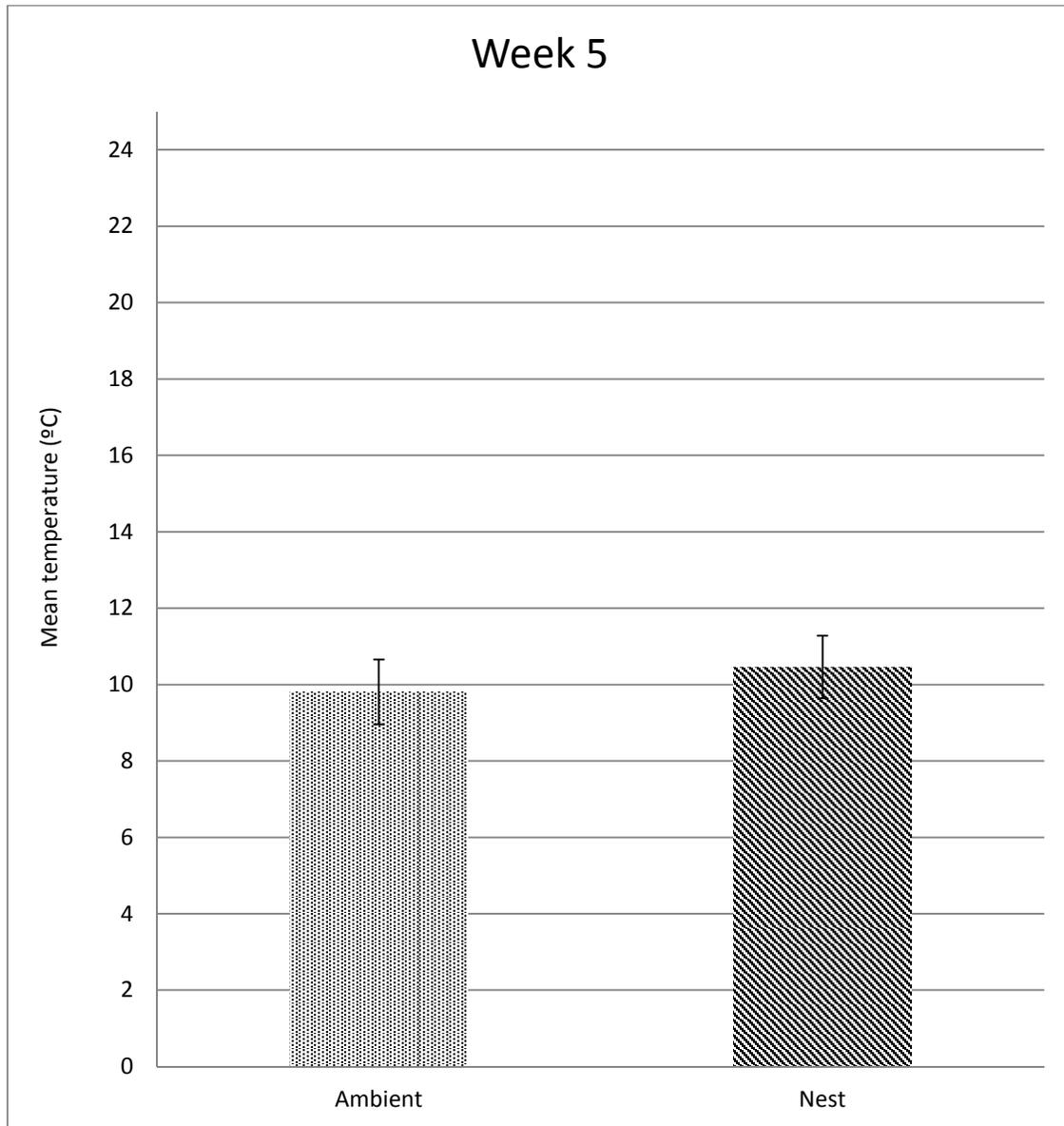
Fig. 3.6. Mean (± SE) weekly ambient and *Linepithema humile* (Mayr) nest temperature at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) week 5 in 2012. There was no significant difference of mean weekly temperature between ambient and nest at P=0.005. (Means were analyzed by T-test)
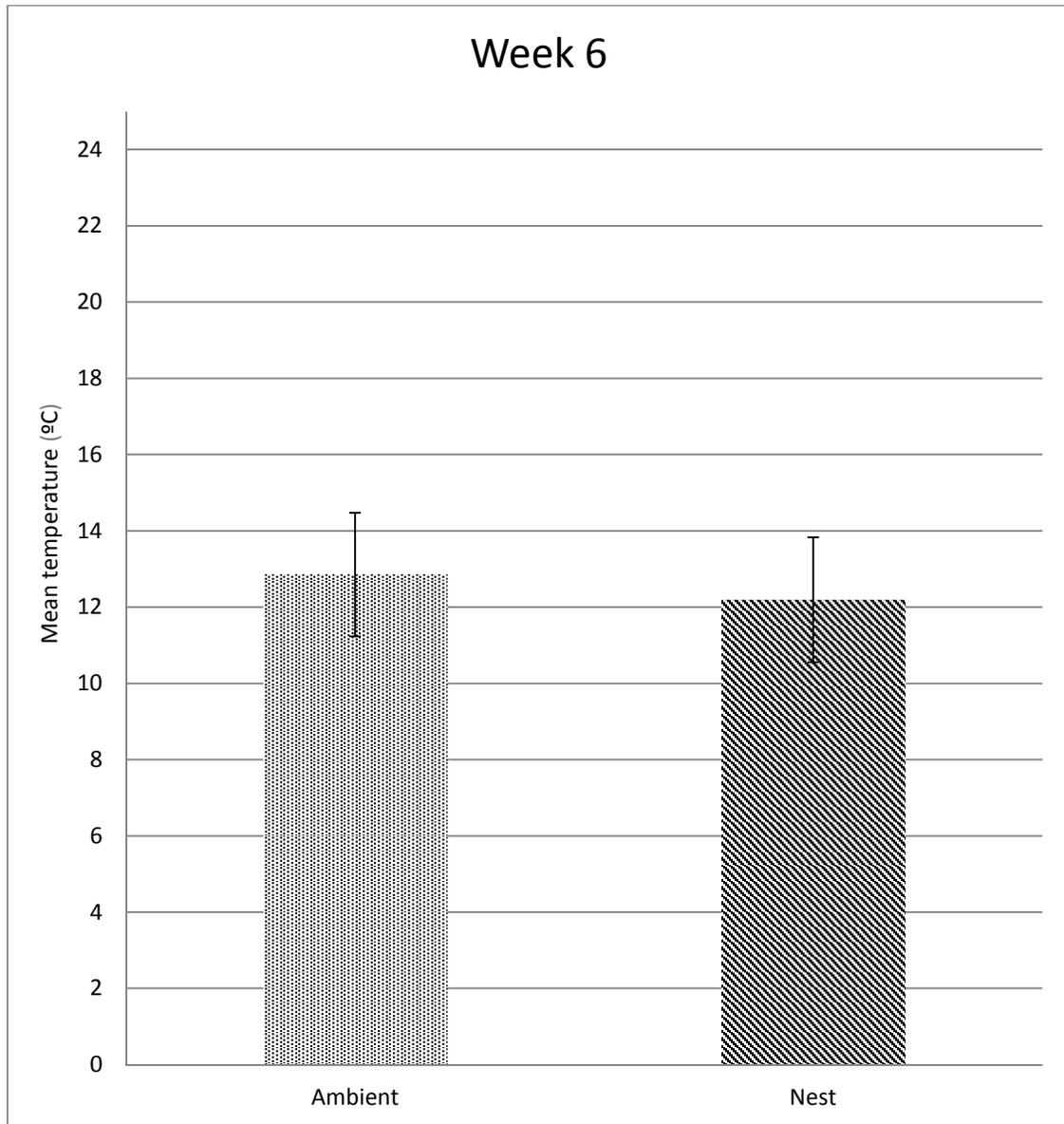
Fig. 3.7. Mean (± SE) weekly ambient and *Linepithema humile* (Mayr) nest temperature at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) for week 6 in 2012. There was no significant difference of mean weekly temperature between ambient and nest at P=0.71. (Means were analyzed by T-test)
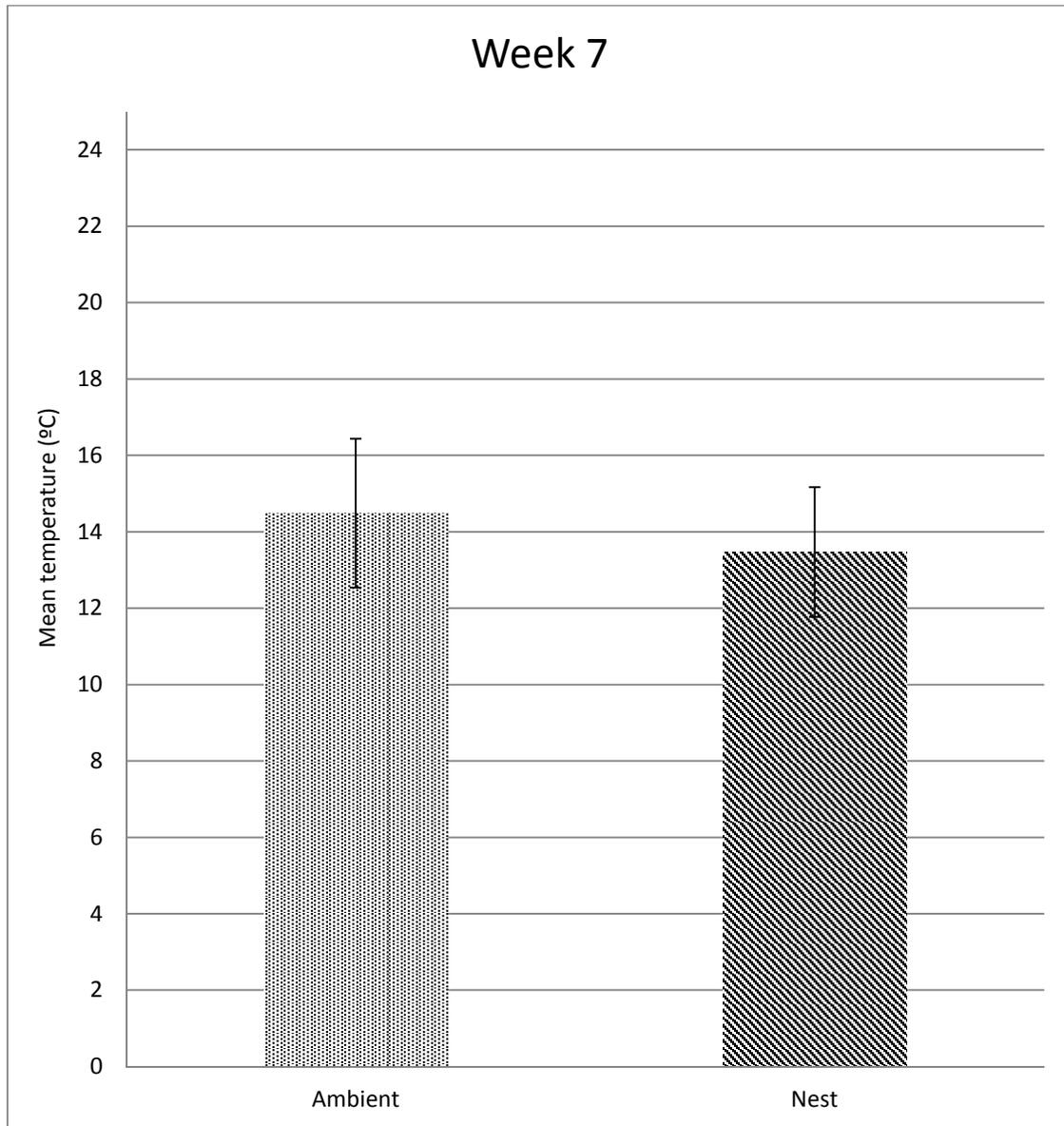
Fig. 3.8. Mean (± SE) weekly ambient and *Linepithema humile* (Mayr) nest temperature at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) for week 7 in 2012. There was no significant difference of mean weekly temperature between ambient and nest at P=0.5. (Means were analyzed by T-test)
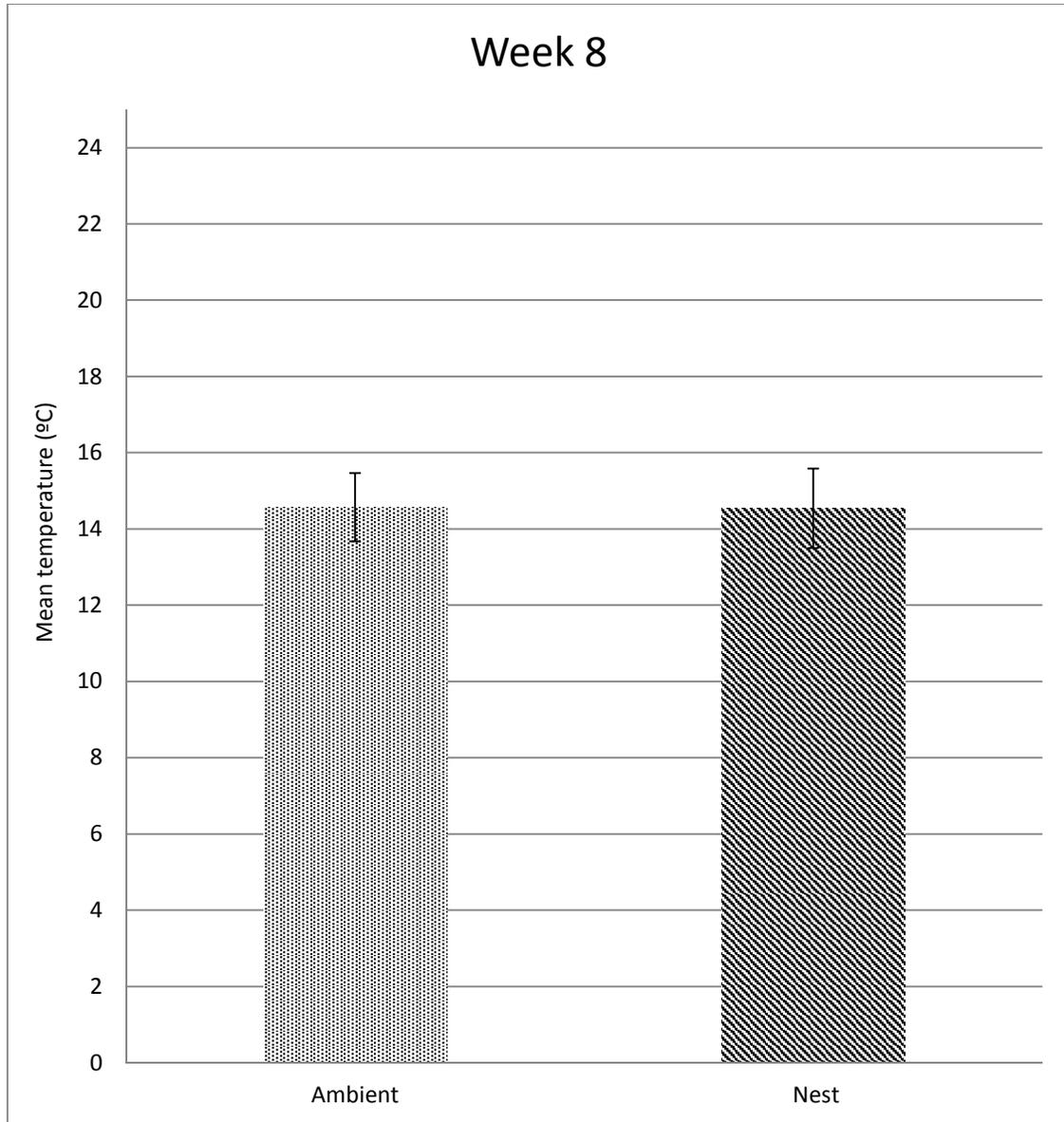
Fig. 3.9. Mean (± SE) weekly ambient and *Linepithema humile* (Mayr) nest temperature at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) for week 8 in 2012. There was no significant difference of mean weekly temperature between ambient and nest at P=0.815. (Means were analyzed by T-test)
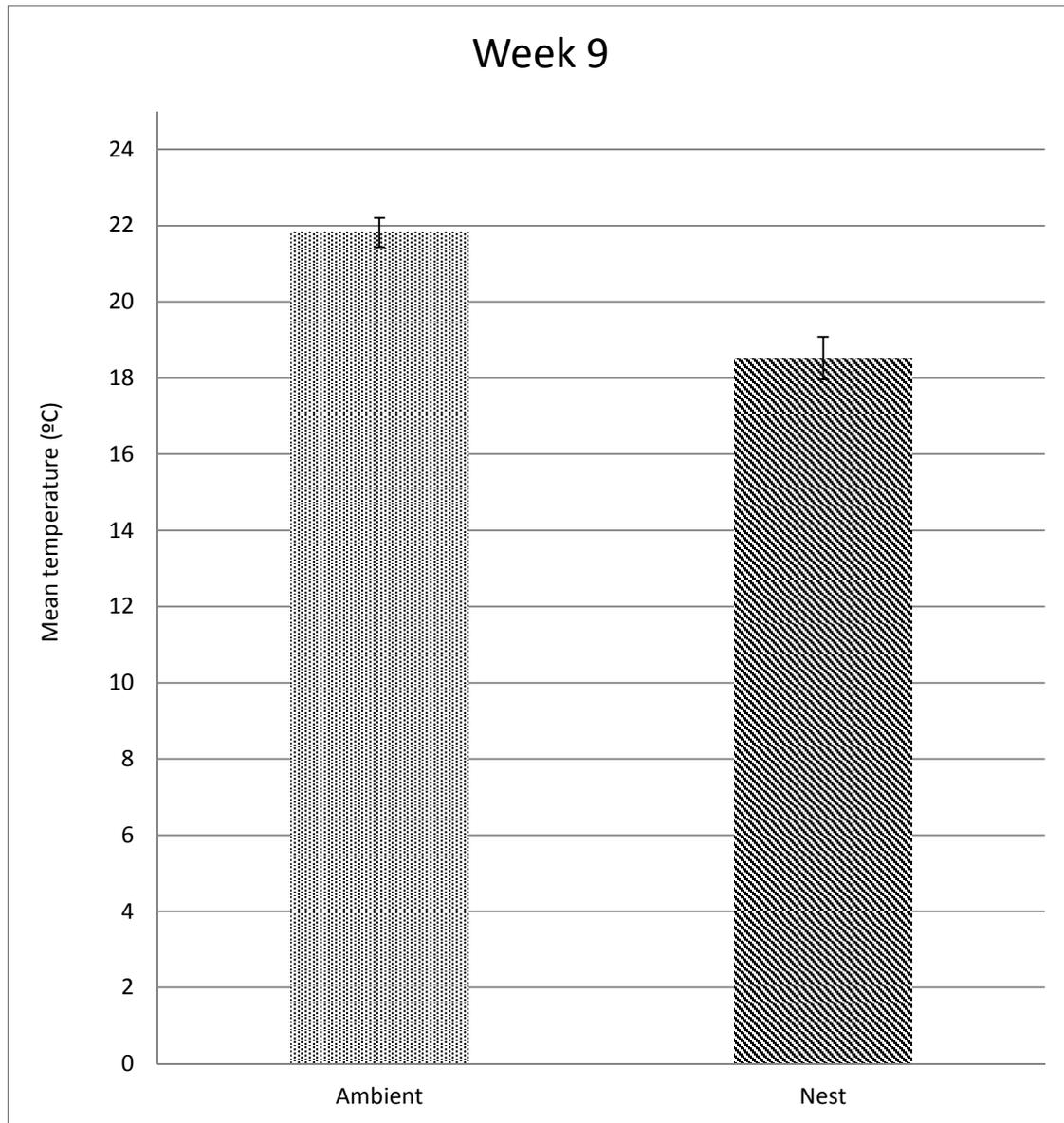
Fig. 3.10. Mean (± SE) weekly ambient and *Linepithema humile* (Mayr) nest temperature at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) for week 9 in 2012. There was no significant difference of mean weekly temperature between ambient and nest at P=0.007. (Means were analyzed by T-test)
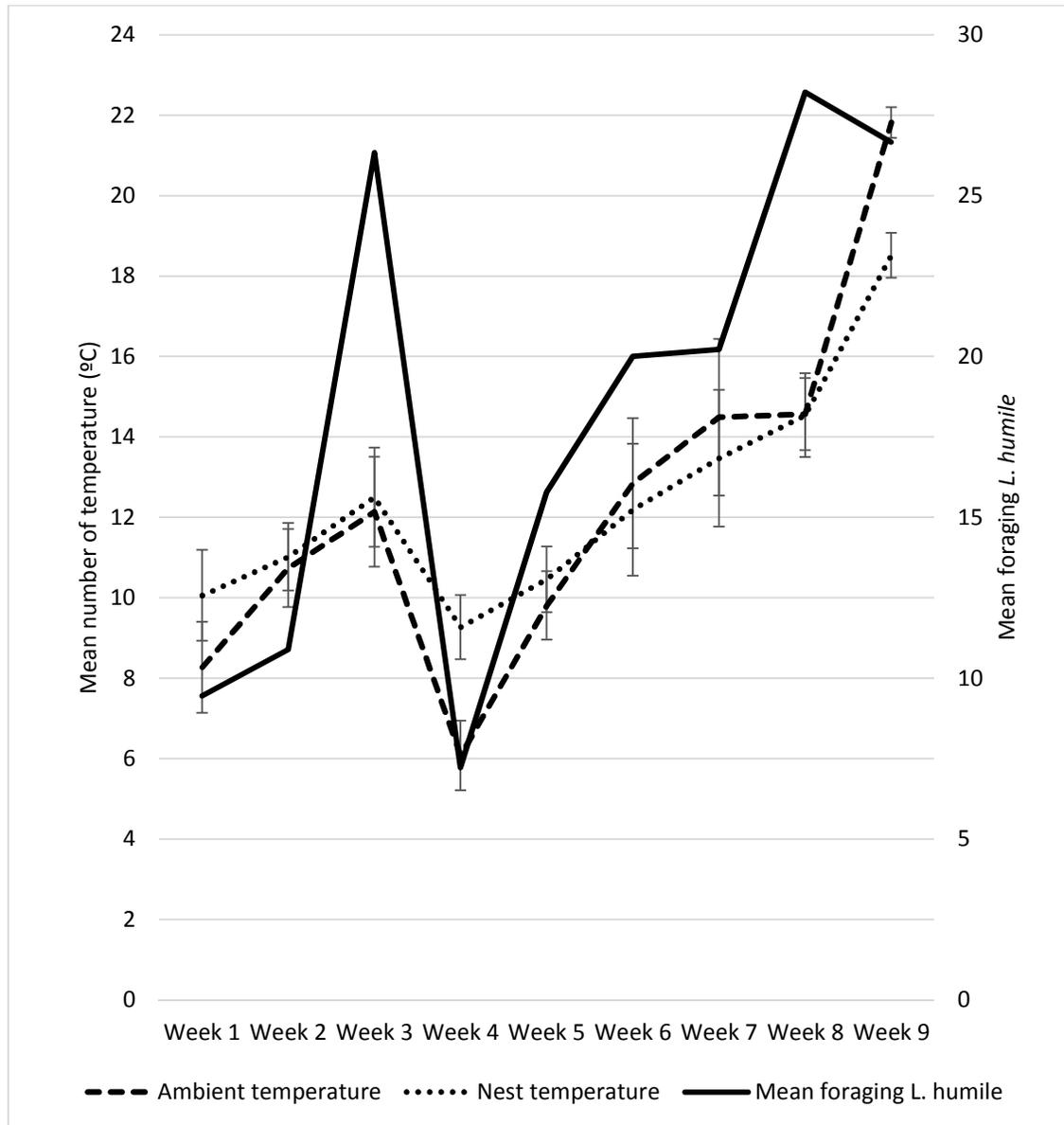
Fig. 3.11. Mean (± SE) weekly ambient temperature and foraging activity of *Linepithema humile* (Mayr) workers at Lake Greenwood State Park during 3 month period (From January 2012 to March 2012). A solid, square dot, and dash dot line represents mean ambient temperature, mean nest temperature. The *L. humile* mean numbers is related to the ants passing a point in 30 seconds.

CHAPTER FOUR

Evaluation of Early Season Bait Strategies for *Linepithema humile* Control in South

Carolina State Park Natural Areas

Introduction

Linepithema humile (Mayr), the Argentine ant, is an invasive, foraging ant

species. Argentine ants are native to South America. However, *L. humile* has established

on six continents and many oceanic islands (Suarez et al. 1999). In the United States, *L.*

humile is wide spread in Arizona, Missouri, Indiana, Maryland, Oregon, and Washington.

However, *L. humile* is known to be well established throughout the Southeast, California,

and Hawaii in United States (Mallis 2011). *Linepithema humile* is gradually dispersing

throughout the southern and western states (Buczkowski et al. 2004; Holway 2000;

Barber 1916). *Linepithema humile* was first recorded in New Orleans, in the United

States, by Edward Foster in 1891 (Barber 1916). While coffee ships discharged their

cargo, *L. humile* may have been introduced to the United States (Newell & Barber 1913).

Linepithema humile invading natural habitats can reduce native ant diversity, and

additionally change native species diversity (Heller et al. 2006). They are unicolonial, by

which they have weak territorial boundaries between colonies and multiple

interconnected nests (Suarez et al. 1999 and Giraud et al. 2002). They make multiple

nests with multiple queens (Brightwell and Silverman 2007). They are an aggressive

foraging ant and replace other ant species in areas where they are established (Heller et

al. 2006). They are likely to make a nest in areas of suitable moisture, and close to food,

and often is hidden except for entrances (Mallis 2011, Vega and Rust 2001). Argentine

ants continue to be a pest in South Carolina State Parks of the Piedmont region, invading the campsites of recreational campers (Ellis 2009). According to Ellis (2009), in a 2007 and 2008 survey about the *L. humile* problem at Lake Greenwood State Park (LGSP), many campers complained about Argentine ant problems. Campers used their own chemical products to control *L. humile* with little to no success for control, but with the potential for environmental contamination by the pesticides in the park. The severe *L. humile* problems were one of the reasons that some campers reported they would not return to the park (Ellis 2009).

There are many types of insecticides for controlling *L. humile* including spray and bait formulations. Sprays are usually barrier insecticides that suppress ant foragers only or keep ants from entering a structure. These formulations mostly kill foraging workers rather than larvae and queens in a nest, thus, ant populations can recover quickly. Broad spectrum spray insecticides also can kill beneficial insects (Nelson and Daane 2007). Sprays must often be reapplied at least once per month or more often, due to a short residual time (Nelson and Daane 2007). In 2007, Ellis performed test sprays, combined with granular ant bait insecticides. Insecticidal sprays alone compared to insecticidal sprays combined with granular ant baits were evaluated for control. In these studies, granular bait combined with sprays did not significantly reduce ant numbers when compared to untreated control sites (Ellis 2009).

Linepithema humile prefer liquid-based foods. Major food sources for *L. humile* include carbohydrate-rich sources such as honey dew (Rust et al, 2000). About 99% of food *L. humile* brought to nests from citrus trees was in liquid form (Markin 1970).

Therefore, the development of liquid carbohydrate bait is often the most effective means for control of *L. humile* (Rust et al. 2000). Gel baits also can be effective compared to granular baits, but overall, liquid baits are the best formulation for controlling *L. humile* (Silverman and Roulston 2001).

In previous research of the optimal bait station placement distance at LGSP natural areas, it was determined that the optimal distance of bait station placement was 10 m apart but 20 m bait placement were also viable and more effective in reducing cost and labor to implement. Based on this information, the objective of this study was to evaluate early-season, liquid baiting to control *L. humile* populations at LGSP natural areas in Ninety Six, SC using liquid-based bait station placed at 20 m intervals. It was hypothesized that the mean number of *L. humile* at the un-treated control sites would be significantly different than the mean number of *L. humile* at a treatment site where bait with insecticide were used at LGSP.

Material and Methods

A study was conducted to evaluate bait station placements 20 m apart for the control of *L. humile* in a natural area (picnic area) of LGSP (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N). The KM AntPro liquid bait stations (KM AntPro LLC; P.O. Box 967, Nokomis, FL 34275) were used and placed from 21 March to 13 June 2012. Before treatment areas were assigned, ant numbers were evaluated once a week, from January to the middle of March. Ant numbers were counted on 30 trees where the individual number of *L. humile* crossed an arbitrary line (in both up and down directions) for 30 seconds (Moreno et al. 1987). If a tree had more than one trail, the ant trail with

the greatest number of individuals was counted and recorded. When the mean number of ants counted was approximately 50 on the foraging trails of the 30 trees, day-time temperatures were consistently reaching 15°C or higher. On 21 March, three monitoring areas (an area with no stations (natural control (NC)), a control area with stations containing only sugar water (bait control (BC)), and a treatment area with stations containing sugar water and an insecticide (bait treatment (BT)) were established. Each area was approximately 1,600 m^2 in size. The peripheries of all three areas were at least 72 m apart to avoid the interaction of *L. humile* foraging from one area to another area (Cooper et al. 2008). Nine bait stations were placed in three rows at 20 m apart, based on a previous test in the BC and BT areas. In the BC area, each station contained 200 ml of 25% sugar water. The BT area had the same station placement, but stations were filled with 180 ml of 25% sugar water mixed with 20 ml Maxforce Quantum Ant Bait (0.03% imidacloprid, Bayer Environmental Science, Kansas City, MO). Maxforce Quantum Ant bait was chosen for this study because the active ingredient, imidacloprid, had been successfully used to control *L. humile* in a grape vineyard (Daane et al. 2006).

Each area, NC, BC and BT, contained 10 of the original 30 trees used for monitoring ant activity. All ants counted on foraging trails in each area were summed weekly and then averaged to provide a mean number of ants. For reporting and statistical evaluation, ant numbers were combined to obtain the mean number of *L. humile* for each area from 21 March through 15 October 2012. Bait stations were available to foraging *L. humile* in the BC and BT areas from 21 March through 13 June 2012. Each week, remaining liquid bait in each station in the BC and BT areas was measured and replaced with fresh liquid bait. After 13 June, bait replacement ceased but the weekly monitoring

of ant activity continued. The mean number of *L. humile* in a foraging trail for each treatment continued to be recorded from April to June 2013. The numbers were averaged to obtain the mean number of *L. humile* for each treatment area. Data were analyzed at each sampling time by using analysis of variance. Mean separation was done using Fisher's LSD.

Results

Before chemical bait was placed in the BT area at LGSP, the mean number of *L. humile* was 65 in the NC area, 51.8 in the BC area, and 50.8 in the BT area (Figure 3.1.). The mean number of *L. humile* among the three treatments was not significantly different during March 2012 (F=0.86, P= 0.455).

The mean number of *L. humile* in the 10 foraging trails per treatment area during week 5 (April) indicated there was still no significant difference between the BC and BT areas (F= 2.67, P= 0.088). The mean number of *L. humile* was 98 in the NC area, 58.26 in the BC area, and 43.13 in the BT area (Figure 3. 2). Even though there was no significant difference in the mean number of *L. humile* in NC and BC areas, the mean number decreased in the BT area.

The mean number of *L. humile* in the 10 foraging trails per area throughout May indicated that NC area was significantly greater than BT area (F= 3.84, P= 0.034). The mean number of ants was 144.32 in the NC area, 123.22 in the BC area, and 53.26 BT area (Figure. 3.3).

The mean number of *L. humile* in the 10 foraging trails per area counted throughout June indicated that the BT area was significantly greater than the NC and BC areas (F= 12.22, P= 0.0002). The mean number of ants was 148.45.4 in the NC area, 147.7 in the BC area, and 42.68 in the BT area (Figure. 3.4). When the mean number of *L. humile* at BC area was significantly different than the NC and BC areas for May and June, bait replacement was stopped.

After the replacement of bait was stopped, the mean number of *L. humile* in the 10 foraging trails per area observed throughout July indicated there was no significant difference among treatments (F= 16.31, P= 0.0001). The mean number of ants was 153.38 in the NC, 146.2 in the BC, and 42.25 in the BT areas (Figure. 3.5). This mean number of *L. humile* was the highest of the entire season in the NC area.

The mean number of *L. humile* in the 10 foraging trails per treatment area throughout August indicated that NC and BC areas were significantly greater than BT area (F= 23.05, P= 0.0001). The mean number of ants was 140.35 in the NC, 125.5 in the BC, and 13.2 in the BT areas (Figure. 3.6). The mean number of *L. humile* decreased in all areas between July and August.

The mean number of *L. humile* in the 10 foraging trails per treatment area throughout September indicated that the BT area was significantly different than that of the NC and BC areas (F10.9, P=0.0003). The mean number of ants was 125.4 in the NC, 140.6 in the BC, and 12.6 in the BT areas (Figure. 3.7). The BT and NC areas once again had a decrease in the mean *L. humile* number for September, when compared to August. However, the mean number of *L. humile* in the BC area increased (Figure 3.9.).

The mean number of *L. humile* in the 10 foraging trails per area over October indicated that the BT area was significantly different than the NC and BC areas. The mean number of ants was 82.3 in the NC, 83.7 in the BC, and 18.3 in the BT areas (Figure. 3.8). This data indicated the mean ant number in NC and BC areas decreased, while the BT area increased, as compared to the mean numbers in September (Figure 3.9.).

The mean number of *L. humile* in the 10 foraging trails per area during week 54 (March 2013) indicated that there was no significant difference among the three treatment areas (F= 0.18, P= 0.8386). The mean number of *L. humile* was 11.4 in the NC, 10.5 in the BC, and 9.3 in the BT areas (Figure. 3.10).

The mean number of *L. humile* in the 10 foraging trails per area during week 55 without liquid bait treatment (March) in 2013 indicated that the BT area was significantly different than the NC areas (F= 4.2, P= 0.0257). The mean number of *L. humile* was 65.7 in the NC, 37.8 in the BC, and 19 in the BT areas (Figure. 3.11). This data indicated the mean ant number in the NC area increased as compared to the mean number in week 54 of April data.

The mean number of *L. humile* in the 10 foraging trails per area during week 59 (May 2013) indicated that there was a significant difference of the mean number of *L. humile* between the NC and the BT area (F= 11, P= 0.0003). The mean number of *L. humile* was 102.3 in the NC, 39.5 in the BC, and 27.4 in the BT areas (Figure. 3.12). This data showed that the mean number of *L. humile* in the NC area had a larger increase than other treatment areas, as compared to the April 2013 mean number.

The mean number of *L. humile* in 10 foraging trails per each area during week 65 (June 2013) indicated that the BT area was significantly different than the NC and BC areas (F= 5.59, P= 0.0093). The mean number of *L. humile* was 146.3 in the NC, 104.2 in the BC, and 61.5 in the BT areas (Figure. 3.13). This data indicated that the mean number of *L. humile* increased in all treatment areas when as compared to the mean numbers from May 2013.

Discussion

Early spring is a difficult time for *L. humile* to collect a naturally occurring sugar water source, so it is an ideal time to get ants to focus on foraging on sugar source bait. According to Nelson et al. (2007), bait deployment date is important because ant colonies develop more brood in the early spring. Thus, there is a better chance to transfer toxic bait to larvae and prevent such colony growth. For this study, the KM AntPro Insect Control System was used to reduce evaporation and microbial growth. Because liquid bait was replaced with fresh bait every week, mold problems in the bait were not encounter. If baits were replaced on a less frequent basis, 0.15% citrus acid mixed with baits would help prevent mold (Nelson and Daane 2007). The KM AntPro station also helped to prevent wildlife and park visitors from tampering with or consuming product (Silverman and Brightwell 2008). However, tampering from wildlife or park visitors still occurred due to curiosity. Additionally, Maxforce Quantum Ant bait was chosen for this study because of the active ingredient, imidacloprid. According to Daane et al. (2006), liquid bait containing imidacloprid had been successfully used to control *L. humile* in a grape vineyard. Imidacloprid is a slow acting insecticide (Daane et al. 2006) and needs

time to affect nests of *L. humile*. After foraging, workers bring bait sources to the nests and share the food with brood, queens, and other workers, the bait would then accumulate in the brood, queen and workers' body. Furthermore bait is a lower toxicity and safer insecticide than a spray insecticide (Nelson and Daane 2007).

In this study, the liquid bait study was performed to evaluate a specific distance of bait station placement at LGSP in 2012 to control *L. humile* (Figure. 3.13). With follow-up evaluations without baiting in the spring of 2013 the intent was to observe the effect of the 2012 one year later. It was concluded that an early season liquid baiting strategy, with a specific placement distance of 20 m for the bait stations was an effective control method to decrease *L. humile* (Mayr) at LGSP in 2012 and into 2013. To determine the best time to set up bait stations, the mean number and nest temperature of *L. humile* had been previously monitored during January and February 2012. Preliminary data indicated that when the temperature continued to be more than 15°C, *L. humile* began to actively forage.

At the start of field evaluations on 21 March 2012, the mean numbers of foraging *L. humile* among the three areas were not significantly different. After baits were placed in the BC and BT areas, the number of *L. humile* were summed and averaged by month. In April, the mean ant counts in the NC area were significantly greater compared to the BC and BT areas (Fig. 6). By May, the mean ant numbers in the BT area were significantly lower than the NC and BC areas and remained this way for the duration of the field study. In June the mean ant counts peaked in the BC area. In July the mean ant counts peaked in the NC area. During June and July, the mean ant counts in the NC and

BC areas were over 3 times greater than the mean ant counts in the BT area. By the end of the study on 15 October, the mean ant counts in the NC and BC areas were over 4 times greater than the mean ant counts in the BT area. While there was some fluctuation, mean ant counts in the NC and BC areas tended to decline from August to October. The reason for this decreasing trend in mean ant counts was not clear. However, lower *L. humile* activity has been reported in late summer or early fall in other studies (Daane et al. 2006).

Counting *L. humile* trail numbers was performed again from April to June 2013. This data collection was performed one year post-treatment to determine if the BT area still had lower mean foraging activity than the NC and BC areas. In the first week of April the mean ant counts in the BT area was not significantly different from the NC and BC areas. Because the mean temperature was 12°C, this might have affected the foraging activity. However, temperatures increased the following week, when the mean temperature was 22°C. The mean number of ants in the NC area was significantly greater than those at BC and BT areas in the month of April. During May and June, the mean ant count in the NC and BC area was significantly greater than the BT area.

In conclusion, I was able to determine optimum placement of sugar-water bait stations containing imidacloprid. Stations were placed at 20 m intervals for foraging ants starting in March and this treatment was effective in reducing *L. humile* numbers between April and October in a natural area of LGSP. For this study, only one insecticide product was examined. In the future, a study that investigates other active ingredients for ant baits

such as thiamethoxam and boric acid (Cooper et al. 2008) versus imidacloprid would be

beneficial.

References

Barber, E. R. 1916. The Argentine ant: Distribution and control in the United States. U.S.D.A. Bulletin 377: 1-23.

Brightwell R. J. and J. Siverman. 2007. Argentine ant foraging activity and interspecific competition in complete vs. queenless and broodless colonies. Insectes Sociaux. 54: 329-333.

Buczkowski G., E. Vargo and J. Silverman. 2004. The diminutive supercolony: The Argentine ants of the southeastern U.S. Mol. Ecol. 13: 2235-2242.

Cooper M., Daane K. M., Nelson, E. H., Varela, L. G., Battany, M. C., Tsutsui N. D. and Rust M. K. 2008. Liquid baits control Argentine ants sustainably in coastal vineyards. California Agriculture. October – December. 177-183.

Daane, K. M., K. R. Sime, B. N. Hogg, M. L. Bianchi, M. L. Cooper, M. K. Rust, and J. H. Klotz. 2006. Effects of liquid insecticide baits on Argentine ants in California's costal vineyards. Crop. Prot. 25: 592-603.

Ellis, B. R. 2009. Managing *Linepithema humile* (Mayr), the Argentine ant, in South Carolina state park campgrounds. M.S. Thesis, Clemson University. 93 pp.

Giraud T, Pedersen J. S., and Keller J. 2002. Evolution of supercolonies: the Argentine ants of southern Europe. Proceedings of the National Academy of Sciences 99, 6075–6079.

Heller N., Gordon D. 2006. Seasonal spatial dynamics and causes of nest movement in colonies of the invasive Argentine ant *(Linepithema humile)* Ecol. Entomol. 31: 499-510.

Holway, D. A. and T. J. Case 2000. Mechanisms of dispersed central-place foraging in polydomous colonies of the argentine ant. Anim. Behav. 59 (2), 433-441.

Mallis, A. 2011.10[th] Edition. Handbook of Pest Control [ed.] Stoy Hedges. Franzak and Foster Company. Cleveland, OH.

Markin, G. P. 1970. Food distribution within laboratory colonies of the Argentine ant, *Iridomyrmex humilis* (Mayr). Ins. Soc. 17: 127-158.

Moreno, D. S., P. B. Haney, and R. F. Luck. 1987. Chlorpyrifos and diazinon as barriers to Argentine ant (Hymenoptera: Formicidae) foraging on citrus trees. J. Econ. Entomol. 80: 208-214.

Nelson, Erik H. and Daane, Kent M. 2007. Improving liquid bait programs for Argentine ant control: Bait station density. Environ. Entomol. 36 (6): 1475-1484.

Newell, W. and T. C. Barber. 1913. The Argentine ant. U.S.D.A. Bur. Entomol. Bull.

Rust, M. K., D. A. Rejerson, E. Pain, and L. J. Blum. 2000. Seasonal activity and bait preferences of the Argentine ant (Hymonoptera: Formicidae). J. Agric. Urban Entomol. 17: 12.

Silverman J. and T. H. Roulston. 2001. Acceptance and intake of gel and liquid sucrose compositions by the Argentine ant (Hymenoptera: Formicidae). J. Econ. Entomol. 94: 511- 515.

Silverman J. and R. J. Brightwell. 2008. The Argentine ant: Challenges in managing an invasive unicolonial pest. Annu. Rev. Entomol. 53: 231- 252.

SAS Institute Inc. 2011. *SAS/STAT® 9.3 User's Guide*. Cary, NC: SAS Institute, Inc.

Suarez A. V., Tsutsui N. D., and Holway D. A. 1999. Behavioral and genetic differentiation between native and introduced populations of the Argentine ant. Biol. Invasions. 1, 43– 53.

Vega, S. J. and M. K. Rust. 2001. The Argentine ant- a significant invasive species in agricultural, urban, and natural environments. Sociobiology 37: 3- 25.
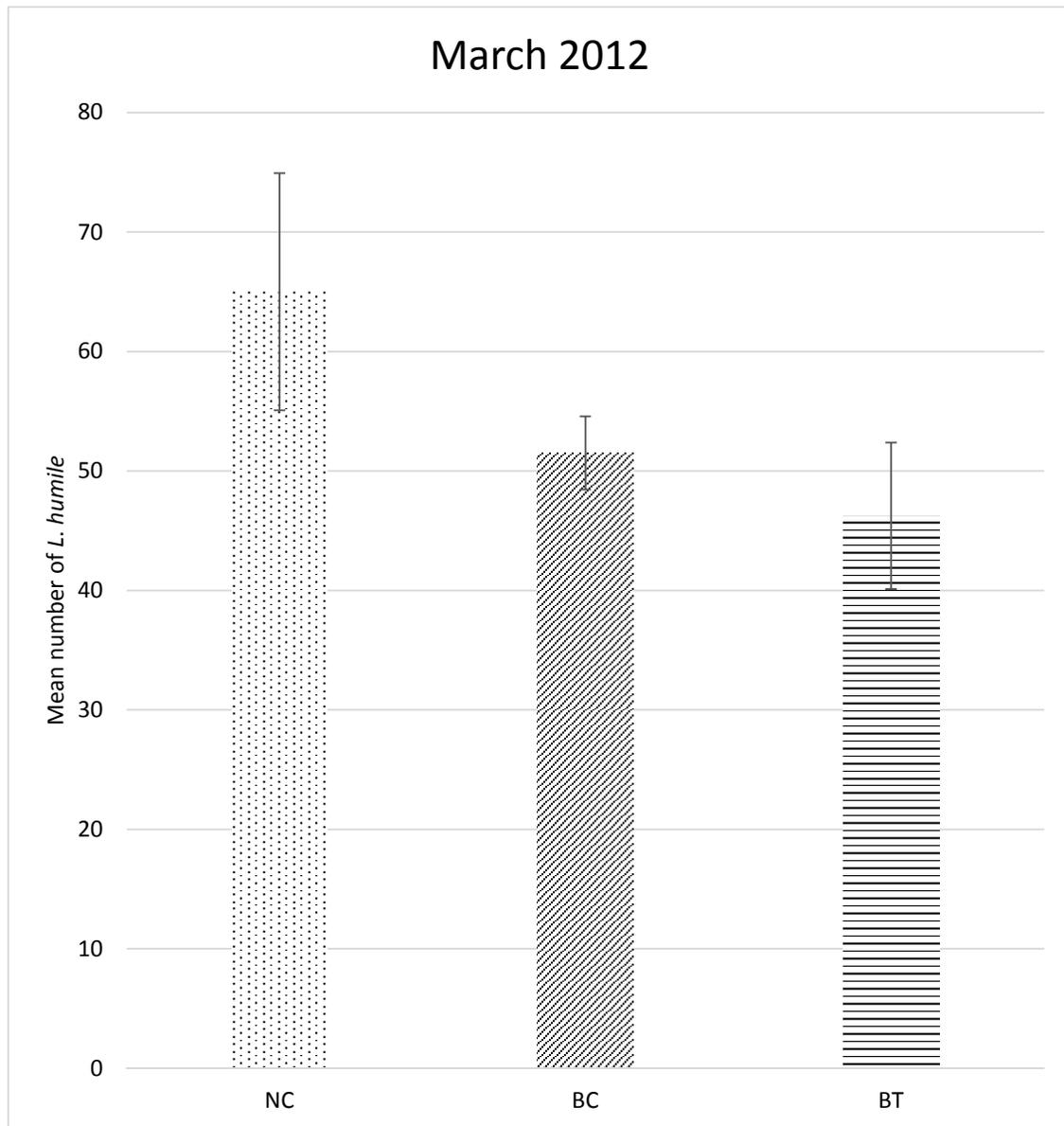
Fig. 4.1. The mean (± SE) number of *Linepithema humile* (Mayr) present in a foraging trail at Lake Greenwood State Parks (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) during week 1 of treatment (March in 2012). Three monitoring areas were established with an area with no stations (natural control (NC)), a control area with stations containing only 300 ml sugar water (bait control (BC)), and a treatment area with stations containing 300 ml sugar water and 0.03% imidacloprid insecticide (bait treatment (BT)) were established. Data were analyzed at each sampling time by using Analysis of variance. Mean separation was done using Fisher's LSD.
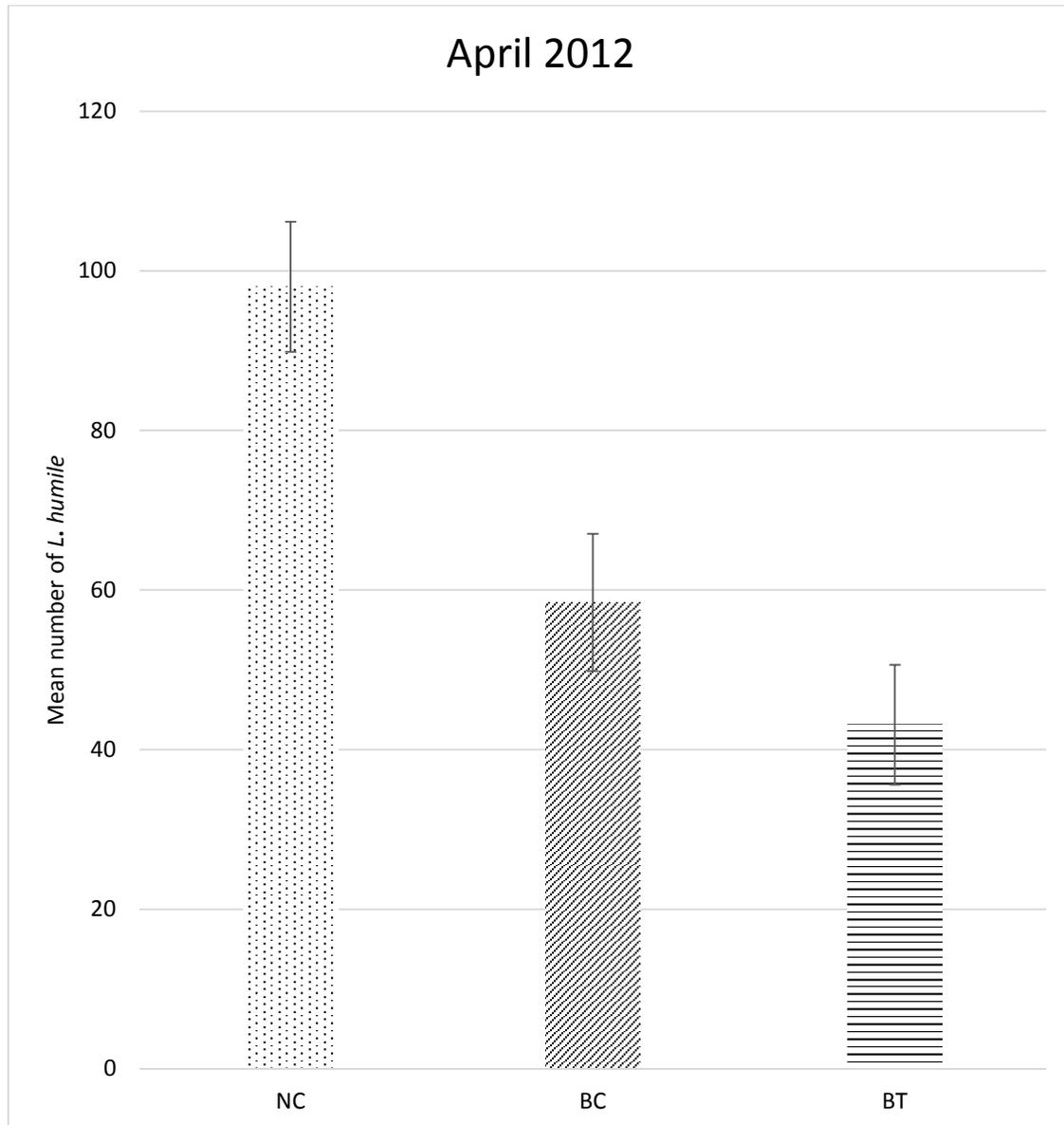
Fig 4.2. The mean (± SE) number of *Linepithema humile* (Mayr) present in a foraging
trail at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34°
11'58. 7904N) during week 5 of treatment (April in 2012). Three monitoring
areas were established with an area with no stations (natural control (NC)), a
control area with stations containing only 300 ml sugar water (bait control (BC)),
and a treatment area with stations containing 300 ml sugar water and 0.03%
imidacloprid insecticide (bait treatment (BT)) were established. Data were
analyzed at each sampling time by using Analysis of variance. Mean separation
was done using Fisher's LSD.
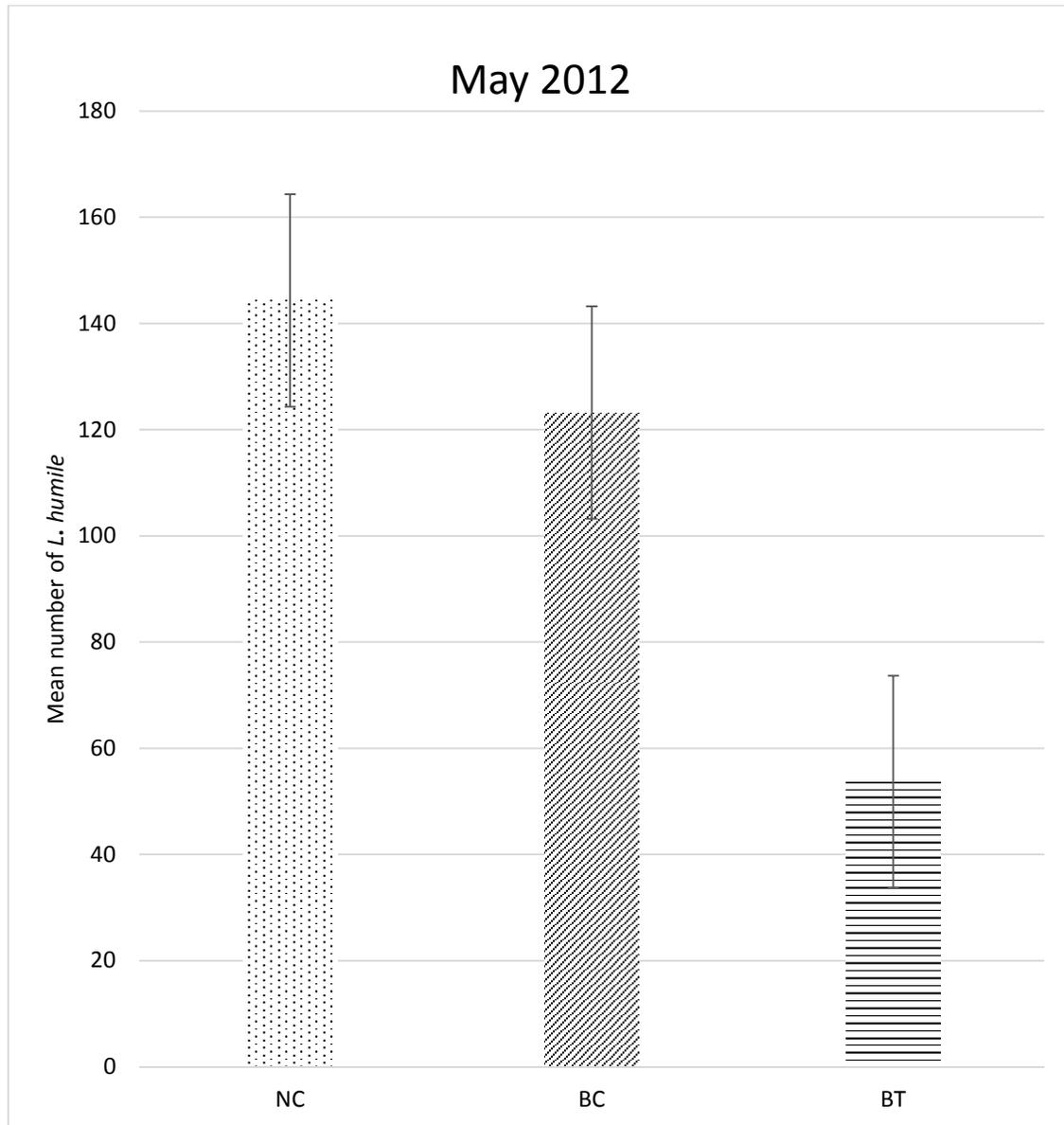
Fig 4.3. The mean (± SE) number of *Linepithema humile* (Mayr) present in a foraging trail at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) during week 7 of treatment (May in 2012). Three monitoring areas were established with an area with no stations (natural control (NC)), a control area with stations containing only 300 ml sugar water (bait control (BC)), and a treatment area with stations containing 300 ml sugar water and 0.03% imidacloprid insecticide (bait treatment (BT)) were established. Data were analyzed at each sampling time by using Analysis of variance. Mean separation was done using Fisher's LSD.
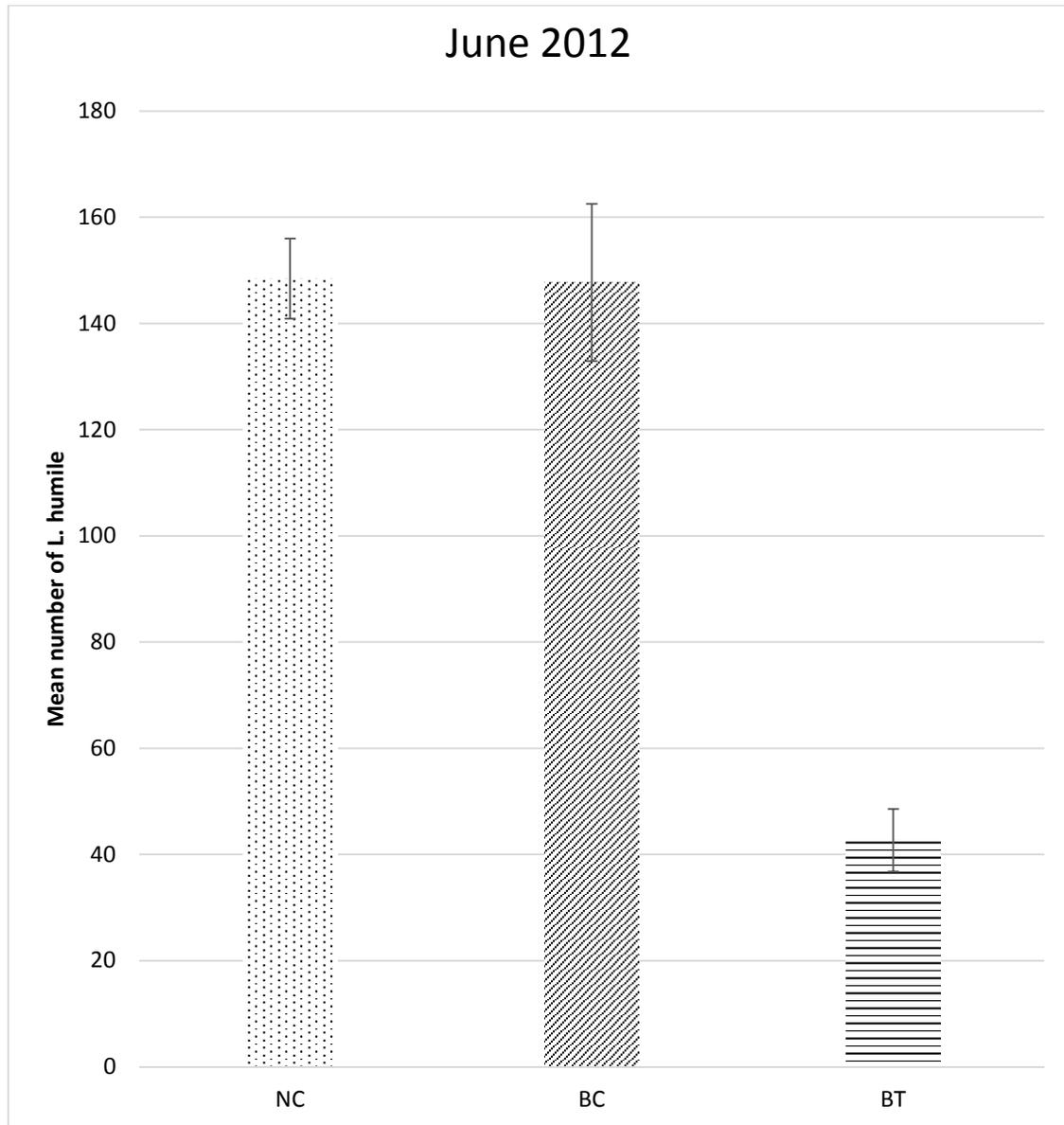
Fig 4.4. The mean (± SE) number of *Linepithema humile* (Mayr) present in a foraging trail at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) during week 12 of treatment (June in 2012). Three monitoring areas were established with an area with no stations (natural control (NC)), a control area with stations containing only 300 ml sugar water (bait control (BC)), and a treatment area with stations containing 300 ml sugar water and 0.03% imidacloprid insecticide (bait treatment (BT)) were established. Data were analyzed at each sampling time by using Analysis of variance. Mean separation was done using Fisher's LSD.
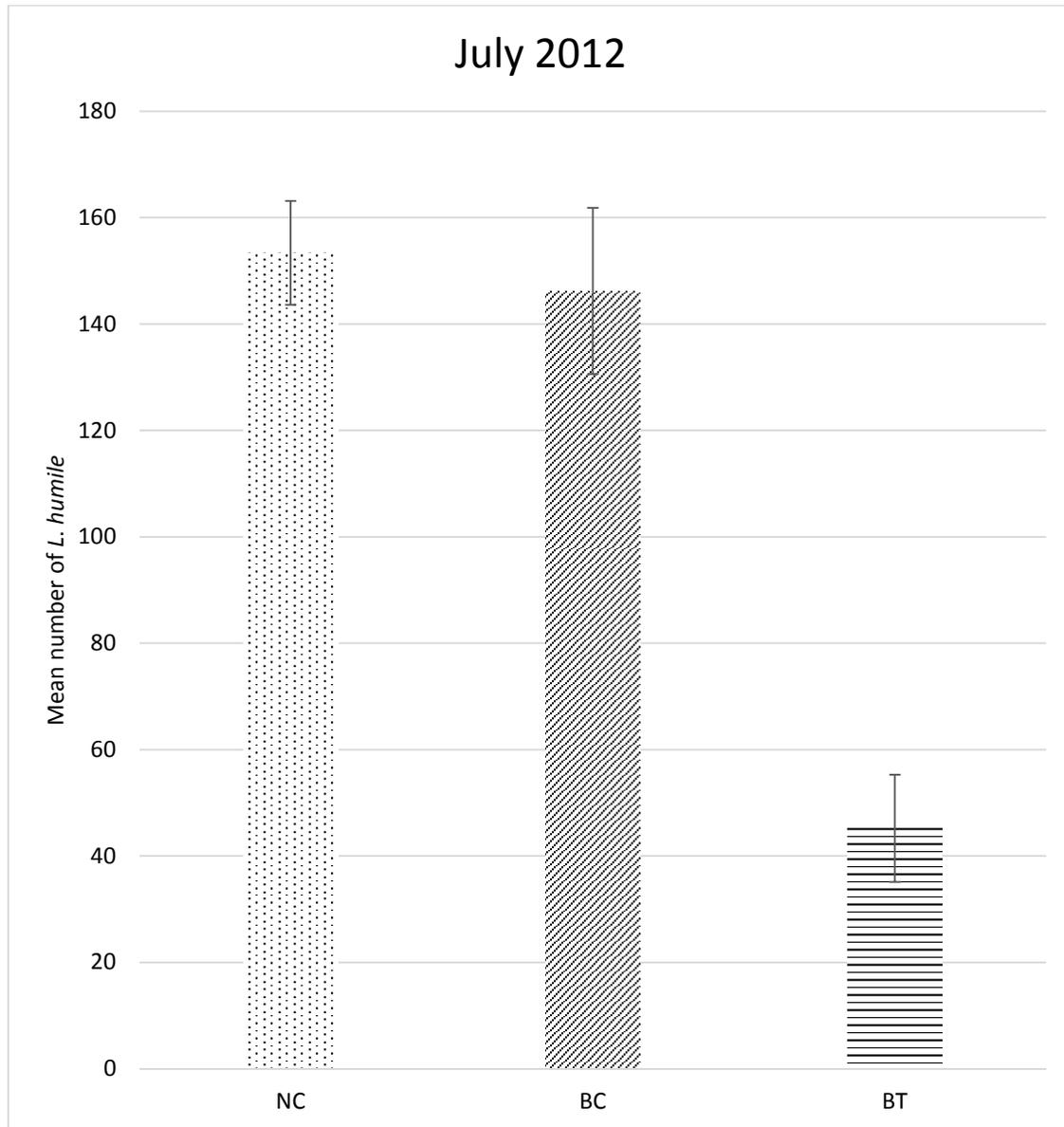
Fig 4.5. The mean (± SE) number of *Linepithema humile* (Mayr) present in a foraging trail at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) during week 17 of treatment (July in 2012). Three monitoring areas were established with an area with no stations (natural control (NC)), a control area with stations containing only 300 ml sugar water (bait control (BC)), and a treatment area with stations containing 300 ml sugar water and 0.03% imidacloprid insecticide (bait treatment (BT)) were established. Data were analyzed at each sampling time by using Analysis of variance. Mean separation was done using Fisher's LSD.
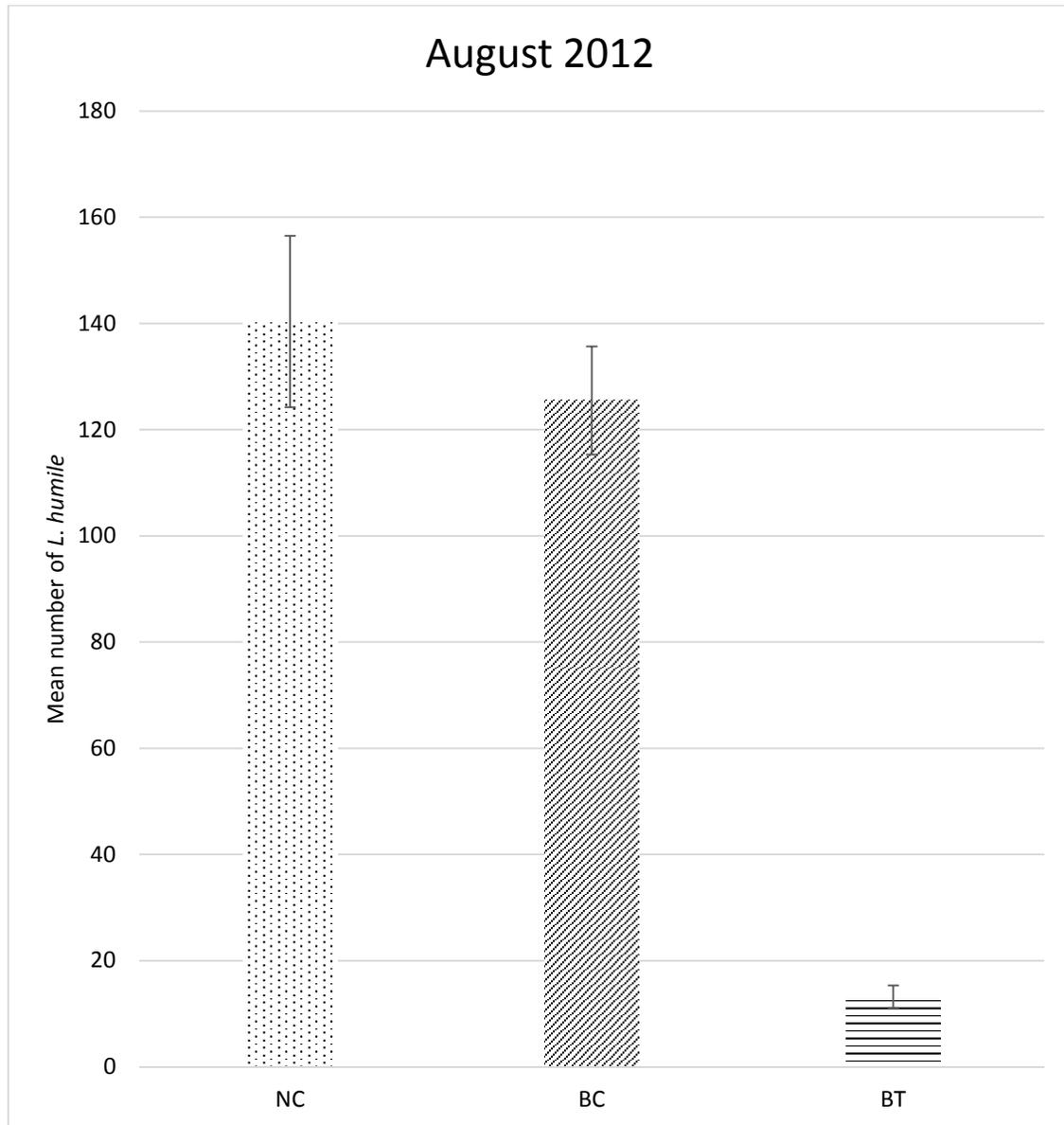
Fig 4.6. The mean (± SE) number of *Linepithema humile* (Mayr) present in a foraging trail at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) during week 20 of treatment (August in 2012). Three monitoring areas were established with an area with no stations (natural control (NC)), a control area with stations containing only 300 ml sugar water (bait control (BC)), and a treatment area with stations containing 300 ml sugar water and 0.03% imidacloprid insecticide (bait treatment (BT)) were established. Data were analyzed at each sampling time by using Analysis of variance. Mean separation was done using Fisher's LSD.
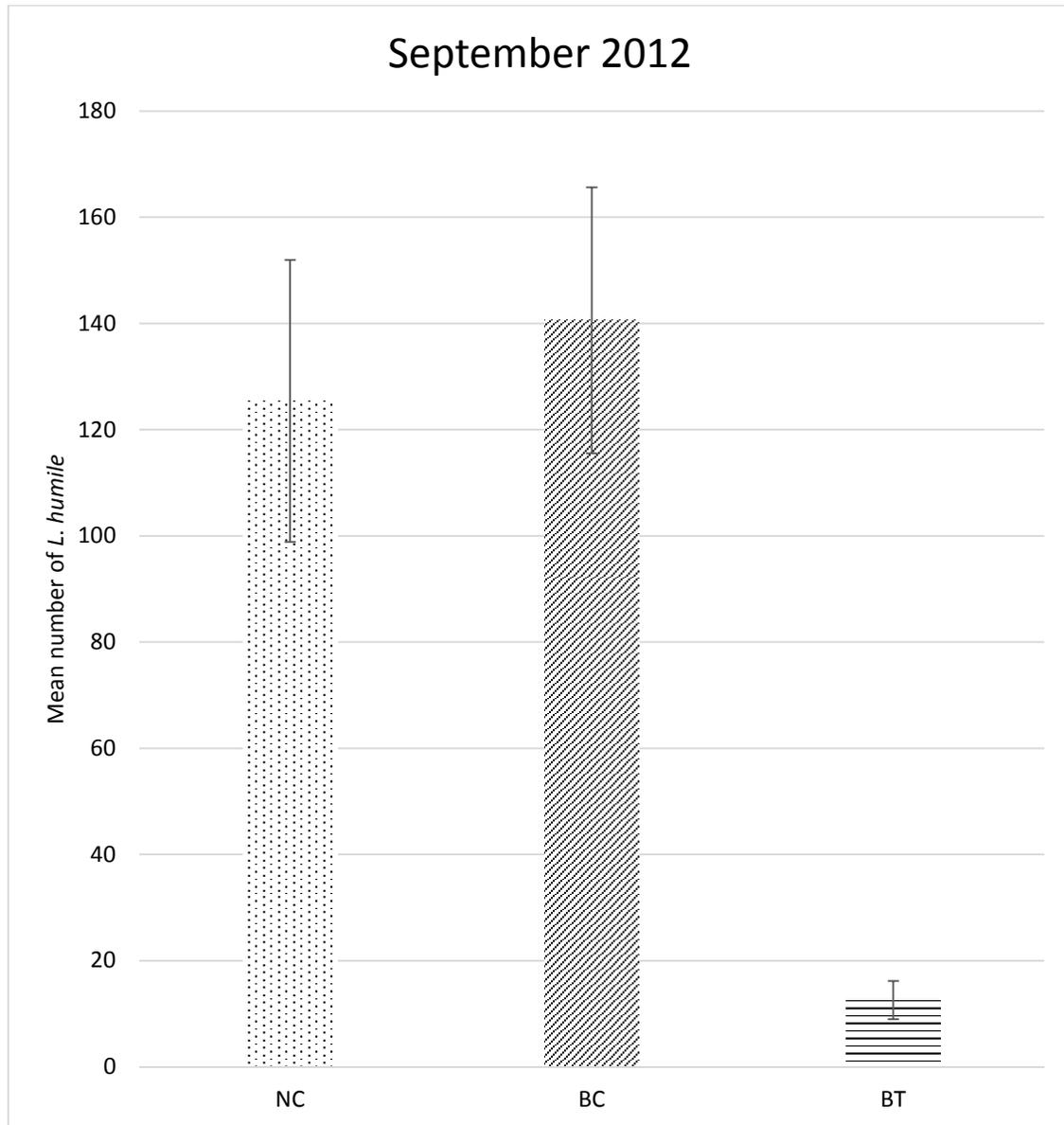
September 2012

Fig 4.7. The mean (± SE) number of *Linepithema humile* (Mayr) present in a foraging trail at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) during week 26 of treatment (September in 2012). Three monitoring areas were established with an area with no stations (natural control (NC)), a control area with stations containing only 300 ml sugar water (bait control (BC)), and a treatment area with stations containing 300 ml sugar water and 0.03% imidacloprid insecticide (bait treatment (BT)) were established. Data were analyzed at each sampling time by using Analysis of variance. Mean separation was done using Fisher's LSD.
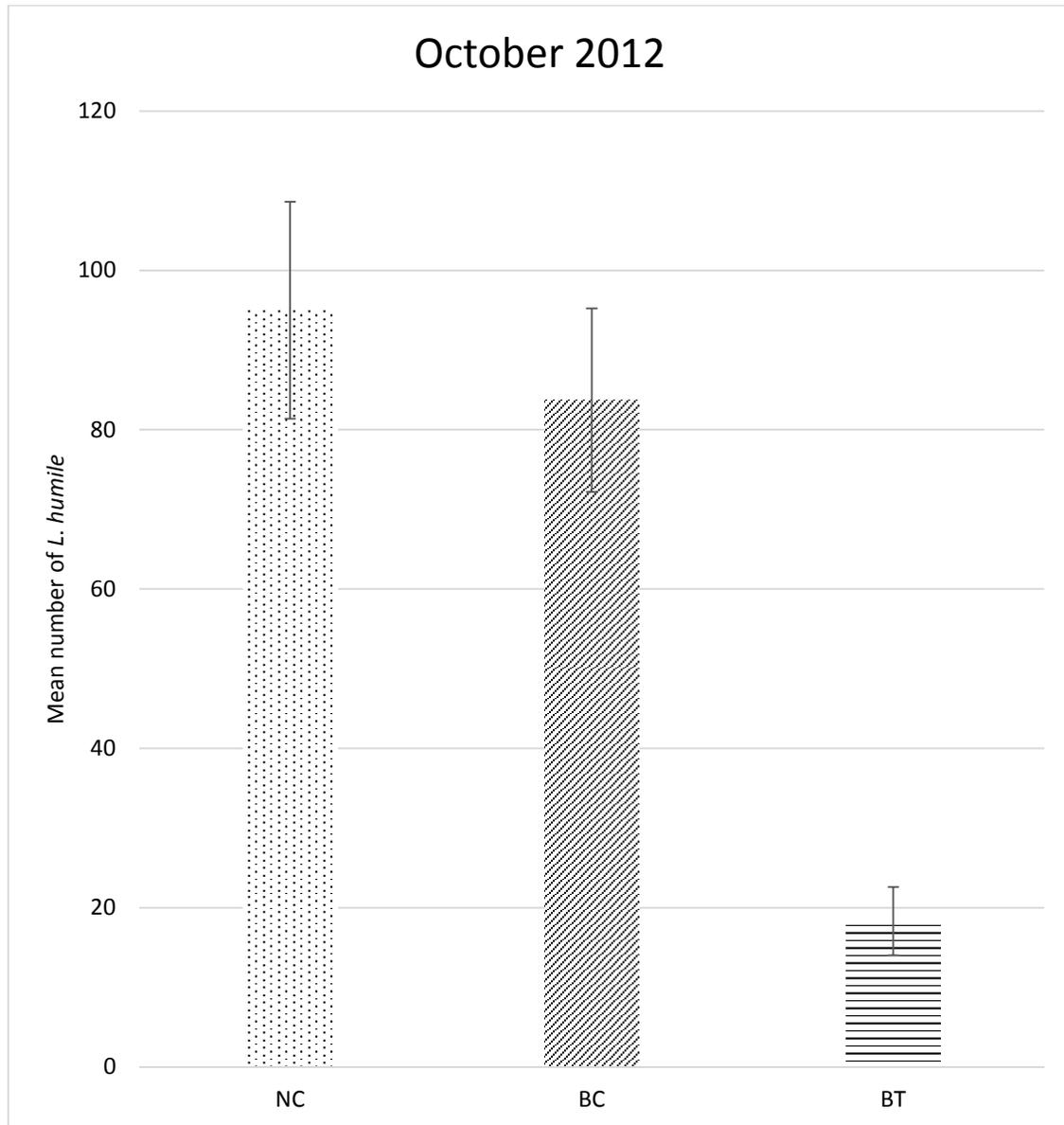
Fig 4.8. The mean (± SE) number of *Linepithema humile* (Mayr) present in a foraging trail at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) during week 30 of treatment (October in 2012). Three monitoring areas were established with an area with no stations (natural control (NC)), a control area with stations containing only 300 ml sugar water (bait control (BC)), and a treatment area with stations containing 300 ml sugar water and 0.03% imidacloprid insecticide (bait treatment (BT)) were established. Data were analyzed at each sampling time by using Analysis of variance. Mean separation was done using Fisher's LSD.
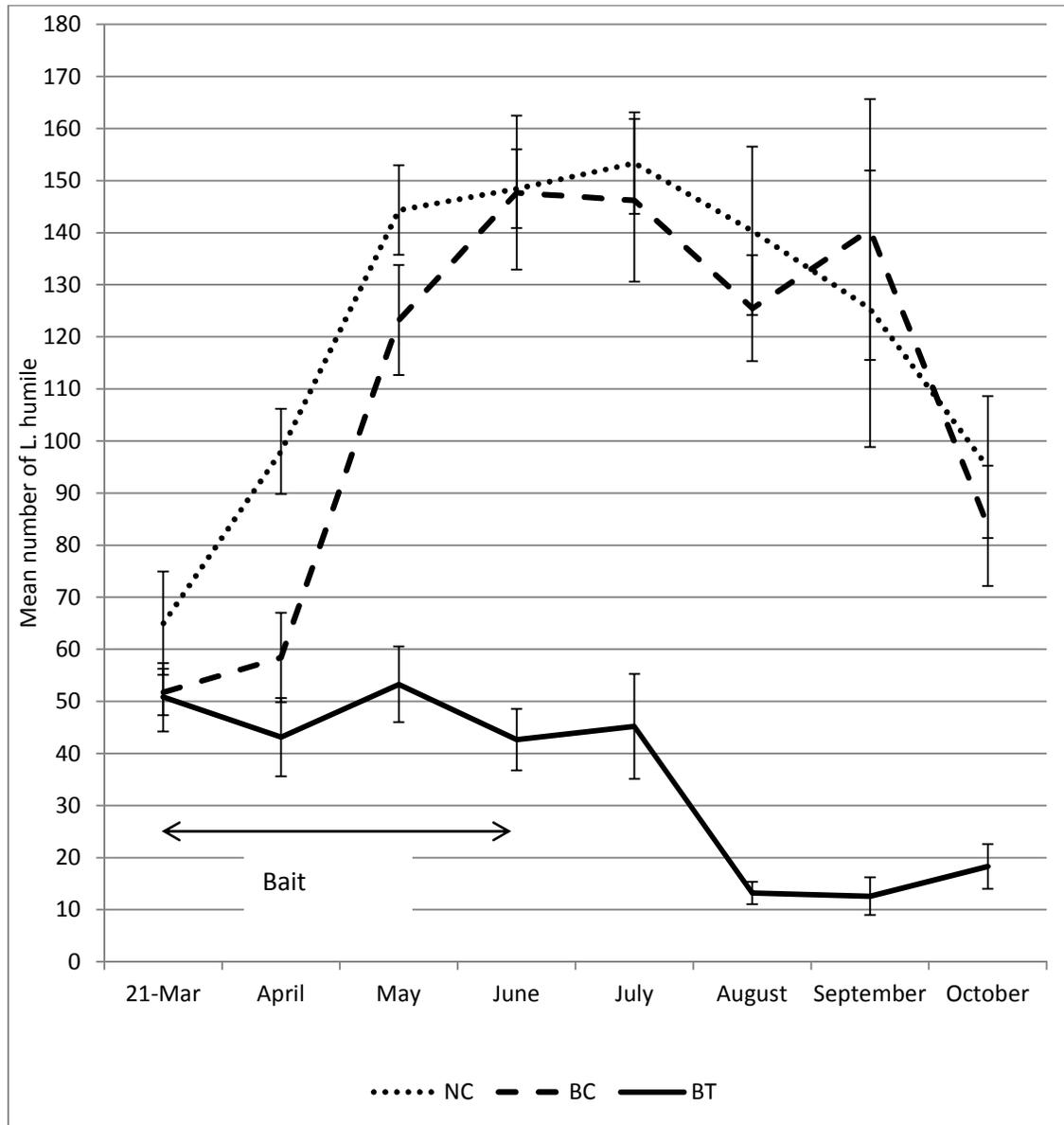
Fig 4.9. Evaluation of control of *L. humile*, from 21 March to 15 October 2012 using bait stations placed 20 m apart in a natural area at Lake Greenwood Sate Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N), SC. Three monitoring areas were established with an area with no stations (natural control (NC)), a control area with stations containing only 300 ml sugar water (bait control (BC)), and a treatment area with stations containing 300 ml sugar water and 0.03% imidacloprid insecticide (bait treatment (BT)) were established. The mean (± SE) number of *L. humile* present on 10 foraging trails per site per month are represented. Data were analyzed at each sampling time by using Analysis of variance. Mean separation was done using Fisher's LSD.
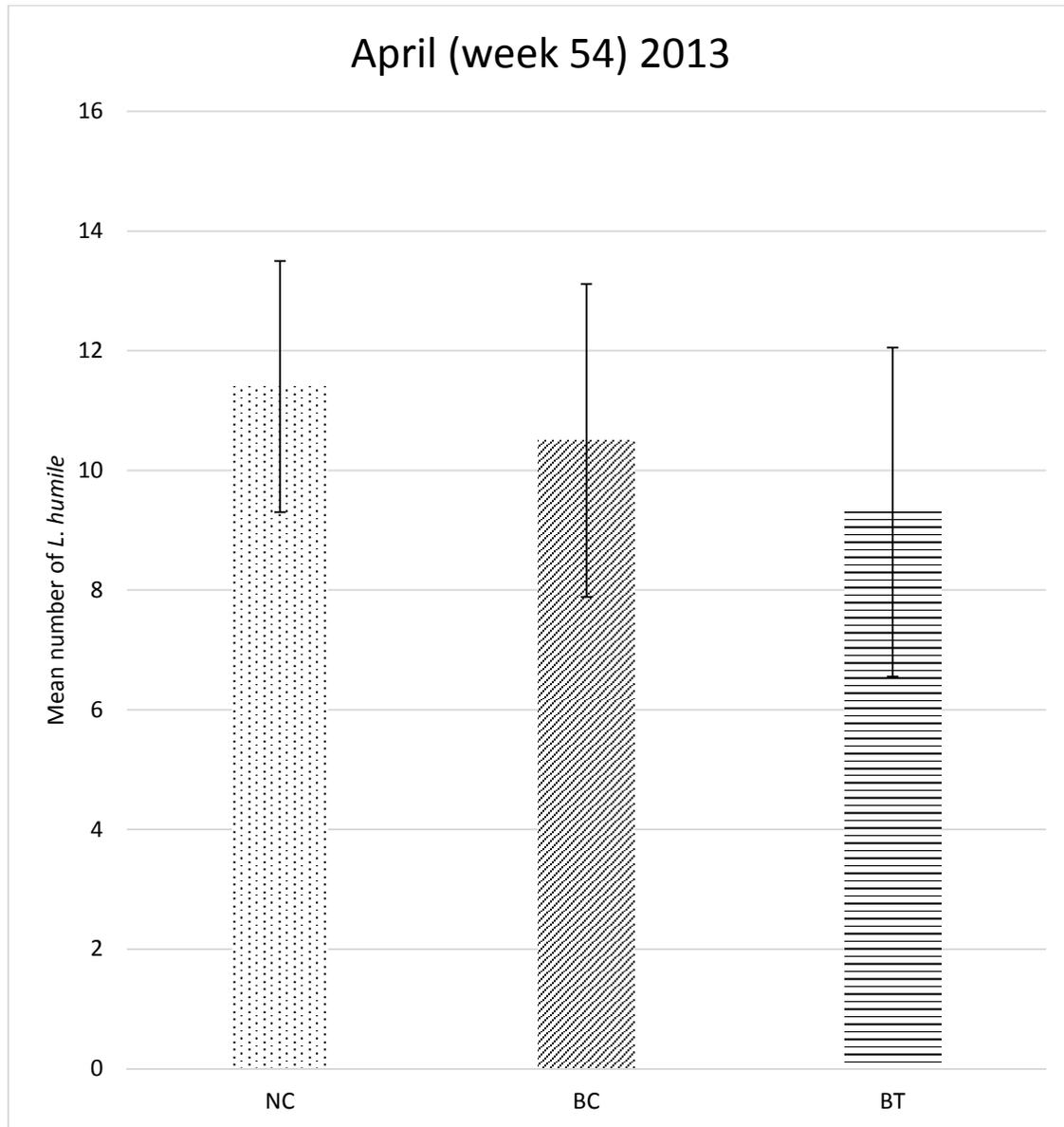
Fig. 4.10 The mean (± SE) number of *Linepithema humile* (Mayr) present in a foraging trail at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) during week 54 (April in 2013) without treatment. Three monitoring areas were established with an area with no stations (natural control (NC)), a control area with stations containing only 300 ml sugar water (bait control (BC)), and a treatment area with stations containing 300 ml sugar water and 0.03% imidacloprid insecticide (bait treatment (BT)) were established. Data were analyzed at each sampling time by using Analysis of variance. Mean separation was done using Fisher's LSD.
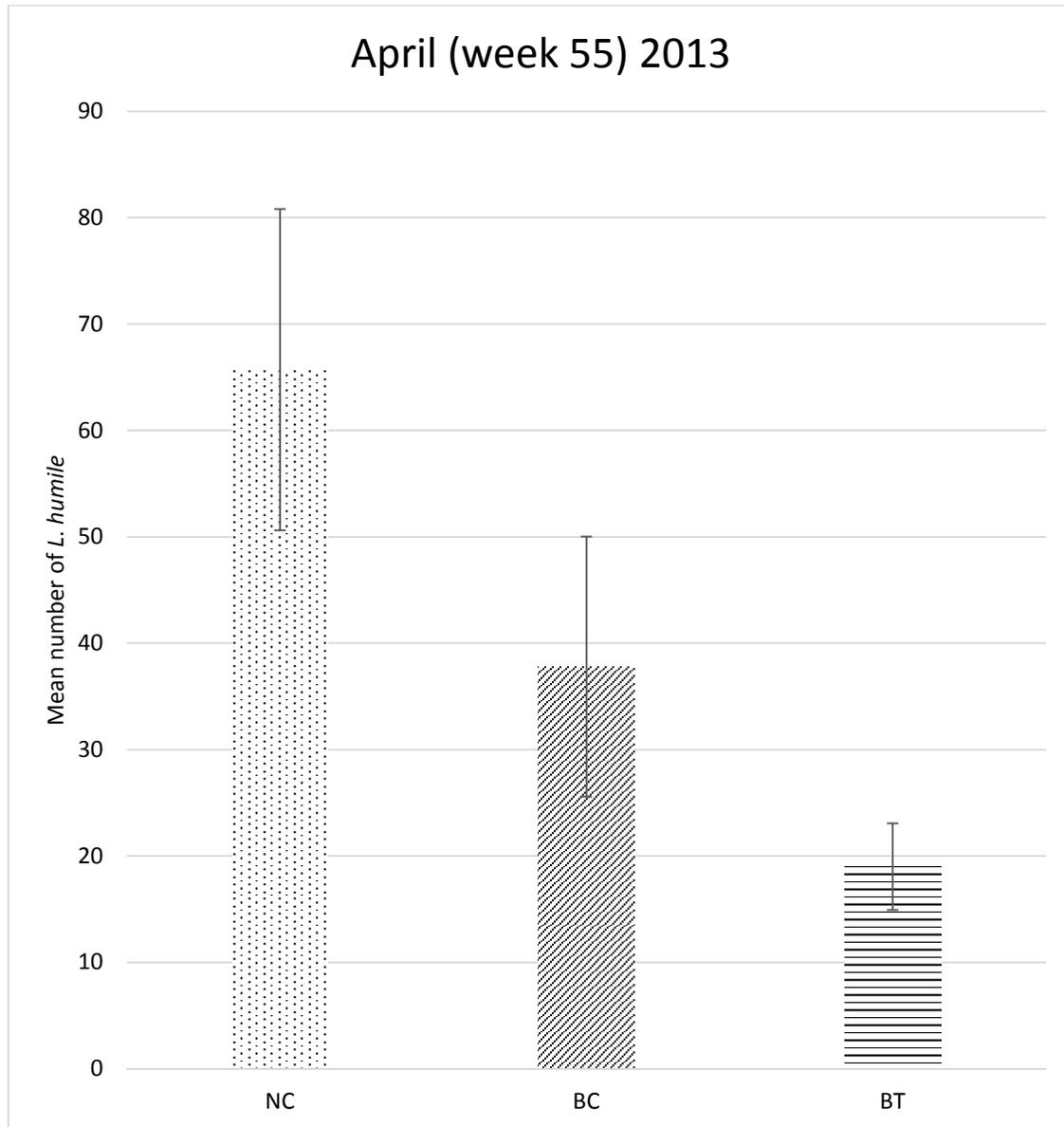
Fig. 4.11 The mean number of *Linepithema humile* (Mayr) present in a foraging trail at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) during week 55 (April in 2013). Three monitoring areas were established with an area with no stations (natural control (NC)), a control area with stations containing only 300 ml sugar water (bait control (BC)), and a treatment area with stations containing 300 ml sugar water and 0.03% imidacloprid insecticide (bait treatment (BT)) were established. Data were analyzed at each sampling time by using Analysis of variance. Mean separation was done using Fisher's LSD.
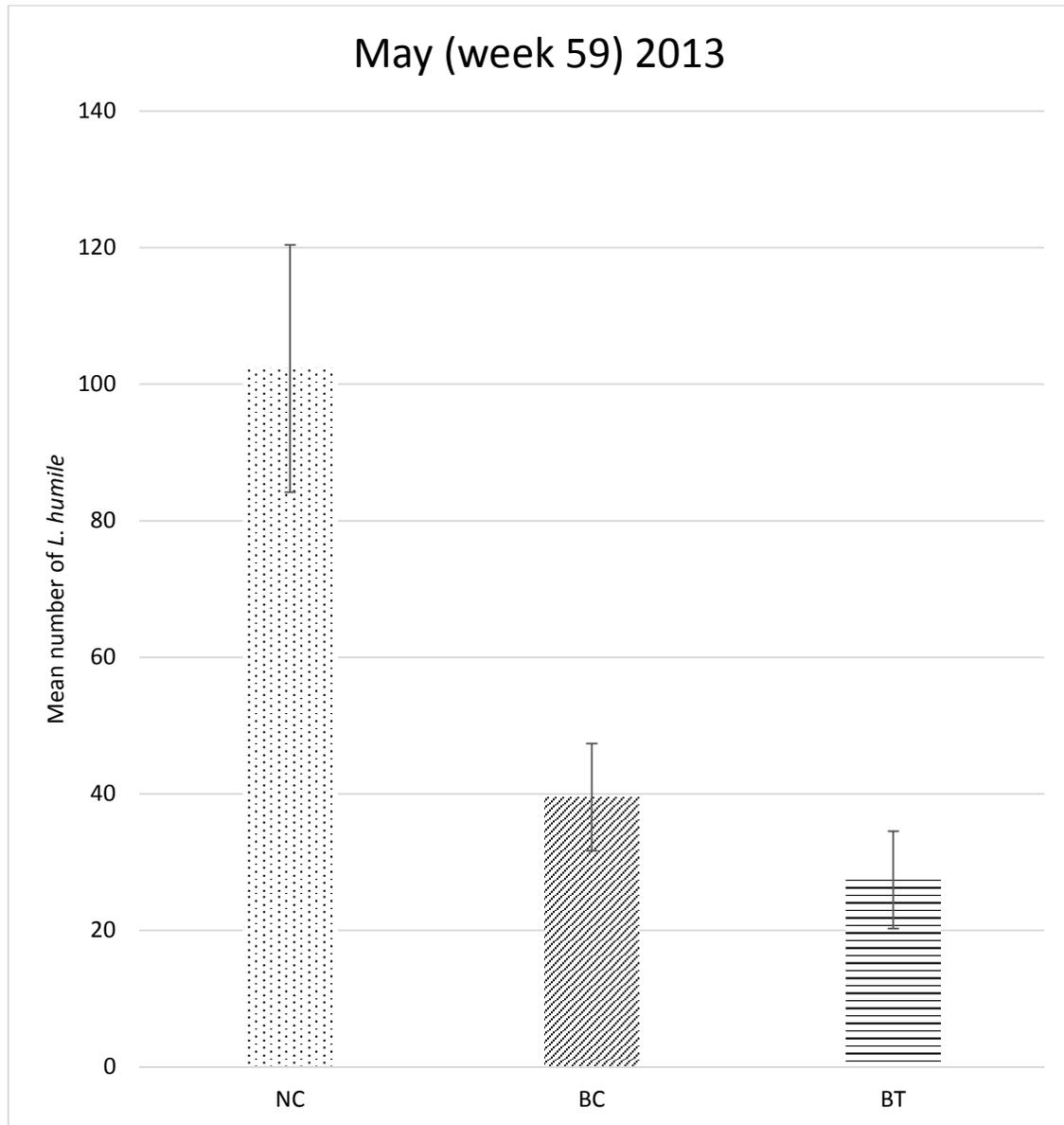
Fig. 4.12. The mean number of *Linepithema humile* (Mayr) present in a foraging trail at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) during week 59 (May in 2013). Three monitoring areas were established with an area with no stations (natural control (NC)), a control area with stations containing only 300 ml sugar water (bait control (BC)), and a treatment area with stations containing 300 ml sugar water and 0.03% imidacloprid insecticide (bait treatment (BT)) were established. Data were analyzed at each sampling time by using Analysis of variance. Mean separation was done using Fisher's LSD.
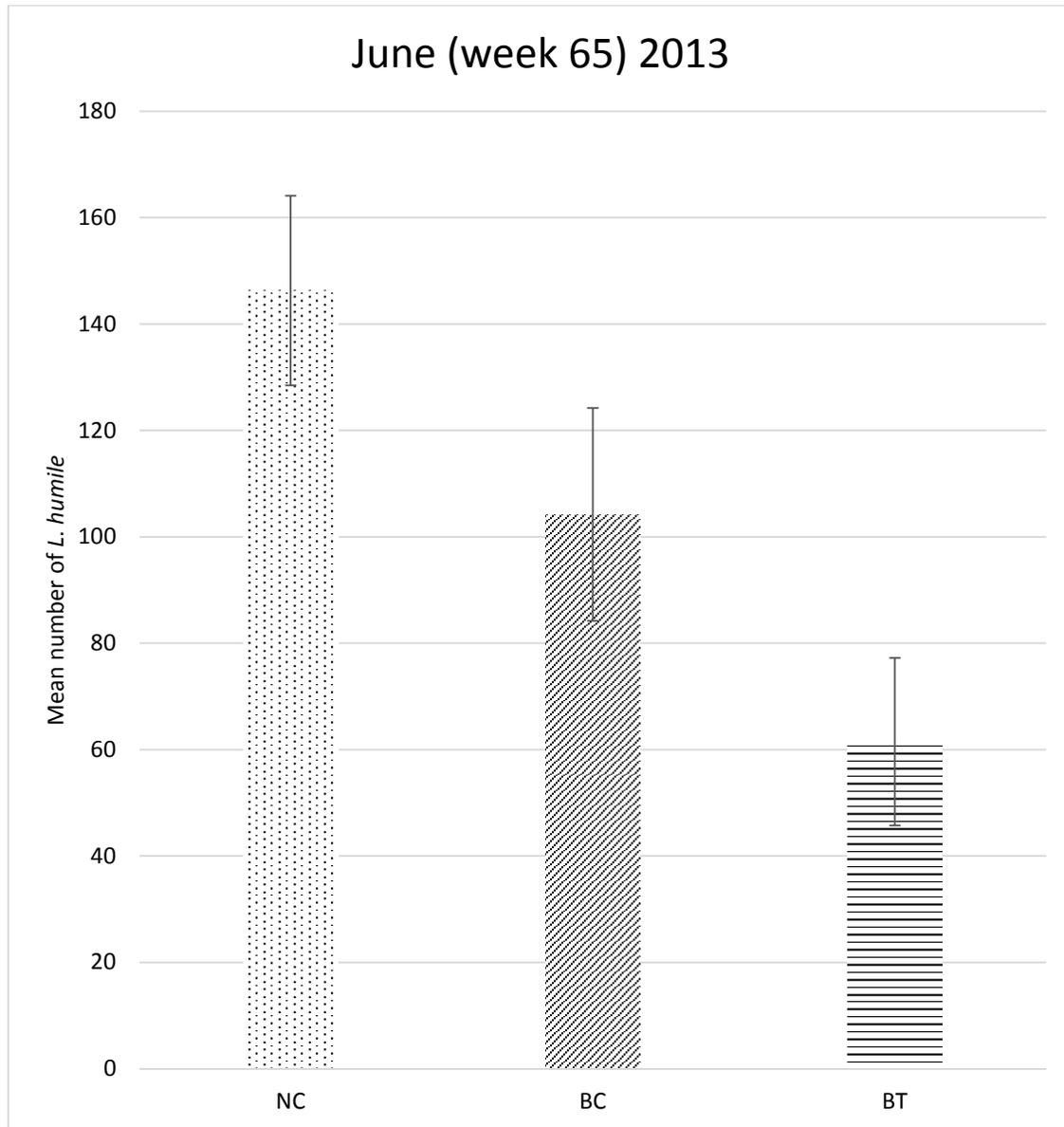
Fig. 4.13. The mean number of *Linepithema humile* (Mayr) present in a foraging trail at Lake Greenwood State Park (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N) during week 65 (June in 2013). Three monitoring areas were established with an area with no stations (natural control (NC)), a control area with stations containing only 300 ml sugar water (bait control (BC)), and a treatment area with stations containing 300 ml sugar water and 0.03% imidacloprid insecticide (bait treatment (BT)) were established. Data were analyzed at each sampling time by using Analysis of variance. Mean separation was done using Fisher's LSD.

CHAPTER FIVE

Conclusions and Future Research Needs

Linepithema humile (Mayr), the Argentine ant, is a foreign invasive pest that has spread throughout the United Stated and is a problem in natural and managed habitats of the Piedmont region of South Carolina. Even though *L. humile* is an urban pest, it causes ecological problems in natural habitats by displacing native ants such as in state park natural areas. *Linepithema humile* foraging patterns and distance travelled, as well as foraging activity and liquid bait control were studied in urban areas. However, *L. humile* has not been studied extensively in natural areas. The primary objectives of this study were to 1) determine the foraging distance of *L. humile*, 2) calculate the effective distance for the placement of bait stations for control, 3) assess over-wintering nest temperatures and foraging activity of *L. humile* in relation to temperature, and 4) evaluate early season liquid bait placement to control *L. humile* at Lake Greenwood State Park (LGSP) (Ninety Six, SC; 81° 58 '0.8868' W, 34° 11'58. 7904N).

The purpose of the first study was to detect ants carrying technical grade rabbit immunoglobin (IgG) protein marker by using the double antibody-sandwich ELISA test (DAS-ELISA) and thereby determining the foraging distance of *L. humile* and calculate the optimal placement of bait stations that would be needed for control of this species. The optimal protein marker concentration for detection in individual *L. humile* was 0.01 mg/ml mixed in a 30% sugar water solution. As *L. humile* was fed with protein marker for three days in laboratory conditions, the mean absorbance of an individual ant was significantly different from the control stations only containing 30% sugar water. It

showed that the optimal retention time of *L. humile* was three days. The retention time study of protein marker in individual ants under field conditions also showed that the mean absorbance was significantly different than the control up to three days. After the concentration and retention time was determined, foraging distance of *L. humile* was evaluated. The mean absorbance of individual ants was significantly different than the control up to 15 m. However, the protein marker could be detected in ants collected at 20 m and even up to 30 m. Detection of the marker sharply decreased after 25 m. Since the mean absorbance in ants at 20 m was 10 times higher than the control, this distance was considered a practical foraging distance for detecting ants. After determining the concentration, retention time, and foraging distance, suitable bait station placement distances (10 m or 20 m) were tested to reduce labor time and supplies. The mean absorbance was significantly different between bait stations placed at 10 m and 20 m. The mean absorbance of 10 m bait station placement sample was almost two times higher than 20 m bait station placement sample. However, it was determined that *L. humile* could forage for food up to 20 m based on this study. For applying this technique to control *L. humile* population, I recommend that bait station placement is effective and cost efficient at 20 m intervals.

The second study was conducted to determine over-wintering nest temperatures and foraging activity of *L. humile* in relation to relative ambient temperatures. Furthermore, mean temperature and foraging activity were evaluated to determine a suitable date for early season bait placement. The *Pinus spp.* at LGSP provided not only access to food resources, such as tree feeding insects producing honeydew, but also nest locations in close proximity to water. Mean nest temperature range was less variable than

mean ambient temperature range throughout this study. This suggested that nest temperature is more stable than ambient temperature. When the mean ambient temperature slowly increased from January to March, the mean foraging number of *L. humile* also gradually increased. When the mean daytime temperature continued to be over 15°C, foraging activity of *L. humile* was more active. In this study, this occurred on 15 March 2012 and was considered the optimal time to place bait stations in natural areas for foraging *L. humile*.

The third test of this study was conducted to evaluate early-season liquid baiting to control *L. humile* populations at Lake Greenwood State Park picnic area in Ninety Six, SC. Because ant colonies started active foraging in spring, bait deployment date was important to control *L. humile* in the early spring. Thus, there is a better chance to transfer toxic bait to larvae and prevent colony growth if there is early access to bait. When foraging activity of *L. humile* averaged over 25 ants past an evaluation point in 30 second and temperatures were continuously above 15°C in early spring, three treatment areas (natural control (NC), control with bait station containing only 180 ml of 25% sugar water (BC), and stations filled with 180 ml of 25% sugar water mixed with 20 ml Maxforce Quantum Ant Bait (0.03% imidacloprid, Bayer Environmental Science, Kansas City, MO) were assigned to natural areas. Baiting began on 20 March 2012. Each area, NC, BC and BT, contained 10 of the original 30 trees used for monitoring ant activity. All ants counted on foraging trails in each area were summed weekly and then averaged to provide a mean number of ants. In June 2012, when the mean number of foraging ants was significantly different in control and treatment areas, baiting was discontinued. However, the mean number of *L. humile* continued to be recorded from 20 June 2012 to

15 October 2012. The mean foraging number of workers was also recorded from April 2013 to June 2013, even though no bait had been applied since June 2012. In the spring of 2013, there was still a significantly lower number of ants in the BT area. I conclude that early season liquid baiting, with specific placement distance, was an effective method for controlling *L. humile*.

In the future, a study evaluating second year baiting would be beneficial. Because *L. humile* populations in the previously baited area gradually increased in 2013, early season baiting in 2013 might have produced an even more effective control method. Another study evaluating bait placement at either 15 m or even 30 m could be done in future tests. This study could determine whether the length of time it takes to bait and control *L. humile* is affected by bait distance. An additional study evaluating similar field sites for a specific distance with liquid baits could also be beneficial.

Supporting Information for Chapter One

Email requesting confirmation of topics discussed with Stan Hutto

Subject: RE: Argentine ant update

From: Stan Hutto <shutto@scprt.com>

Date: Wed, September 3, 2008 11:42 am

Hope this helps,

Stan Hutto

Resource Management Biologist

SC Department of Parks, Recreation & Tourism

1205 Pendleton St.

Columbia, SC 29201

Phone: (803) 734-0532

Fax: (803) 734-1017

-----Original Message-----

From: brittar@CLEMSON.EDU [mailto:brittar@CLEMSON.EDU]

Sent: Sunday, August 31, 2008 7:30 PM

To: Stan Hutto

Subject: Argentine ant update

Stan:

I just wanted to send a quick update on where I am in my research. After September 7th I will be done with the actual surveying of ants from treatments at each park. I also had a few questions that I hoped you could fill in the blanks for. I think the answers to these will help put the project into perspective for people and add a bit more depth to my presentations and thesis. If after reading these questions you think of other points I may have left out, I would appreciate anything you have to add that you feel is important information (financially important or just other facts). Thank you again for all of the help you have provided over the past couple of summers. Once all of the data is put together I will get back in touch with you and let you know how it turned out.

1) Is there a set amount of money allocated (for the state, for each park, any way you can answer) for pesticide treatment in the parks?

There is not a set amount of money allocated for pesticide control in State Parks. Although we have established a Budget category for the parks to request funding for any pesticide/herbicide related project. This includes, termite & pest control contracts on park structures as included in this budget. As far as funding for argentine ants or any pest goes, if a pest causes a significant or potentially significant

Email cont.

impact to revenue generation we have been able to fund as needed to protect the visitor experience and revenue generation.

2) How long has the parks system been putting out Tempo or other chemicals to combat the ants? (I guess an estimate of how long the ant problem has been going on).

We have been working on argentine ants for the last 3 years. We tried several baits including several granular type baits and the liquid bait Terro-PCO with little relief. The first chemical that gave any relief was Tempo. It was used as a barrier. After working with the Clemson Entomology department we began investigating additional chemicals and are currently using Premise, Phantom, Termidor and Tempo depending on the location and conditions of the site.

3)How many parks in the state would you say are having problems with Argentine ants?

Seven parks including Hamilton Branch, Hickory Knob, Baker Creek, Calhoun Falls, Lake Hartwell, Lake Greenwood and Dreher Island State Parks.

4)Is it possible to say how much Tempo and Phantom and any other pest chemical each park receives and how much they actually use in a year?

Tempo is chemical we have used at all sites on an as needed basis to spray around camping pads. I would estimate we have used the following amounts of concentrate over the past 3 year period:

Tempo 8.64 liters

Termidor 624 ounces

Premise 22.5 ounces of the 75 WP

Phantom 108 ounces.

It is hard to put a yearly total on use as the initial treatment with termidor on nests is the largest application. Then each spring just as the ants are becoming active we spot treat any new nests found during the survey. We also treat any new nests throughout the season. All treatments are mapped to insure no more that 2 treatments with Termidor in the same area per year. These follow up treatments are greatly reduced to probably no more that 8 gallons of mix a year. We have used the Premise as an initial treatment along hard surfaces like walks and roads. We have not used this product to date as a retreatment. We have used the phantom as needed to spray the interior and exterior bases of comfort stations, cabins and loge rooms. Tempos has always been used on an as needed basis especially on sites where we have not used large scale spraying with the other chemicals. To date we have treated campgrounds at Dreher Island, Lake Hartwell and the lodge and cabin areas as well as portions of the campground at Hickory Knob with Termidor & Premise

Email cont.

5) How much money was spent at Dreher during the "eradication" attempt? What all was used chemical wise and how/where? Have there been complaints or even small problems with the Argentine ants since the big treatment there?

Approximately $2000 was used in chemicals at Dreher Island during the eradication phase this was for Termidor, Premise and Phantom. Both Campgrounds were completely treated and required only minimal follow-up that same year. Termidor was applied throughout the entire area to all ant nests, Premise was used along the edges of all hard surface roads and walkways and also to spray in cracks within the hard surfaces. Phantom was used on the bases of interior and exterior comfort station walls. Prior to the treatment we had received in excess of 150 complaints and issued refunds in excess of $1500. Since the initial phase complaints we have received a total of 3 complaints, 2 of which were immediately after the initial treatment where some nests had been missed. Follow-up treatment in 2007 cost $200. Of note is the fact that there was a change in park managers from 2007 to 2008. In the early summer of 2008 it has been noted complaints were rising. An investigation into the situation revealed that with park management turnover, treatment for ants complaints had revert to using tempo rather than locating nests and treating with Termidor due to the immediate action of Tempo. Park staff has since been reeducated and ant populations are again under control with minimal treatment.

6) Approximately, how much revenue do campers and day trippers supply to the park budget?

That's a hard one. I don't have access to figures per user group. Gross revenue for Dreher island is probably in excess of $600,000 per year. But that would include all sales including gas and boat ramp fees, marine fees, villa & camping users, park entrance fees etc. There are in excess of 125 campsites at Dreher island that rent from $15-$21 per night depending on site and season.

Thanks again,

Brittany (Russ) Ellis

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Revenue Data from Lake Greenwood State Park

1) How much revenue do campers/day trippers/etc. bring into the park each year? If you could find the numbers for the past couple of years that would be great too, but if not that's fine.

Our Fiscal years run from July 1 - June 3oth. This past FY (fiscal year 08) we brought in $270,318.00 in camping and $59, 711 in admissions. The year before (FY 07) we brought in $233,342.00 in camping and $55,941 in admissions. The year before that (FY 06) we brought in $213,834.00 in camping and $54890.00 in admissions.

2) Is it possible to find the number of refunds given in the past year(s) and their total(s)?

I do not have refund totals for any year other then last year. The estimated refund amount for FY 08 is $565.54

Fayette R. Yenny

Manager, Lake Greenwood State Recreation

Area SC Department of Parks, Recreation &

Tourism 302 State Park Road

Ninety Six, SC 29666

Phone: (864) 543-3535

www.southcarolinaparks.com

Revenue Data from Calhoun Falls State Park

1) How much revenue do campers/day trippers/etc. bring into the park each year? If you could find the numbers for the past couple of years that would be great too, but if not that's fine.

04-05--269,000.00

05-06--332,000.00

06-07--371,000.00

07-08--418,000.00

David Drake

Park Manager, Calhoun Falls State Recreation Area

SC Department of Parks, Recreation & Tourism

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