OPTIMIZATION OF BAIT COMPONENTS FOR *Nylanderia pubens* (FOREL)

By

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To my family and friends who have helped me through this process along with my professors and my lab mates for their humor and all the good times, and to my ants who taught me what crazy feels like

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Components for granular bait for *Nylanderia pubens* (Forel) were assessed through choice experiments in laboratory and field environments. Experimental colonies consisted of ants collect in field environments and maintained in the laboratory. The components tested where: granular size, matrices, additives (sugar, oils and insect tissue), insect tissue preferences, and three insecticidal active ingredients. The preferences of *N. pubens* were determined by removal by number and by weight of the bait components from the foraging arenas. Differences in preferences between laboratory and field environments that were observed were attributed to environmental factors and colony developmental differences. *N. pubens* chose granular bait sizes that corresponded with their head and body sizes (0.850-1.00 mm). The matrix chosen for the carrier of the bait was a carbohydrate-proteinaceous carrier that was easily spreadable and absorbent enough for the addition of additives (dog food). Insect tissue experiments indicated that *N. pubens* prefers live forms of cricket tissue (53% by weight removed) which led to the addition of cricket tissue, to enhance the attractiveness of the carrier. Fipronil, imidacloprid and indoxacarb were added topically to the formulated bait matrix. The rate and percent of ant mortality was assessed. Fipronil caused faster and

higher percent mortality through days 6-14. LD_{50} for fipronil was about 4, whereas imidacloprid and indoxacarb's percent mortality never exceeded 35% over the 14 days the experiments were conducted.

CHAPTER 1
PRELUDE

In 2004, the European Environmental Agency defined the term invasive species as any non-native species which threatens ecosystems, habitats or other species (Frank et al. 2004). Among invasive species, ants are the most destructive to ecosystems once established (Kenis et al. 2009). The impacts to the ecosystems occur when the invaders disrupt the previously established balance among other species. Instances of this disruption to the ecosystem can be seen in many polygynous ant invasive species such as the Argentine ant, *Linepithema humile* (Mayr) and *Solenopsis invicta* Buren. *L. humile* disrupts native ant species, along with many mammals, avians and reptiles (Kenis et al. 2009). A major factor determining an ant's pest potential is in its ability to form numerically large, ecologically dominant colonies, a trait that *L. humile* shares with *Nylanderia pubens* (Forel) (Tsutsui et al. 2003).

Nylanderia pubens is referred to as a nuisance pest; however the economic impact of this invasive species is not yet completely known (MacGown et al. 2010). Millions of these ants accumulate in electrical equipment causing them to fail and short circuit (Drees et al. 2009). Additionally, these ants have caused tens of thousands of dollars in damage to property and remedial costs (Nester et al. 2010). In the Jacksonville, FL Zoo, the numbers of *N. pubens* got so high that their sheer amount caused the zoo train to halt because of the safety hazard posed by the ants occluding the train track (Calibeo-Hayes et al. 2010). *N. pubens* also poses a threat to apiculture business. In Texas, *N. pubens* invaded at least 100 bee hives in 2009 to raid the brood and colonize the hive (Harmon 2009). In St. Croix, *N. pubens* was blamed for crop

destruction due to mealy bug tending on fruit trees and aphid tending on coconut trees (Wetter et al. 2008).

Nylanderia pubens is especially hard to kill because of their high numbers, their foraging strategies and patterns, and their weak foraging on most traditional ant baits. The current control method for this pest species has been insecticidal sprays that appear to decrease the numbers of ants for short periods of time but do not solve the overall problem. The application of insecticidal sprays has been recommended for nest areas and along foraging trails (Warner et al. 2010), but with this approach *N. pubens* simply uses their fallen comrades as a bridge, thus burring the insecticide under the fallen ant bodies and making it useless (Drees et al. 2009).

Granular baits, however, may provide a longer term and more thorough solution. Baits can be broadcast over larger areas which takes advantage of the scatter-pattern of foraging that *N. pubens* displays. Baits work by taking advantage of ant biology such as social grooming and trophallaxis. Once the bait is discovered the foraging ants pick of the bait and take it back to the colony where it can reach the brood and queen, the brood digests the bait and transfer the toxicant to the rest of the colony. The use of granular baits in the control of pest ant species provides a mechanism for using very little insecticide thereby reducing the amount of insecticide in the environment (Hooper-Bùi et al. 2000). Components of granular baits consist of an attractant, carrier and active ingredient (Stanely 2004). Optimal granular bait should: 1) display delayed toxicity, 2) be transferred easily from one ant to the next, 3) use an active ingredient that is non-repellent on the bait matrix, and 4) be formulated to the ant species that needs to be controlled (Stringer et al. 1964, Hooper-Bùi et al. 2000).

The optimal bait for *N. pubens*, with the added active ingredients, should be, when finished: easy to carry, widely accepted by the foraging ants, and slow-acting enough so the active ingredient can be spread throughout the entire colony, making it to the queens. The objectives of this study were intertwined: 1) to tests components of granular bait, in order to formulate carrier size, carrier and additive for enhancement of the carrier and 2) the addition of insecticidal active ingredients to the carrier, to include their attractiveness to *N. pubens*, rate of mortality and over-all percent mortality for possible control of *N. pubens*.

CHAPTER 2
REVIEW OF LITERATURE

Family

Nylanderia pubens (Forel) belongs to the subfamily of ants known as Formicidae. The subfamily Formicidae is a very common and widespread group (Triplehorn et al. 2005). The major traits of the family Formicidae which separates them from the other insects in the order of Hymenoptera is the formation of the pedicel of the metasoma, which can be one or two segmented and have an upright lobe appearance, elbowed antennae, and eusociality (Triplehorn et al. 2005).

Almost all insects in the family Formicidae have a caste system, with the exception of some lesser ants, the morphology of the castes can be classified as: monomorphism (workers all the same size), Monophasic allometry, (nonisometric growth, two sizes connected by median class size), Diphasic allometry (increase in size leads to larger major class), Triphasic and tetraphasic allometry and complete dimorphism (two very distinct size groups) (Hölldobler et al. 1990). These castes do have a similar traits among them, they are all ruled by a single queen (monogyny), although in some species there can be multiple queens (polygyny).

Insects in the family Formicidae are found almost anywhere on earth. They have the unique ability to adapt quickly to their environments, and build nests in almost anything. The location of the nesting site does depend on the area they reside in and the species of ants in that area. Common nest locations include logs, plant cavities, leaf litter, under potted plants, in housing structures, and in the ground.

Classification

Nylanderia pubens can be a difficult species to acquire literature on due to the numerous proposed common names and the recent reclassification into a new genus. *N. pubens* was initially described by Forel as *Paratrechina pubens* in 1893 and identified in Florida by Deyrup et al. in 2000. In 2002, a potentially similar, if not the same species, was identified in Texas as *Paratrechina* near *pubens* (Meyer and Gold 2008). In 2010, a new description transferred *P. pubens* into the genus *Nylanderia* (LaPolla et al. 2010, 2011). The new recognized scientific name became *Nylanderia pubens* (Carlton et al. 2012). In some literature, *N. pubens* has been described as *N. fulva*, which was originally thought to be a subspecies of *N. pubens* but was raised to the status of a species by Trager in 1984 (Trager 1984, Carlton et al. 2012). The confusion between the names *N. pubens* and *N. fulva* arose because Forel misidentified the American specimen as *P. pubens* when it should have been *P. fulva*. Creighton (1950) made a taxonomic listing of the current ant species in North America, where he identified this mistake and corrected it. LaPolla's lab at Towson State University is currently working on the taxonomic status of *N. pubens* to see whether or not it is truly *N. fulva* (Carlton et al. 2012). There is still some debate on the valid scientific name of *N. pubens*. For the purpose of this work *Nylanderia pubens* will be used.

Common names used for *N. pubens* vary as much, if not more, than the scientific names. *Nylanderia pubens* are called crazy ants in some regions due to their erratic movements once they are disturbed. Another name comes about because course hairs cover the thorax of *N. pubens*. This identifying factor for the species has led some to use the common name hairy crazy ants (Wetterer et al. 2008). Other proposed common names include: Caribbean crazy ant, due to the belief that these ant originate in the

Caribbean (Warner et al. 2010), and Rasberry crazy ant, after the pest control operator that first observed the ants in Texas (Carlton et al. 2012). Because there has been no ESA-approved common name, the local names used for this ant species will likely vary throughout the country.

Origin and Distribution

Nylanderia pubens is an invasive species that is quick spreading and hard to eliminate once established in an area. *N. pubens* was believed to either originate from the St. Vincent, Lesser Antilles or in South America (Trager 1984, Meyer 2008). The first recorded introduction of *N. pubens* to the United States was in Miami, Florida in 1953 (Trager 1984). The next mention of this species was in 1990 in a hospital and at two other locations in around Miami, Florida (Klotz et al. 1995). In 2000, *N. pubens* was spotted at the University of Miami running up and down trees. From 2000 to now, these ants have spread up the coasts of Florida.

Seven years after the first observation in Harris County, Texas in 2002, *N. pubens* had spread to isolated spots in 14 counties in Texas (Drees et al. 2009). In 2009, Hancock County, Mississippi, reported large numbers of the Texas variety of *N. pubens* (MacGown et al 2010). In 2010, *N. pubens* was discovered in Port Allen, Louisiana, which represented the first record of this species in Louisiana. In 2011, *N. pubens* was identified in Calcasieu Parish, Louisiana (Hooper-Bui et al. 2010, Carlton et al. 2012). Within a period of less than 10 years, *N. pubens* colonies have been established in four states and are continuing to spread throughout the southern United States.

Colony Structure

Unicoloniality, or super colonies, are characterized by many wide spread but interconnected colonies that may contain one or many queens (Tsutsui et al. 2003).

Super colonies are formed by budding, which occurs when one or many queens abandon the original colony to form a separate one. The original colony can be the origin of many colonies in a super colony; ants from the separate colonies do not display aggression toward the ants in other colonies in the same area (MacGown et al. 2010). The lack of aggression appears to be a resource allocation tactic by ants that form super colonies. If no energy is wasted in defending the nest from their sister colonies, more resources can be dedicated to defense from other ants, colony growth, and foraging (Tsutsui et al. 2003). *N. pubens* fits this model because they are a polygynous species with anywhere from 8-40 queens in their colonies, they form large super colonies in areas where they have been found, and they show a lack of aggression toward other colonies of *N. pubens* in their area (Tsutsui et al. 2003, Warner et al. 2010).

Nests sites for the colonies can be found in multiple outdoor locations and occasionally indoor locations. *N. pubens* prefer moist areas and will nest under and in almost anything. They have been found outdoors in soil, rotting wood, in and under potted plants, in vehicles, and in various outdoor structures (MacGown et al. 2010). Inside buildings, *N. pubens* have been seen to occupy places such as inside computers and moist areas. Following the model of *L. humile*, another polydomous ant species, *N. pubens* may follow a nest dispersion model referred to as central-place, which occurs when a colony of ants places its nest sites closer to a food source to save on resources (Holway et al. 2000).

Description

N. pubens queens are 4.0 mm or longer, males are 2.4-2.7 mm, which is not much larger than the workers, which are 2.0-2.4 mm and monomorphic. All castes are reddish

brown in color, their thoracic region is covered in thick pubescence. *N. pubens* have one petiolar segment and they do not sting but can spray formic acid (Warner et al. 2010, Hooper-Bùi et al. 2010, MacGown et al. 2010, LaPolla et al. 2011). Distinguishing characteristics of this species are the striped light and dark appearance of gaster after feeding has taken place, and the length of their antennal scape which is nearly twice the width of their head with 12 segments on the antennae and no clubs (Warner et al. 2010).

Foraging and Feeding

Nylanderia pubens have an omnivorous diet. In nature, they can be seen tending to hemipterous insects and in nectaries of plants, along with foraging for insect tissue (Creighton 1950, MacGown et al. 2010). Like other ant species the foraging adults cannot eat solid foods; they must return to the nest and place the solid food on the brood to be digested and redelivered to the workers through trophallaxis. In other invasive ant species, foraging adults have been observed to allocate proteinaceous food to the larvae and the queens (Cassill et al. 1995).

Food granules have been observed in the nest cells of *N. pubens*, not located directly next to the brood (personal observation). This may indicate that they store food for later use. Most ant species that store food use seeds, although protein storage has been a observed in the ant species *Solenopsis invicta* (Buren) (Gayahan et al. 2008). *N. pubens* is a tropical ant species, which means weather may have a strong effect on foraging behaviors. *N. pubens* have not been observed foraging heavily in weather colder than 15.6 ˚C (Warner et al. 2010, Calibeo-Hayes et al. 2010).

Pest Status

Nylanderia pubens falls under the European Environmental Agency's definition of an invasive species (Frank et al. 2004). *N. pubens* is similar to other invasive ant species in having numerically large, ecologically dominant colonies, with multiple queens which causes them to out-compete native species for resources (Tsutsui et al. 2003).

Damages reported thus far include economic losses due to the disruption of business such as the train stopping in the Jacksonville, FL Zoo, destruction of electrical equipment, and property damage such as damage to livestock, death of rabbits in Little Fountain, St Croix, crop destruction as a side effect of insect tending, and the destruction of 100 bee hives in Texas (Wetterer et al. 2008, Calibeo-Hayes et al. 2010, Drees et al. 2009, Harmon 2009 and Nester et al. 2010).

Control

Controlling *N. pubens* is difficult due to their high numbers and weak foraging on most traditional ant baits. Therefore, pest control operators say that typical control methods for a number of ant species will not work on *N. pubens* (Nester et al. 2010). Control methods previously tried include sprays, which reduce the numbers of ants but fail to completely control the ants. In Texas, the growth inhibitor Esteem® 0.86% was applied by spraying it in an area, but the study indicated that this application decreased the ants seen in the treatment area, but not all of *N. pubens* in the area were killed (Nester et al. 2010). Drees et al. (2009) suggested using Termidor® SC Termiticide/Insecticide (9.1% fipronil) sprayed on the outside perimeter of a building infested with *N. pubens*. For turf grass, they suggested the use of TopChoice™

Insecticide (0.0143% fipronil), and, for ants entering the house, use of Phantom® Termiticide-Insecticide (21.45% chlorfenapyr).

Louisiana scientists suggested using an overall integrated pest management approach for *N. pubens* (Hooper-Bui et al. 2010). Their approach includes six parts: 1) monitoring for the ants, 2) sanitation practices, 3) disruption of foraging on trees and structures, 4) destruction of visible nests, 5) use of small particle baits, and 6) repeating the entire process after 12 weeks. In step five, they indicated that, in Texas, *N. pubens* prefers Whitmire Advance Carpenter Ant Bait (abamectin B1 0.011%) in small particles. This bait along with liquid bait stations that have been tested for palatability can be effective. The authors did not include information on the efficacy of the baits in controlling the ants.

CHAPTER 3
CHOICE BASED EXPERIMENTS OF GRANULAR BAIT COMPONENTS

Introduction

Nylanderia pubens has been established in Florida since the 1950's (Trager 1984). In recent years, this invasive ant species has quickly risen to the status of pest in many areas in and around Florida. Colonies of *N. pubens* can be in the thousands if not millions. This characteristic of their biology causes them to be hard to control and allow them to out-compete native species and push them out of their environment (Warner and Scheffrahn 2010).

The current approach for the control of this ant species is to use insecticidal sprays. This method has proven to be not very effective due to the sheer numbers of these ants. Because of the lack of effectiveness in controlling this ant species, a different method of control should be developed, such as granular bait.

There is little information on the food preferences of *N. pubens*. In the 1950's Creighton observed that these ants preferred honeydew, plant nectar, and insect tissue. The purpose of this study was to develop a granular bait matrix, with the idea that an active ingredient could be applied to it and used for *N. pubens* control. This knowledge could be used in the future to develop a more effective control method for this invasive species of ant.

Materials and Methods

Insects

Colonies of *N. pubens* were collected in from three sites in Gainesville, FL: 4821 Northwest 6th Street (The Rancher), between SW 5th St and SW 3rd ST (Depot). Moistened corrugated cardboard nest cells (25.4 cm x 15 cm) were placed in areas with

high numbers of foraging ant trails. Nest cells were collected after 2 wk, and ants were shaken off the cells into a tray. Collected ants were placed in gardening trays (13 cm H x 39 cm W x 52 cm L) with the inner sides lined with Insect-a-SLIP® (BioQuip Products, Rancho Dominguez, CA) to prevent ant escape. Each tray, depending on numbers of ants, contained 1-5 nest cells (Petri dishes [100 mm x 15 mm] with plaster on the bottom, and the lid covered with yellow cellophane). Food for the ants consisted of fresh orange slices, honey, ground cat food (Purina cat chow-naturals plus vitamins & minerals, Nestle Purina, St. Louis, MI), live insects, water and 10% sugar-water supplied weekly. Live insects consisted of crickets (*Acheta domesticus*, (Linnaeus)), American cockroaches (*Periplaneta americana,* (Linnaeus)), and mealworms (*Tenebrio molitor*, Linnaeus).

Satellite colonies were established by placing 1.5 g of *N. pubens* (3 queens, brood and workers) into a (33.5 cm x 24 cm) container (RubberMaid take along, RubberMaid, Fairlawn, Ohio) with the inner sides coated with Insect-a-SLIP®. One nest cell, a water vial, a sugar-water vial, four food trays, and a 60 ml soufflé container for waste disposal from colony were placed in each container. A waste container was utilized to clear the satellite colonies of food wastes. Because ants may be on top of spent food items, these items were placed into the waste container and ants were allowed to move out before waste was removed from the satellite colonies. The diet for the satellite colonies was identical to the diet used for parent colonies. Each satellite colony was allowed to acclimate for a minimum of 72 h before use in experiments and then starved for 24 h by removing all food. Healthy satellite colonies were randomly selected for experiments.

Granular Size

Dog food (Purina One healthy puppy food, Nestle Purina pet care company) was baked at 100°C for one hour and allowed to cool for 30 minutes to eliminate potential insect or mite infestations. Dog food was ground into small granules with a coffee grinder and sieved into four sizes. Stacked soil sieves (2.0 mm, 1.40 mm, 1.18 mm, 1.00 mm and 0.850 mm openings [Fisher Scientific Inc., Pittsburg, Pa.]) were used to separate granules that passed through the larger and were retained by the smaller size sieves. Granules were given designations for the sieve size which retained them. Granule sieve sizes used were 1.40 mm, 1.18 mm, 1.00 mm and 0.850 mm.

Additives

Additives used were soy-bean oil (Eden Organic, Clinton, Michigan), 25% corn syrup (ACH Food Companies, Memphis, TN) in water solution, and cricket slurry. Additives (0.2mL) were pipetted onto sieved (1.00 mm) dog food granules (1 g) placed into a soufflé cup (30 ml). Granules were shaken until the additive was evenly distributed and absorbed onto the dog food. Granules were refrigerated and stored for 24 h before use in experiments.

Matrices

The six different food products were tested, along with two different matrices types: Bird feed (Zupreem fruit blend flavor, Premium nutritional products, Shawnee, KS) with 14% protein, reptile feed (Juvenile iguana food growth formula , Rep-Cal research labs, Los Gatos, CA) with 24% protein, Dog Food (Purina one healthy puppy formula, Nestle Purina) with 28% protein, Cat Food (Purina cat chow Naturals plus vitamins & minerals, Nestle Purina) with 38% protein, Dog treats (Waggin’train jerky tenders, Waggin’Train LLC., Anderson, SC) with 65% protein, Tast-E bait (Endres

Processing, LLC., Rosemount, MN) with unknown protein content, and root watering crystals (Agrosoke international, Arlington, TX) protein content N/A. The matrices choices were ground to size in a coffee grinder and granules of size 1.00 mm were used (Table 3-1).

Insects

Lab-reared 3rd and 4th instar common house crickets, (*Acheta domesticus* (Linnaeus)), were fed to *N. pubens* as immobilized live, dried, or freeze-killed insects. All the insects were immobilized by removing their legs using a razor blade to cut the legs at the trochanter. Dried crickets were prepared by baking them at 100°C for an hour and then allowing them to cool for 30 minutes before use. Freeze-killed crickets were held in a freezer for 24 hours and were allowed to thaw for 30 minutes prior to the experiment. Non-insect controls were dog food granules (1.00 mm granules) which were weighed out to match the weight of the live crickets used in experiments.

Cricket Slurry

Laboratory reared crickets were ground into a pulp material in a Cuisinart food processor (Cuisinart, East Windson, NJ) then macerated using a 2-ml Pyrex® tissue grinder (Cardinal Health, Dublin, OH). Water (0.5 ml) was added for every 1 g of cricket in the grinding process. The product of the grinding was cricket slurry and applied immediately to the dog food granules. A 0.2 ml aliquot of “cricket slurry” was added to every 1 gram of 1.00 mm sieved dog food granules and mixed using a 30-ml soufflé container until the granules were saturated.

Foraging Arenas

Either 4-way or 6-way foraging arenas were constructed. Foraging arenas consisted of a Petri dish lid (4-way: 100 mm x 15 mm, 6-way: 150 mm x 25 mm)

mounted on three 2.5 cm vial cap lids in a stable triangle base design for the 4-way arenas, or supported by a another Petri dish hot-glued to the foraging arena, for the 6-way arenas. Four 1.5 cm holes were drilled around outer walls of the Petri dish base in a compass fashion (N, E, S, W,) this allowed entry and access to the wooden applicator. A 0.5-cm hole was drilled into the center of the foraging arena, and a 2.5-cm wooden applicator was hot glued to the bottom of the foraging arena at the opening of the 0.5-cm drilled hole. The outside of the foraging arena was coated with Insect-a-SLIP®. This only allowed for one entry on to the foraging arena. Ants entered arena from the center by climbing the wood applicator and chose from the four or six choices of food given. The choice position opposite to where the applicator tip connected to the arena, was designated as position one. The following positions (2-4 or 2-6) were designated in a clockwise fashion around the arena. Before arenas were reused for different experiments, they were thoroughly washed and wiped with isopropyl alcohol to eliminate any traces of food items or products tested previously and any ant trail pheromone.

Bioassay

For the laboratory experiments, satellite colonies were chosen at random from those prepared previously. Parafilm (American National Can, Greenwich, CT) squares were folded so the opposite edges (1 mm) were perpendicular to the central part forming small trays. Food choice items were individually counted out and placed onto the parafilm trays. The trays were then placed on to the foraging arena. In each experiment, the food choices were shifted in a clockwise manner for different replications and each food choice was tested in each foraging tray position twice. The ants were given 60 minutes to forage, after which the foraging arenas were removed

and the remaining granules were counted. The experiments were monitored in order to determine food choices were not depleted before the 60 minute foraging-time limit. Eight replications were run for 4-way tests, and 12 replications were run for 6-way tests.

The field bioassays were conducted at the three locations where the ants were collected. Granules of each food choice were counted and placed into a 1.5-mL centrifuge snap-cap vial. Each snap-cap vial was labeled and color coded. A set of four or six food choices were considered a repetition and four repetitions were run in a location at a time. At each location, strong foraging trails with at least three ants wide were identified; a flag was placed to mark the trail. Foraging trails were followed to make sure that each trail was unique and not a branch of another trail. The vials with the food choices were placed along the trails. For each replicate, the marker flag was placed on the opposite side of the foraging trail from the observer. To the left of the marker flag were spot one, and spot two. To the right of the marker flag were spot three, and four. The distance between the snap-cap vials, was 2.5 cm. Each food choice was in every position (1-4), in each field location, twice. Ants in the field were allowed a shorter time (10 min) to forage due to the large number of ants.

After the experiment was completed the snap-cap vials containing the food choices were capped and the vials were returned to the laboratory and placed into a refrigerator freezer (-7 ˚C). The granules were counted after 24 hours when ants picked up in the snap-cap vials were dead.

Analysis

To compare the food choices by percent number of granules removed and percent weight of the granules removed, the data was arcsin square root transformed and

ANOVAs were run in the statistical software JMP (SAS Inst., Cary, NC). Student-Tukeys tests were used to compare means.

Results

The number and weight of different sizes of granules removed in the granular size experiments were significantly different both in the laboratory (Number: F= 13.7923, P< .0001; and Weights: F= 9.1400 P: <.0003) and in the field (Number: F= 4.1830, P= 0.0081; and Weights: F= 28.5255, P <.0001, respectively).

In the laboratory, 33% of the removed dog food pieces were 1.00 mm, the granule size with the greatest removal, but there was no statistical difference in removal between granules of sizes 1.00 mm, 1.18 mm and 0.850 mm (Fig. 3-1). Based on weights of the granules in laboratory experiments, the dog food size 1.40 mm was the most removed, but there was no significant difference between 1.40-mm and 1.18-mm granules. The field data for number of granules removed showed that the ants removed more 0.850-mm dog food pieces (35% removed), but considering the weights of the varying sizes of dog food pieces removed, the ants preferred the granule size 1.40 mm with 52% removed (Fig. 3-2).

In the experiment with different animal-food granules, the number and weights of granules removed in the laboratory experiments were not significantly different (Number: F= 1.4493, P= 0.2306; and Weights: F= 2.0021, P= 0.1018). In field experiments, both in terms of the numbers and weights of granules, preference for the different granules was statistically significant (F= 58.7638, P < .0001, and F= 72.9351, P <.0001, respectively).

In laboratory experiments, there was no significant difference in the number or weights of the different animal food removed (Fig. 3-3). The field experiments

preference was shown toward dog treat with 53% removal of dog treat pieces or 61% based on the weight of dog treats removed (Fig 3-4).

When dog food granules containing additives were tested in the laboratory, neither the numbers nor weights of different granules removed were significantly different among the removed treatments (F= 2.6967, P= 0.0684; and F= 2.25598, P= 0.0787, respectively) (Fig. 3-5). In the field experiment, however, the numbers and weights of the different granules with additive removed were significantly different (F= 9.7876, P < .0001; and F= 9.2595, P < .0001, respectively) (Fig. 3-6).

In experiments with different forms of cricket, no significant differences were observed in the numbers of crickets removed; however, in the weights removed, significant differences were observed (F= 1.3788, P = 0.2738; and F= 57.2410, P < .0001, respectively). In the field, both number and weights of crickets removed were significantly different among treatments (F= 39.5317, P < .0001; and F= 79.7471, P < .0001, respectively). *Nylanderia pubens* preferred live and freeze killed cricket forms with 47% and 41% of the weight removed in the laboratory experiments (Fig. 3-7) and live cricket with 53% removal in the field (Fig. 3-8).

In matrices experiments with cricket slurry additive, there were significant differences among the treatments both when number of granule and weight of granules were considered both in the laboratory (F= 7.5359, P= 0.0004; and F= 5.5559, P= 0.0028, respectively) and in the field (F= 15.1999, P < .0001; and F= 14.8248 and P < .0001, respectively). The laboratory experiments with different matrices containing cricket slurry additive showed that the ants preferred dog food matrix with cricket slurry additive with 53% removal (Fig. 3-9) of the granules, whereas the field experiments, the

plain dog food, by weight removed, was the most preferred with 50% removal (Fig. 3-10).

Discussion

The purpose of this study was to gain a better understanding of the foraging and food preferences of *N. pubens*, that would allow us to formulate bait for the control of this invasive pest species. Based on field and laboratory observations, *N. pubens* forage on a variety of foods, but prefer nectar-based foods and insect tissue (Creighton 1950). Although *N. pubens* have an erratic foraging behavior in both the field and laboratory setting, when an acceptable food source is found, limited if any, recruitment is seen when foraging trails are established to the source. The foraging trails disappear once the food source is depleted; this was observed in both the laboratory and field settings. Foraging behaviors in the lab and field settings were noticeably different in my experiments. This can be due to a number of factors, such as, different colony needs based on time of year, the significantly larger number of ants in the field than the lab colonies, and potentially, different stage of colony development for laboratory and field colonies.

The National Pest Control Association describes ideal granular bait as containing granules of similar size that can be labor-saving and easily applied to areas when needed. The authors also go on to describe the carrier of the active ingredient as being the most important part of the granular formulation because both the particle size and the materials and components used determine the spreading characteristics, the effectiveness of recruitment and removal of the bait, and the residual life of the active ingredient (NPMA 1965).

Based on food particle size preference experiments done by Hooper et al. (2002), smaller ant species preferred smaller particle sizes to larger ones when given a choice. If the particle size could be matched to the ant species, this could increase the efficacy of the granular bait by providing greater opportunity for more bait to be taken into the colony (Hooper et al. 2002). My data indicates that the 1.00-mm granular size should be used with *N. pubens*. Because the 1.00-mm particle is similar to the size of head of foraging workers of *N. pubens*, this size granule can be easily carried by the ants (Fig. 3-11 & 3-12). This particle size is also easier to work with when adding additives to the dog food matrix.

Based on the weights of the removed pieces of dog food matrix, the granular size 1.40 mm was the most removed. 1.40 mm was not the chosen size of the final granular matrix because, although it was the most weight removed both in the lab and in the field. The ants were observed to have difficulty carrying the larger pieces of dog food, which appeared to cause constraints for the ants removing the larger pieces from the foraging arenas and constraints in trying to bring the 1.40-mm pieces into the small openings of the Petri dish nest cells. This observed difficulty could be due to the small size of this ant species, and their heads (Fig. 3-13). The 1.40 mm dog food particle is approximately 0.40 mm larger compared to the head size of this monomorphic species, whereas the granule size 1.00 mm is closer to the size the head size of *N. pubens* workers. However more active ingredient can be added to a larger particle size allowing more active ingredient to be introduced into the colony. Nevertheless, the ease in transport by the ants navigating the larger granular bait into the multiple nest sites does

not seem feasible due this ant specie's lack of cooperation and the locations of their nest sites can have very small openings.

The matrix chosen for the bait formulation was the dog food matrix. In the matrix choice lab experiment, there was no difference between dog treats and dog food or cat food in terms of number removed. Although dog treat was preferred by ants in the lab and the field experiments, dog treats were not the choice for the final experiment because the dog treats are hard to sieve out to the uniform size, and are not a good porous carrier for active ingredients. Dog food is a preferred bait matrix because it fulfills ant nutrient requirements, is easy to prepare in a uniform granular size and it readily absorbs additives, although this experiment was not designed to for the effects of seasons.

No sugar-based or oil-based additive was chosen to be added to the dog food matrix; because my experiments indicated that there was no significant advantage in adding these ingredients to the plain dog food matrix.

Because ants showed preference to live crickets, cricket slurry was added on to different matrices. Dog food with the addition of cricket slurry was the decided on bait matrix, not only because the worker ants removed more of this formulation then the other formulations, but because this formulation also allows the addition of active ingredients.

Table 3-1. Proteins contents and other characteristics of products used in matrices experiments with *N. pubens.* N/A= not applicable for this product. Unk= unknown.

Food type	Crude protein	Crude fat	Crude fiber	Moisture	Carbohydrates/ minerals
Bird food	14.0%	4.0%	3.5%	10.0%	68.5%
Iguana food	24.0%	1.0%	16.0%	12.0%	47.0%
Dog food	28.0%	16.0%	3.0%	12.0%	41.0%
Cat food	38.0%	13.0%	5.0%	12.0%	32.0%
Dog treat	65.0%	1.0%	0.5%	16.0%	17.5%
Root Crystals	N/A	N/A	N/A	N/A	N/A
Tast-E Bait	Unk	Unk	Unk	Unk	Unk

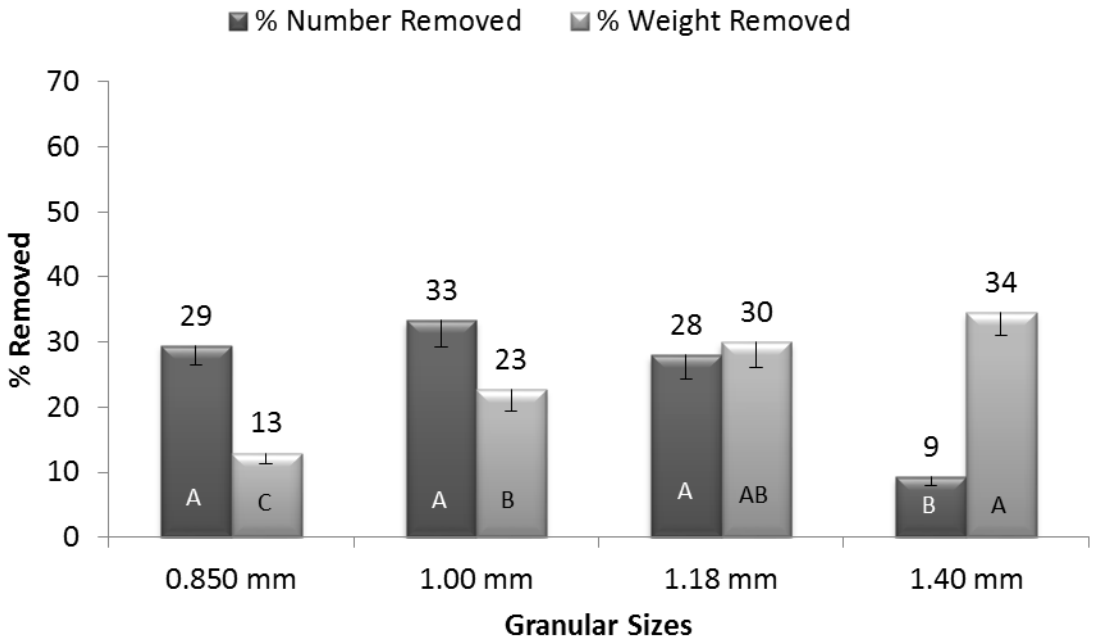

Figure 3-1. Percent number and percent weights of different size granules of dog food removed by *Nylanderia pubens* in laboratory experiments. Means with the same letter are not significantly different. Error bars = SEM.

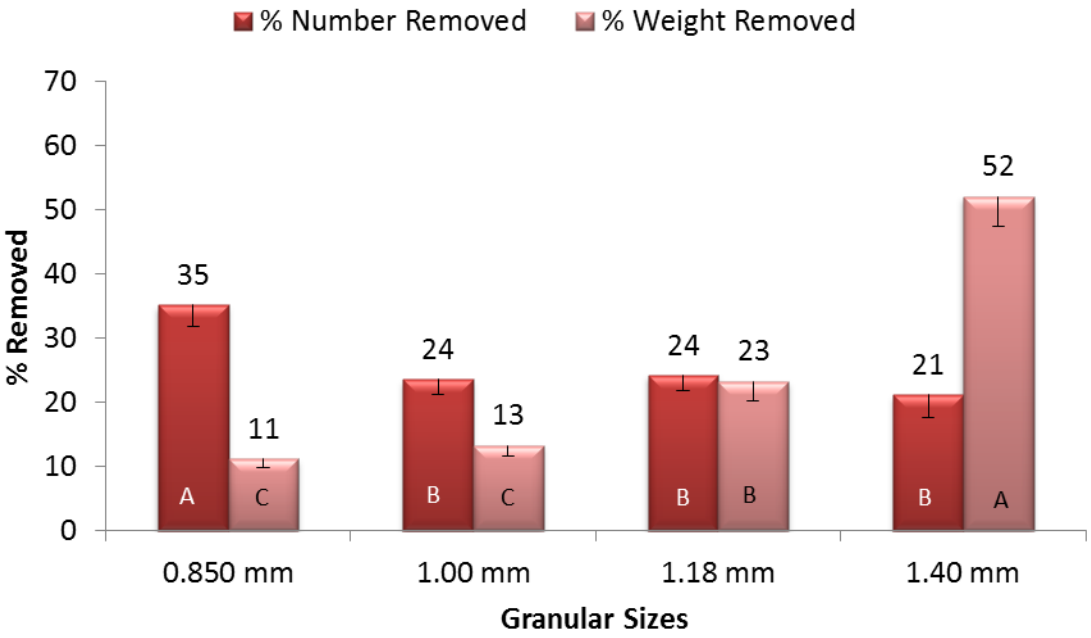

Figure 3-2. Percent number and percent weight of different size granules of dog food removed by *Nylanderia pubens* in the field experiments. Means with the same letter are not significantly different. Error bars = SEM.

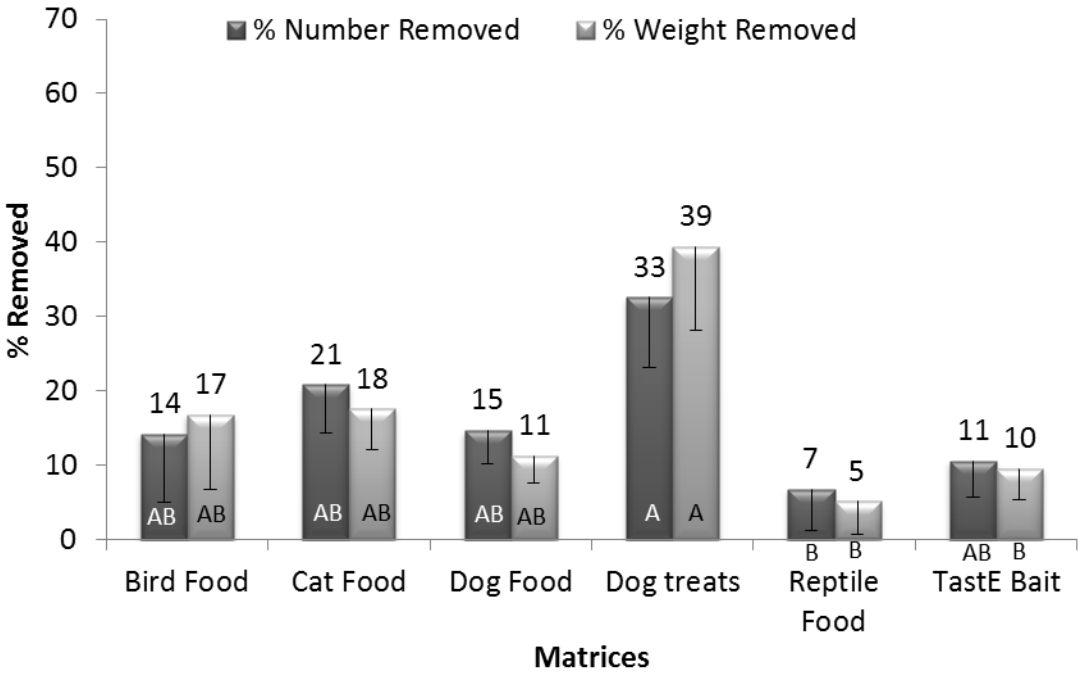

Figure 3-3. Percent number and percent weight of different food matrices removed by *Nylanderia pubens* in laboratory experiments. Means with the same letter are not significantly different. Error bars = SEM.

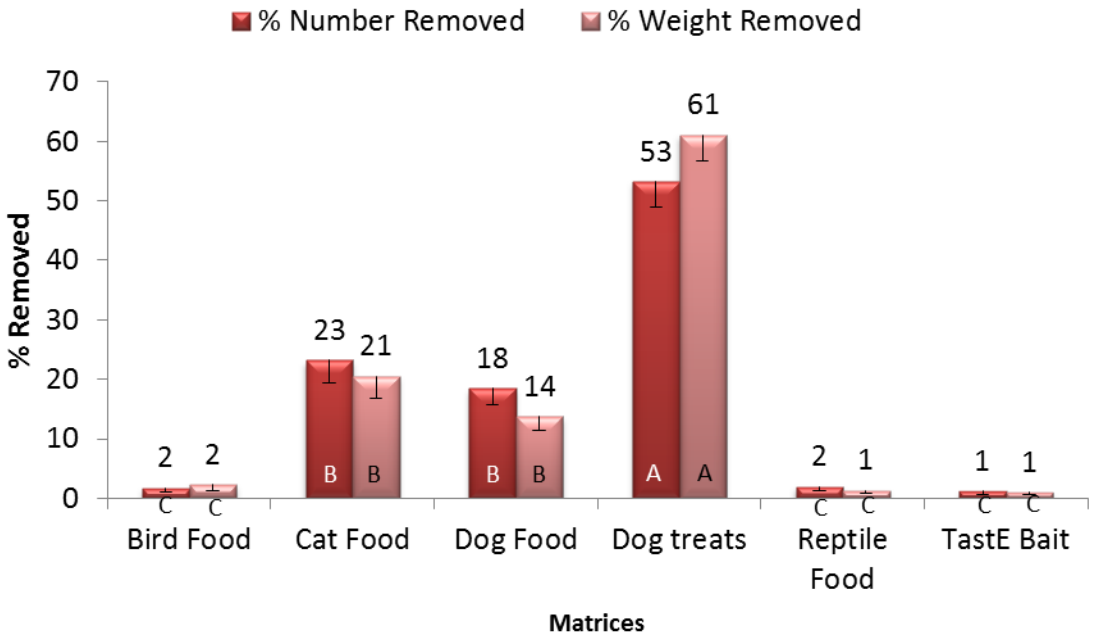

Figure 3-4. Percent number and percent weights of different food matrices removed by *Nylanderia pubens* in field experiments. Means with the same letter are not significantly different. Error bars = SEM.

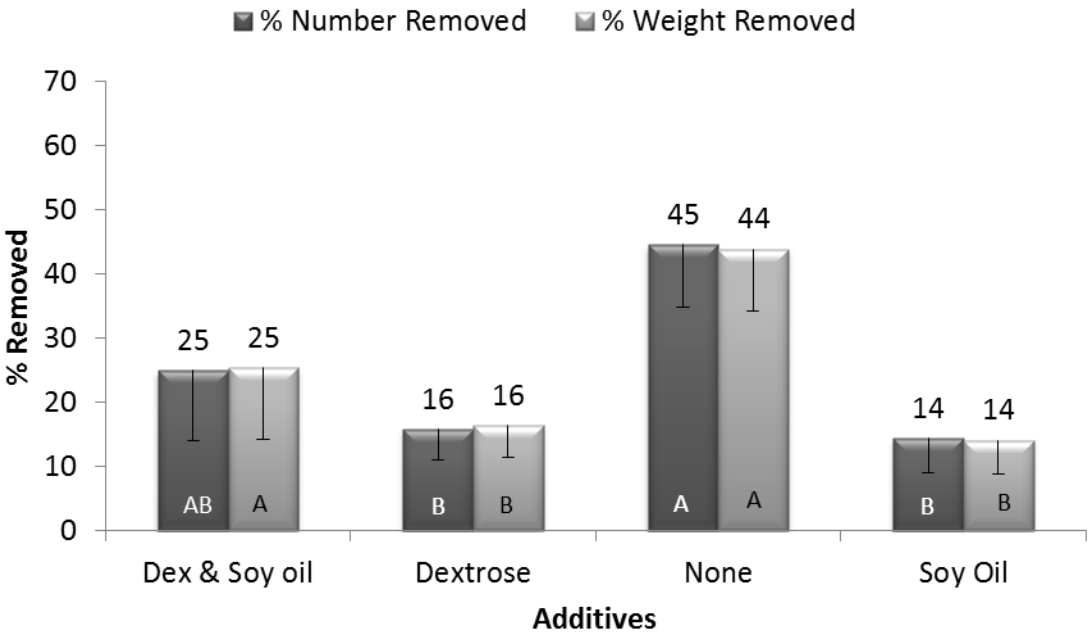

Figure 3-5. Percent number and percent weight of different dog food granular formulations plus additives which were removed by *Nylanderia pubens* in the laboratory experiments. Means with the same letter are not significantly different. Error bars = SEM.

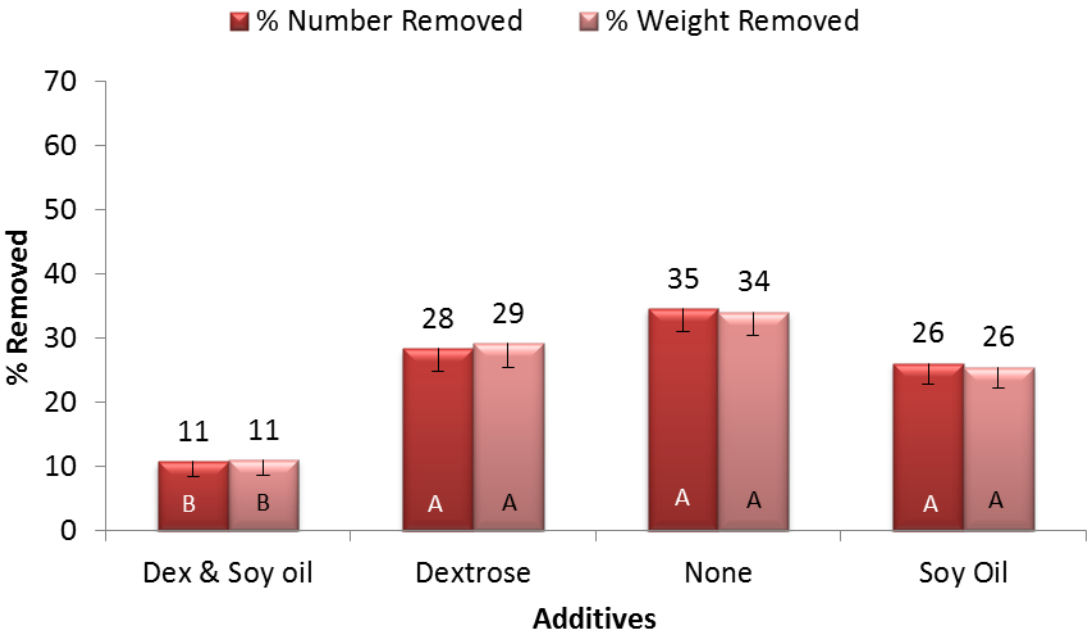

Figure 3-6. Percent number and percent weight of different dog food granular formulations plus additives which were removed by *Nylanderia pubens* in the field experiments. Means with the same letter are not significantly different. Error bars = SEM.

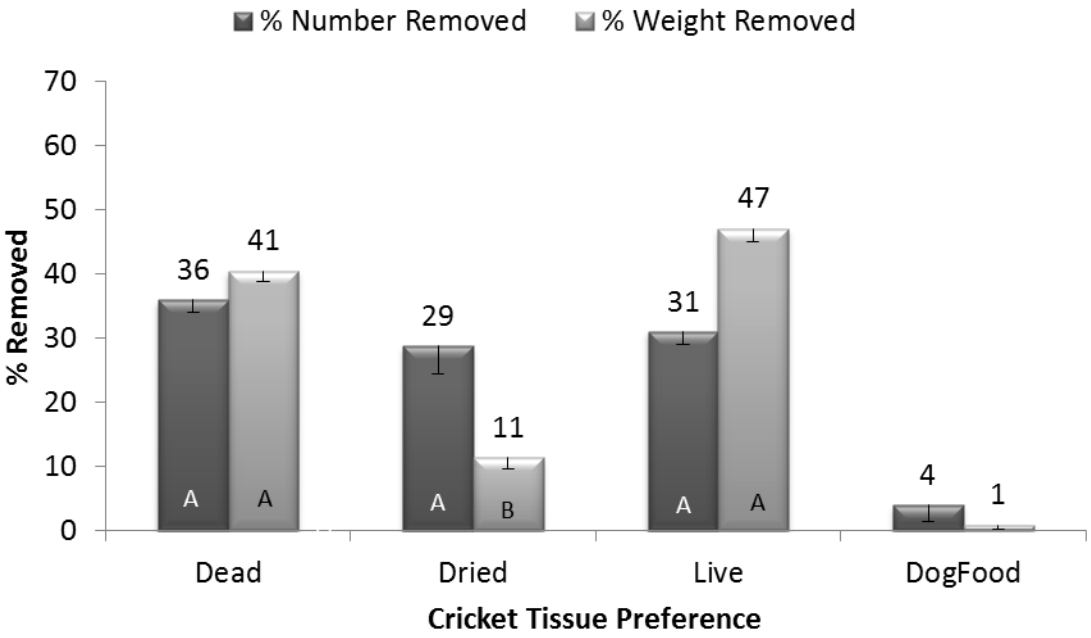

Figure 3-7. Percent numbers and weights of the 3 forms of crickets removed by *Nylanderia pubens* in the laboratory experiments. Dog food was used as a standard treatment but not included in statistics it consists of small granules relative to the size of the crickets. Means with the same letter are not significantly different. Error bars = SEM.

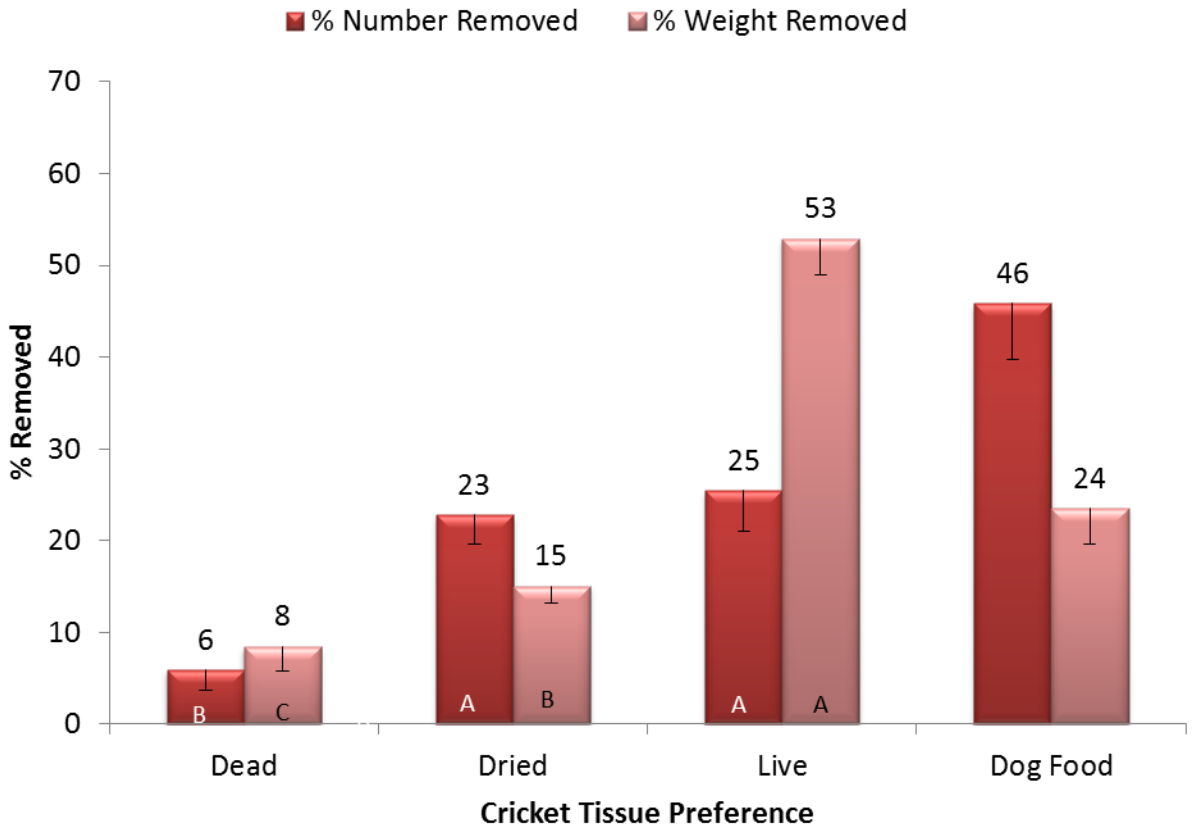

Figure 3-8. Percent numbers and weights of the 3 forms of crickets removed by *Nylanderia pubens* in the field experiments. Dog food was used as a standard treatment but not included in statistics it consists of small granules relative to the size of the crickets. Means with the same letter are not significantly different. Error bars = SEM.

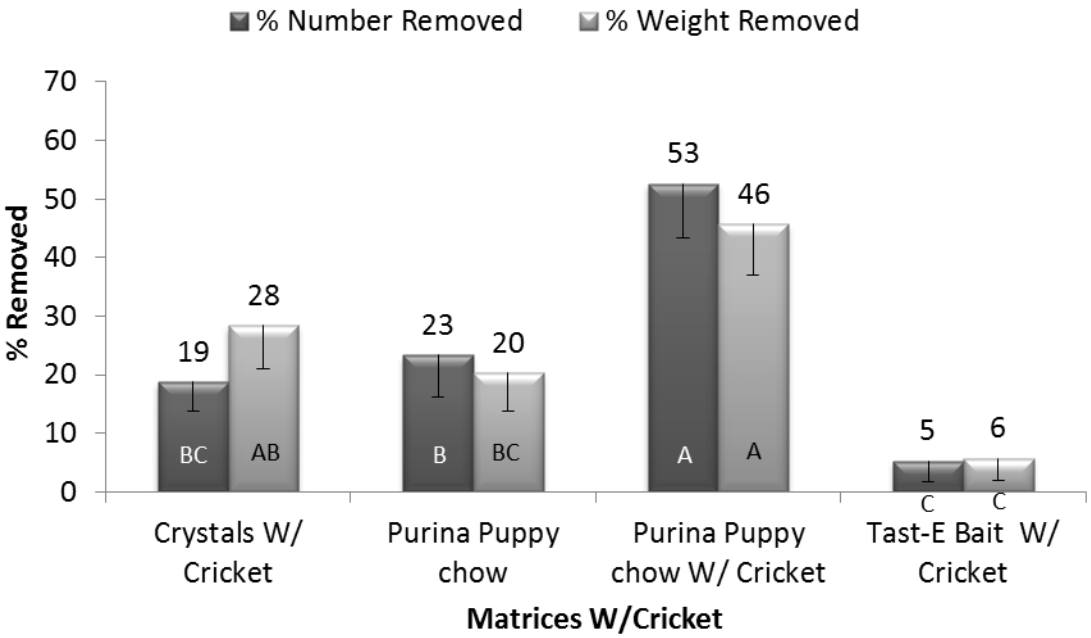

Figure 3-9. Percent numbers and percent weights of granules containing macerated slurry crickets removed by *Nylanderia pubens* in laboratory experiments. Means with the same letter are not significantly different. Error bars = SEM.

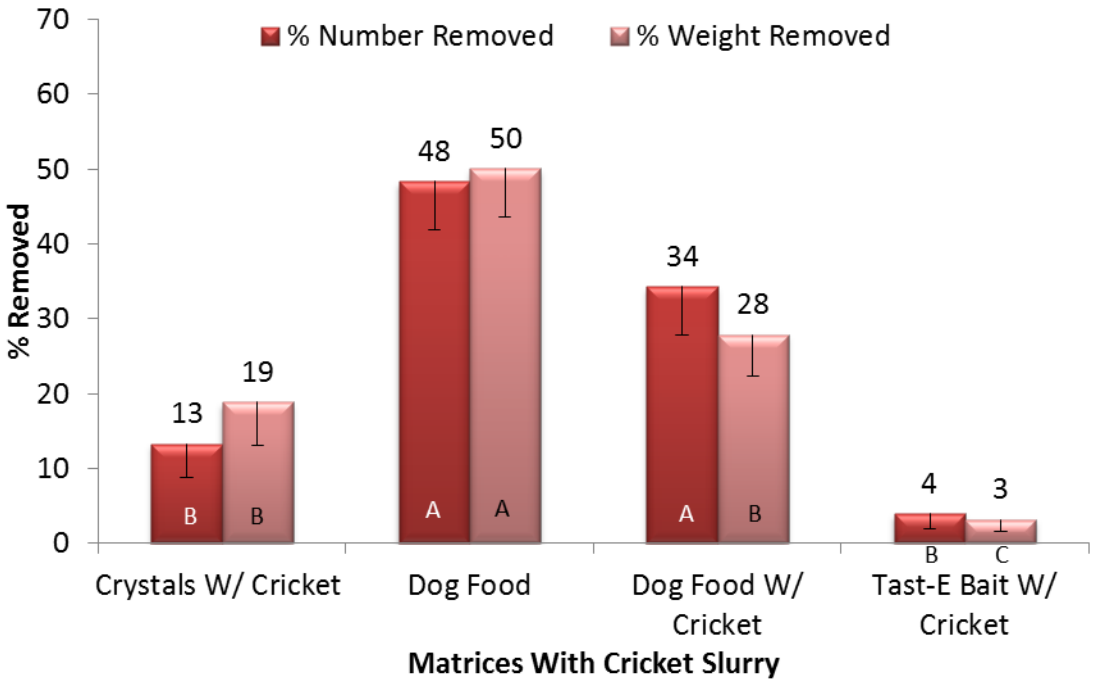

Figure 3-10. Percent numbers and percent weights of granules containing macerated crickets slurry removed by *Nylanderia pubens* in field experiments. Means with the same letter are not significantly different. Error bars = SEM.

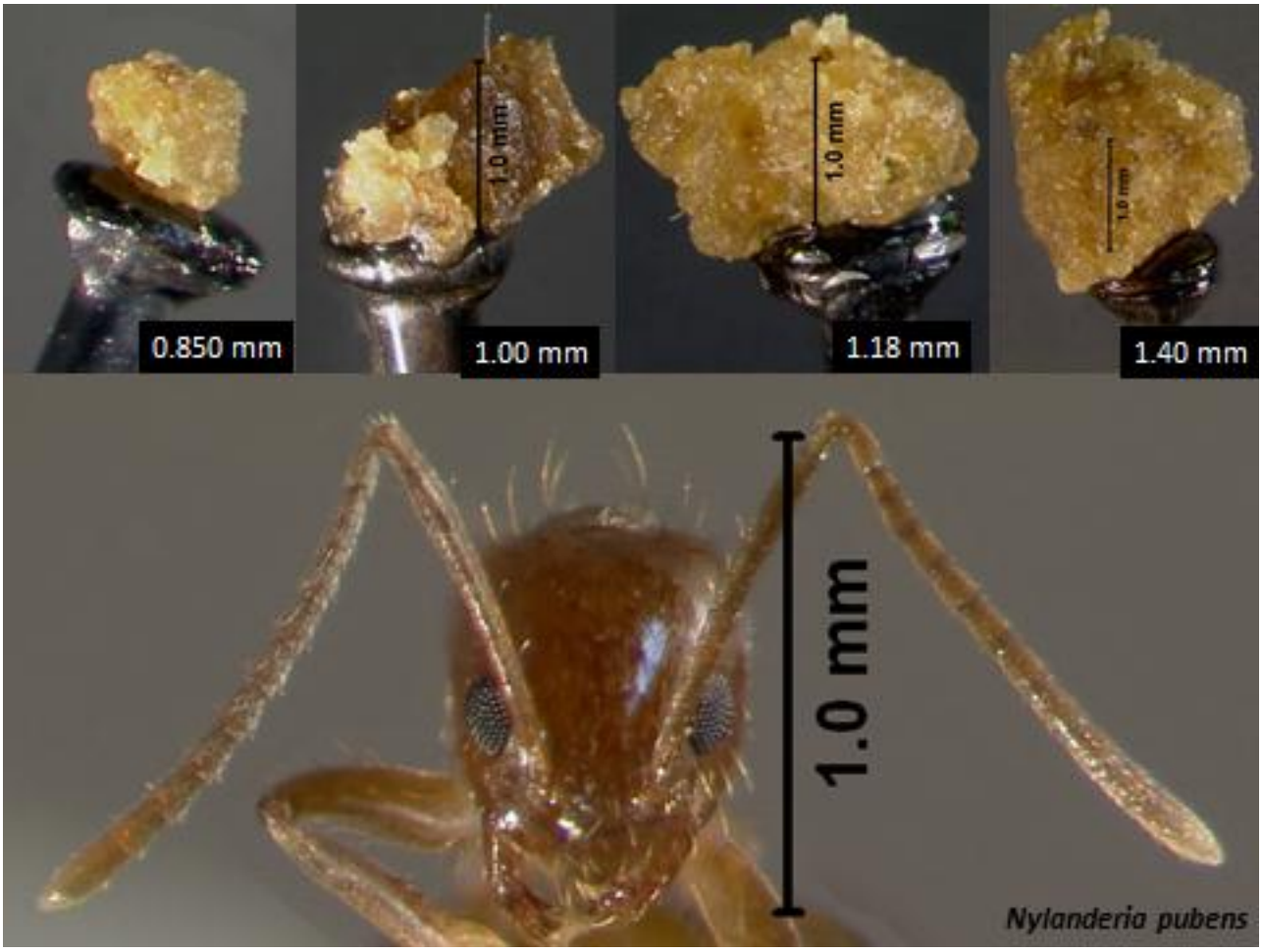

Figure 3-11. Workers head, *Nylanderia pubens*, in comparison with dog food granules used in size preference experiments.

Figure 3-12. *Nylanderia pubens* foraging in a laboratory setting on 1.00-mm dog food granules used in size preference experiments.

Figure 3-13. *Nylanderia pubens* foraging in a laboratory setting on 1.40-mm dog food granules used in size preference experiments.

CHAPTER 4
GRANULAR BAIT MATRIX WITH ADDITIVES AND ACTIVE INGREDIENTS

Introduction

Baits are one of the most effective means of urban pest management because they are easy to work with, can contain little active ingredient, and they capitalize on social ant behaviors such as foraging and trophallaxis (Silverman et al. 2003). Current granular baits on the market are formulated for a number of invasive ant species, but so far none seem to be effective against *N. pubens*. This is because these baits are not specifically formulated for this ant species. For the development of a bait formulation that targets *N. pubens* (chapter 3); active ingredients must be added and the final product tested for its efficacy in controlling this *N. pubens*. The active ingredients to be applied to the formulated granular bait include indoxacarb, imidacloprid and fipronil.

Indoxacarb, S)-methyl 7-chloro-2,5-dihydro 2[[(methoxycarbonyl) [4(trifluoromethoxy)phenyl]amino]-carbonyl]indeno[1,2-e][1,3,4]oxadiazine-4a-(3H)-carboxylate, is considered to be an organophosphate-replacement by the EPA and belongs to the chemical family of oxadiazines . Indoxacarb's insecticidal action blocks the sodium channels in the insect nervous system which affects many of the insects systems such as the digestive system. Indoxacarb is considered to be a reduced risk pesticide, which makes it an optimal choice for application to a formulated bait matrix

Imidacloprid, 1-[(6-Chloro-3-pyridinyl)-N-nitro-2-imidazolidinimine, is a neonicotinoid insecticide in the chloronicotinyl nitroguanidine family (Gervais et al. 2010). Imidacloprid's insecticidal activity affects the central nervous system through several types of post-synaptic nicotinic acetylcholine receptors. This causes nerve impulses to be spontaneously released and subsequently failure of the neuron to

propagate any signal (Gervais et al. 2010). Imidacloprid can be extremely toxic to fish, but application in ant baits should offer little risk to fish.

Fipronil, 5-amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-((1,R,S)-(trifluoromethyl)sulfinyl)-1-H-Pyrazole-3-carbonitrile, is considered a broad-spectrum phenylpyrazole insecticide (Jackson et al. 2009). Fipronil's insecticidal activity is due to the blockage of the essential $GABA_A$-gated chloride channels in the insects central nervous system. This prevents the uptake of chloride ions which leads to excess neuronal stimulation and eventually death (Jackson et al. 2009). Fipronil is considered to be a moderate-to-low risk insecticide, with some environmental concerns because it can be highly toxic to some species of birds and highly toxic to fresh water fish. Termidor®, which contains fipronil, has been utilized to control populations of *N. pubens* populations (Meyer 2008).

The recent increase, spread, and destruction caused by *N. pubens* have pushed the limits of traditional ant control. Because of large numbers of ants, and multiple queens, this pest species becomes a neighborhood problem, not just an individual property problem, and one that has not been successfully controlled by the traditional spray and bait control methods. The purpose of this study is to evaluate a granule bait formulation that targets this pest species with the addition of a slow acting insecticidal active ingredient to allow for distribution of the a.i. within the ant colonies.

Materials and Methods

Insects

Insects used in the experiment were collected, reared, and handled as explained in Chapter 3.

Granular Bait Formulation

The granular bait used to apply the active ingredient was a formulation based on choice experiments in both field and laboratory settings as described in chapter 3. Dog food (Purina One healthy puppy food, Nestle Purina pet care company) was baked at 100°C for one hour and allowed to cool for 30 minutes to eliminate potential insect or mite infestations. The dog food was then ground into small granules with a coffee grinder. The ground up dog food pieces were sieved using different sieve sizes (Thermo Fisher Scientific, Rochester, NY), and the pieces that remained in the 1.00 mm sieve were used in the formulation.

Laboratory reared crickets were ground into a pulp material in a Cuisinart food processor (Cuisinart, East Windson, NJ) then macerated using a 2-ml Pyrex® tissue grinder (Cardinal Health, Dublin, OH). Water (0.5 ml) was added for every 1 g of cricket in the grinding process. The product of the grinding was labeled “cricket slurry” and applied immediately to the dog food granules. An aliquot of 0.2 ml of cricket slurry was added to every 1 gram of 1.00-mm sieved dog food granules and mixed using a 30 ml soufflé container until the granules were saturated.

Active Ingredients

Active ingredients applied to the formulated bait matrix were chosen from current ant baits on the market that target other ant invasive species. The active ingredients that were chosen for these experiments were: indoxacarb, fipronil, and imidacloprid (Table 4-2). The amounts of active ingredient to be added to the formulated bait matrix were based on commercial granular ant baits containing the same active ingredient but further dilution of the active ingredients was necessary to obtain maximum foraging by *N. pubens* in preliminary experiments. Indoxacarb (DuPont™, Wilmington, DE) was

diluted with water, to a final concentration of 0.0225 % in the formulate bait. This dilution was based on Advion® Fire Ant Bait, 0.045% active ingredient (DuPont™, Wilmington, DE) granular ant bait on the market. Imidacloprid (Bayer Environmental Science, Research Triangle Park, NC) was diluted with water, to the concentration of 0.015% in the formulated bait, based on Maxforce® Quantum Ant Bait, which contains 0.03% active ingredient (Bayer Environmental Science, Research Triangle Park, NC). Fipronil (BASF Corporation, Research Triangle Park, NC) was diluted with water, to a final concentration of 0.000225%, based on Maxforce® FC Fire Ant Bait, (0.00045% active ingredient) (Bayer Environmental Science, Research Triangle Park, NC). The control was formulated granular ant bait 0.00288% water added.

To apply the active ingredient to the formulated granular bait matrix, for every 1 g of matrix, weighed out in a 60-ml soufflé container, and 0.20-ml aliquots of the diluted active ingredients were pipetted onto the matrices in the soufflé container. The mixtures were shaken until saturation of the bait matrix occurred. The formulated bait matrices with active ingredients were placed into the refrigerator until were used.

Bioassay

Individual test colonies were prepared by pulling 300 worker ants, two queens and approximately 100 pieces of brood were pulled from laboratory colonies of *N. pubens*. The ants were pulled using an aspirator and included similar numbers of foraging and nurse workers (150 of each). Nurse ants were taken from the nest cells in the laboratory colonies, and most of the nurse ants pulled were carrying brood. An attempt was made to get a minimum amount of 100 pieces of brood per individual test colony.

Once the ants were pulled from the originals colonies, they were placed into a (14.5 cm x 2.5 cm) Petri dish testing arenas. The Petri dish lid had a 2.0-cm hole drilled

into its center, which was covered with a 2.5-cm stainless steel 40 wire cloth dish (Small Parts Inc, Miami, FL) hot glued to cover the hole on the lid. This hole allowed for air flow into the arenas. Each Petri dish testing arena included two 1.5-mL centrifuge snap cap vials (Thermo Fisher Scientific, Rochester, NY), which had the lids removed with scissors. The lidless centrifuge snap caps served as water vial and sugar-water vials which were held in place by folded pieces (1 cm X 5 cm) of Parafilm (American National Can, Greenwich, CT). The lids of the snap-cap vials were used as dishes for soybean oil (Eden Organic, Clinton, Michigan) and clover honey (Wal-Mart Stores, Bentonville, AR) also offered to each experimental colony. To avoid unnecessary deaths in these liquids, cheese cloth was cut and placed over both the soybean oil and honey snap caps. Also included in the testing arenas was a 3 cm X 9 cm lid from a plastic snap-cap vial (Thornton Plastics, Salt Lake City, UT) containing ground cat food (Purina cat chow-naturals plus vitamins & minerals, Nestle Purina, St. Louis, MI) (Fig. 4-1). The ants were given a 4 cm X 1 cm Petri dish containing a plaster bottom. The lid of the Petri dish was covered with yellow cellophane, and this provided a darkened nest cell for the ants. The ants were allowed to acclimate to the testing arenas for 72 hours before the experiments.

After the acclimation period, the honey, soybean oil, cat food and sugar-water were removed from the testing arenas for two days. After two days, the formulated bait matrices with active ingredient were taken out of the fridge and allowed to reach room temperature before being placed in the experimental colonies. The dead ants in the test arenas were counted and replaced before the experiment was started. Once at room temperature, 90 granules were counted out and weighed on a (41 x 41 x 8 mm)

polystyrene weigh boat (Thermo Fisher Scientific, Rochester, NY). The weighed granules were then placed into the testing arenas. Ants were allowed one hour to forage on the granules. After one hour, the bait granules remaining on the dishes were removed from the testing arenas, weighed and counted. The original food that had been removed was refreshed if any food was low and it was then replaced into the testing arenas. Sugar-water and water were added and the nest cell were moistened every third day afterwards. The ants were observed every other day for 14 days. Numbers of dead ants were recorded and the dead ants were removed from the testing arenas at that time. At the end of the 14 days, the remaining live ants were freeze-killed. The live ants in the control arenas were placed back into the colonies they were originally pulled from.

Analysis

Experimental ant colonies that removed less than 10% of the granules provided, were no included in the analysis of the results. This was done to eliminate colonies that did not show typical foraging behavior. To compare the added active ingredients by consumption and percent mortality, the data was arcsin square root transformed and ANOVAs run were in the statistical software JMP (SAS Inst., Cary, NC). Student-Tukeys tests were used to compare means.

Results

There was no significant difference in the consumption of baits with different A.I.'s (F= 1.3691, P= 0.2824) (Fig. 4-2). Because all baits were consumed evenly, there was no correlation between the amount of bait taken and ant mortality rate. There are significant differences amog the different active ingredient formulations (F=3.5069, P= 0.0353; F= 4.4851, P= 0.0153; F=4.3135, P= 0.0176; F=4.4610, P= 0.0156; F= 4.8206,

P= 0.0116) with fipronil causing significantly greater mortality than the other treatments (Students T-test: α= 0.05, *t*= 2.0930) (Fig. 4-3) than indoxacarb, imidacloprid, and the control baits. Mortality over the 14-day trial period caused a decrease in the numbers of *N. pubens* in all active ingredients treatments. The total percent mortalities for indoxacarb and imidacloprid, over the 14-day experimental period, were 37% and 26%, respectively. These treatments caused less than half the mortality caused by the of fipronil bait, which caused percent ant mortality of 86% over the 14 day experimental period.

Discussion

There is much debate about an effective control method for *N. pubens*. Meyers (2008) tested a variety of insecticidal control methods on this species, but no effective formulation was found. Due to the limited knowledge on nutritional preferences of *N. pubens*, an efficient form of control has not been found. The most common means of controls are insecticidal sprays, other ant granular and liquid baits, which have had limited success in controlling this invasive pest ant species. Because of the inefficiency of current methods of control, a new control method is needed. Research was conducted on including different active ingredients on a bait matrix. The active ingredients chosen had been successful against similar ant species: *S. invicta*, and *L. humile*.

Stringer et al. (1964) proposed that a bait active ingredient should have delayed toxicity, be able to be transferred from one ant to another, and be non-repellent to the foraging ants. Fipronil fits this model by displaying delayed toxicity, was transferred from one ant to another either through trophallaxis or by social grooming, and, as with all of the active ingredients in this experiment, did not discourage foraging from the ants (Fig.

4-2). In Texas, Drees et al. (2009) suggested using Termidor® SC (9.1% fipronil) as an outdoor spray for control of *N. pubens* because they have observed high mortality rates with this product. Fipronil has also proven to give high rates of mortality in formulations for *L. humile* (Hooper-Bùi et al. 2000). The results for indoxacarb and imidacloprid did not confirm past success rates of these active ingredients (Rust et al. 2002, Barr 2003, Oi et al. 2006).

The low rate of mortality with indoxacarb bait was surprising because experiments on *S. invicta* offered Advion fire ant bait (0.0045% indoxacarb) showed a reduction to colony size of 95% by day five (Oi et al. 2006). Over the fourteen-day trial period the total percent of mortality of *N. pubens* in my experiment did not exceed 37%. These results could be due to the lower percent of active ingredient (0.00255%) applied to my experimental granular bait. With further dilution the active ingredient through trophallaxis most of the ants, *N. pubens*, may not have acquired a lethal dose. Fire ant bait is an lipid place on top of a defatted corn cob which could cause the active ingredient to react differently, as seen in deactivation of an active ingredient, from binding to a bait too closely (Cress 1990, Stanley 2004). Deactivation of the active ingredient, indoxacarb, could have been a contributing factor in the ineffectiveness I observed with my experiments. Indoxacarb has been proven to suppress foraging in fire ant experiments, in 48h in field experiments (Barr 2003). The lack of foraging suppression I observed suggests that the dosage of indoxacarb I applied to the bait matrix may have been too low. Klotz et al. (2004) observed *L. humile* actively foraging on a bait but with no reduction on ant numbers, as it was seen in my experiments the authors concluded that the A.I. concentration was too low. More trials should be conducted with different

concentrations of indoxacarb in the baits which containing materials the ants forage readily.

Experiments with *L. humile* proved imidacloprid to be successfully in the suppression of ants in a field setting. Imidacloprid is photosensitive and needs to be protected from the sun in order to be effective in suppressing ants in a field setting (Daane et al. 2008). Shielding for bait stations maybe required for high efficacy. The photo-degradation of the imidacloprid, in Daane et al.'s (2008) trials only took a few hours. My experiments with the same active ingredients were run in a laboratory setting, under a temperature-and light-controlled environment where photo-degradation should have been at a minimum. More experiments should be conducted on the rate of degradation of imidacloprid and its photo sensitivity. In studies with *L. humile*, the rate of colony decline was 50% with dosages of imidacloprid between 0.0005%-0.005% (Rust et al. 2002), but this A.I. was applied at 0.0015% to the granular bait matrix in my experiment. The lack of success in controlling *N. pubens* could have been due to the baiting method. The high numbers of *L. humile* have been controlled by using bait stations with liquid imidacloprid, whereas I used a granular bait with imidacloprid. In previous experiments liquid bait stations, although impractical control of a large infested area, are very attractive to *L. humile*. This is because they *L. humile* prefer, in some studies, liquid sugary food sources offered as observed by Rust et al. (2002).

Several factors may have caused the low bait efficacy I observed: including the low concentration of the active ingredients in the baits, possible deactivation of the active ingredients, and insufficient time for the delayed toxic effect to be expressed with only a 14-day experimental period. These experiments should be observed over greater

amount of time, as seen in experiments by Rust et al. (2002) and, Daane et al. (2008) with *L. humile*, and Collins et al. (1998) with *S. invicta*. A longer observation would provide information on whether or not the diluted active ingredients caused an additional delay in toxicity. Since these experiments took place in a controlled laboratory environment, further research on the effectiveness of the formulated granular ant bait with fipronil should be done in a field environment. This will test the efficacy of this bait in a non-controlled setting thus providing information on the possible limitations of this bait formulation.

Table 4-1. Products used in bait formulations used in laboratory choice and efficacy experiments against *N. pubens* colonies.

Product names	Trade name	Chemical name	% Active ingredient in commerical product	% Active used
Arilon™ Insecticide	Indoxacarb	(S)-methyl 7-chloro-2,5-dihydro-2[[(methoxycarbonyl)[4(trifluoromethoxy)phenyl]amino]-carbonyl]indeno[1,2-e][1,3,4]oxadiazine-4a-(3H)-carboxylate	20.0	0.02250
Premise 2	Imidacloprid	1-[(6-Chloro-3-pyridinyl)-N-nitro-2-imidazolidinimine	21.4	0.01500
Termidor® SC	Fipronil	5-amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-((1,R,S)-(trifluoromethyl)sulfinyl)-1-H-Pyrazole-3-carbonitrile	9.1	0.00023

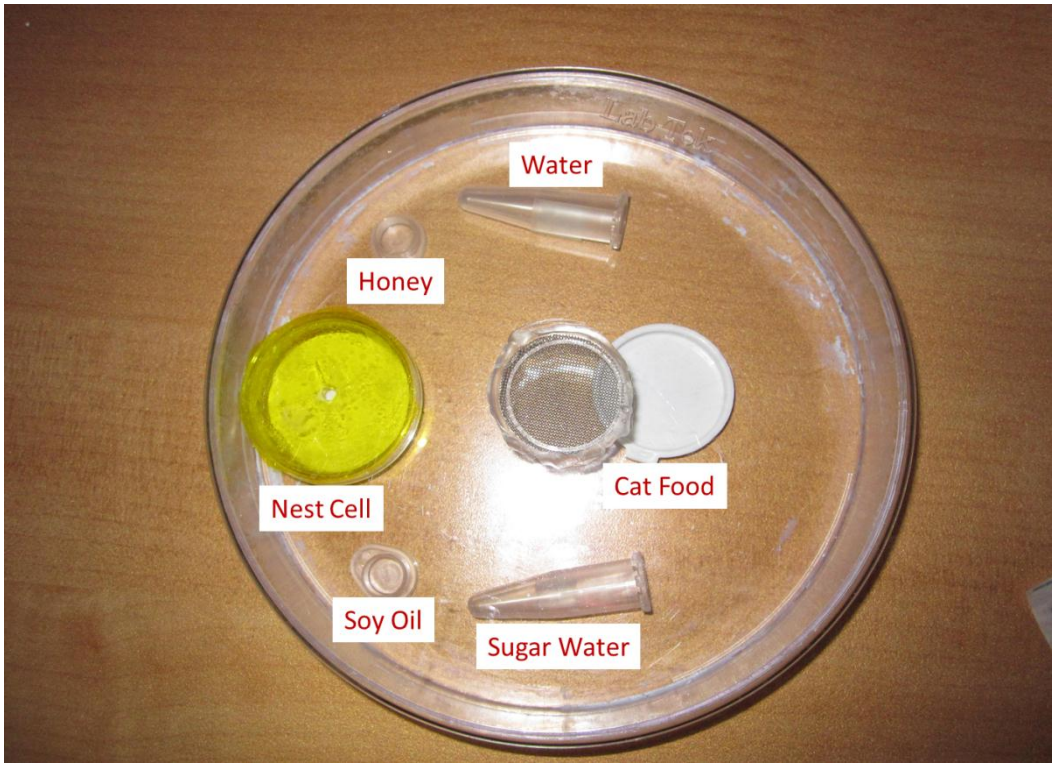

Figure 4-1. Testing arena used for experiments on *Nylanderia pubens* using granular bait matrix applied with active ingredient.

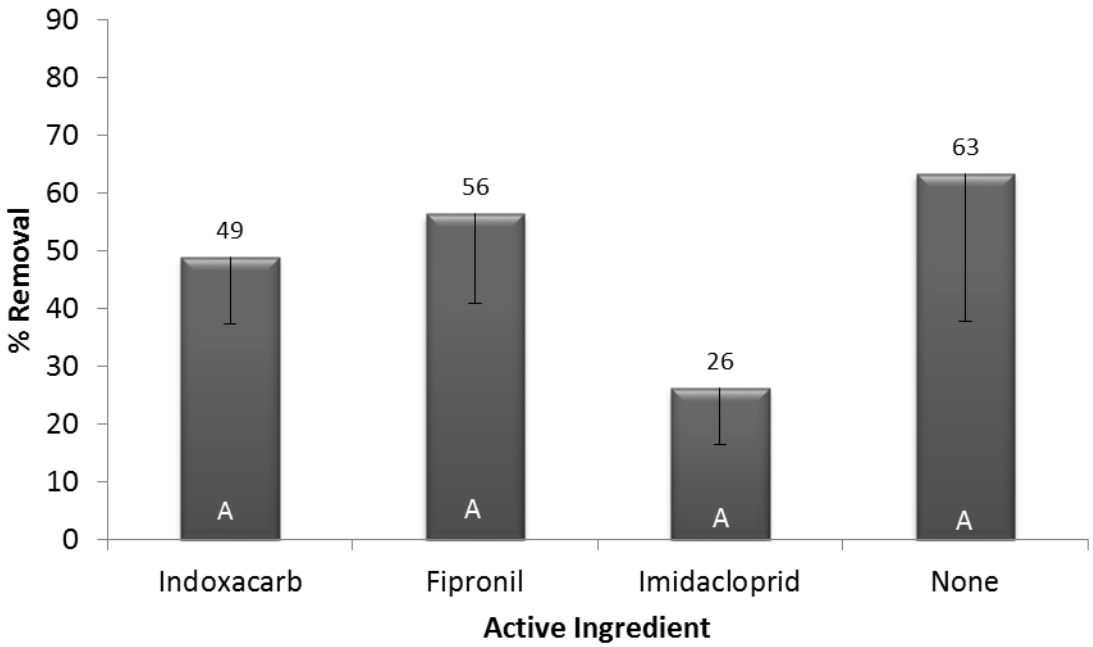

Figure 4-2. Percent removal by *Nylanderia pubens* colonies of granular bait with different active ingredients in laboratory experiments. Means with the same letter are not significantly different. Error Bars= SEM.

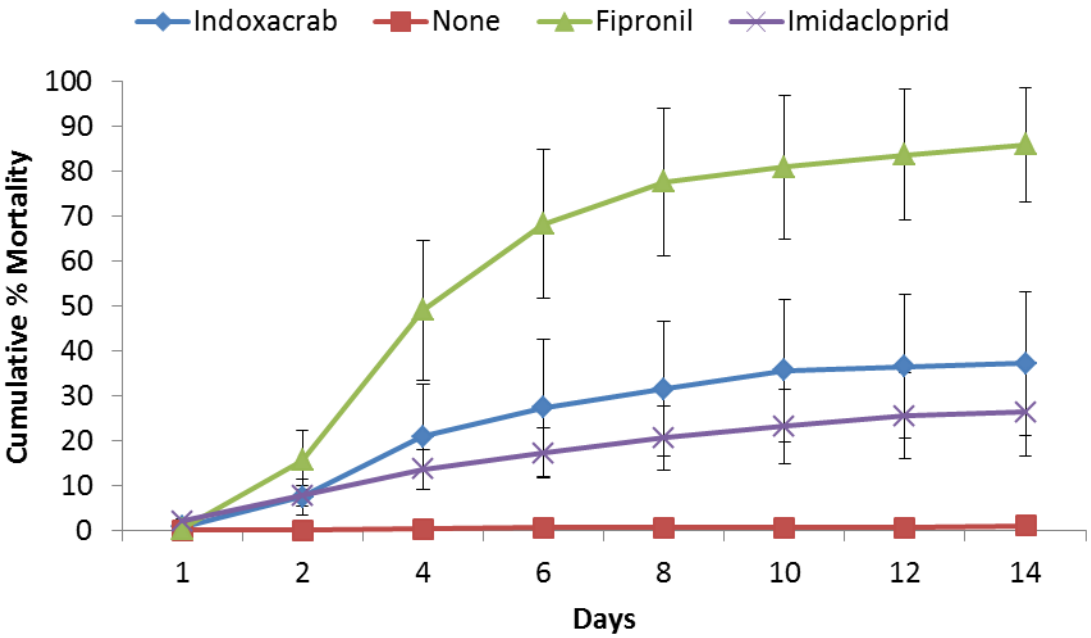

Figure 4-3. Cumulative percent mortality of *Nylanderia pubens*, from laboratory colony fragments, after consumption of granular bait with different active ingredients. Error Bars= SEM.

CHAPTER 5
CONCLUSION

Nylanderia pubens placed in arenas or located in field situations exhibited preferences for certain particles sizes and compositions of food. These preferences were based on both the efficient retrieval of resources and nutritional needs of the colony. In my experiments foraging ants: 1) located the food through random foraging, 2) picked up or fed on food particles, 3) returned to the nest, laying down a pheromone trail to recruit other ants.

Nylanderia pubens did not follow trails precisely in order to recruit large numbers of ants to overwhelm the food source. *N. pubens* appears to follow the foraging strategy outlined by Oster et al. (1978) as trunk-trail foraging rather than mass recruitment. Trunk- trail foraging is when a pheromone scent trail is laid down, but the ants following the trail deviate to forage from the main trail in scattered patterns looking for food (Oster et al. 1978). In my experiments *N. pubens* depleted a variety of particle sizes, they showed a lack of cooperation in removal of food particles, and massive numbers of ants were never observed in an area with high concentrations of preferred food size particles. In my laboratory and field experiments, *N. pubens* foraged on granule sizes 0.850 mm -1.18 mm, the percentages of food particles removed were 28-33% in the lab and 21-35 % in the field, which indicates both scattered pattern of foraging and a lack of mass recruitment to a food source. *N. pubens* style of foraging contrasts the foraging strategy observed in *L. humile*, which employs mass recruitment as their foraging strategy (Roulston et al. 2002). Foragers of *L. humile* have been observed cooperating with other foragers, recruiting large numbers of their foragers to a food source, and depleting preferred food sizes first. In my experiments *N. pubens* was observed not to

follow the foraging strategy employed by *L. humile*, the removal of varying sizes of granules in both the field and laboratory along with their scattered foraging pattern followed what is described as trunk-trail foraging. In my experiments, *N. pubens* trunk-trail foraging behavior appears to not only follow the optimal foraging theory but also follows the head width and body size in relation to food particle theories as well.

On top of foraging strategies, ants also follow patterns based on the food choices they make, those patterns are called foraging theories. In my experiments, the foraging strategies that *N. pubens* followed was the: optimal foraging theory (Nonacs et al. 1990, Roulston et al. 2002).It was also clear that the head width and the overall size of the ants dictates the size particle they pick up (Traniello 1989, Hooper-Bùi et al. 2002).

Ant foraging normally fits what has been described as fitting the optimal foraging theory (Nonacs et al. 1990, Roulston et al. 2002). The optimal foraging theory states that ants should take the biggest pieces of food particles that they can carry, in order to increase their net energy intake per unit of effort (Roulston et al. 2002). In my experiments with granular size removal in the laboratory and in the field, *N. pubens* seemd to fit the optimal foraging theory, because the ants were taking the most amount of calories by returning with the greatest mass of food particle, while spending the least amount of energy. *N. pubens* removed the largest sizes of food particles by weight thus decreasing the number of trips to the food source, which follows the optimal foraging theory.

Nylanderia pubens did not cooperate with one another to remove a food source. This lack of cooperation indicates that although these ants follow the optimal foraging theory for weights of granules removed, more important reasons for their foraging

choices need to be examined to elucidate factors associated with their moving an optimal amount of food back to the colony. In the theories discussed by Hooper-Bùi et al. (2002) and Traniello (1989), the preferences of ant species not only, are, governed by the foraging ant head width, but also determined by the overall size of the foraging ant in relation to the food particles. *Nylanderia pubens* actively foraged on dog food pieces 0.850-1.18 mm in the laboratory, and 0.850 mm predominately in the field. *Nylanderia pubens* has a relatively small head width (0.55-0.64 mm) and body size (2.5-3.0 mm) (Trager 1984, Meyers 2008), which determines the preferred size of food they will remove. Traniello (1989) and Hooper-Bùi et al. (2002), used similar ants species and measured head widths and body sizes in relation to food particle preferences. The head widths and body size of *N. pubens* are similar to the head width and body size of *L. humile* (0.66 mm and 2.0-3.0 mm, respectively) (Wild 2004). Both species preferred granular sizes, 0.840-1.00 mm, whereas the larger, polymorphic ants species *S. invicta* (head widths: 0.45-1.50 mm, body sizes: 2.00- 6.0 mm) preferred the granular sizes: >2.00 mm (Wood et al. 1981, Tschinkel et al. 2003). *N. pubens* is a monomorphic species with an overall size of 2.5-3.0 mm, so the particles that the workers can carry are limited to specific sizes, unlike *S. invicta* that can carry much larger range of particles due to polymorphic nature of the colonies. In analyzing the experiments of Traniello (1989), Roulston et al. (2002), and Hooper-Bùi et al. (2002) on foraging theories, in conjunction with the observations in my experiments, *N. pubens* is small size ant and small head width causes the optimal size food granule for this species to be between 0.850-1.00 mm.

There is a need for the development of insecticidal controls *for N. pubens.* Current methods of control include insecticidal barrier sprays, which have been less than effective in controlling of this species. Baits have the ability to offer a level of control that has not yet been obtained by sprays. The current types of baits out on the market for ant control include gel baits, liquid baits, and solid granular baits. A liquid or gel bait is usually one that requires a bait station and constant reapplication due to the elements. Liquid baits are developed for ants that display mass recruitment to food sources; *N. pubens* lacks mass recruitment strategy, so liquid baits will fail to control this pest species. Granular baits, which can be scattered on the landscape, take advantage of the trunk-trail foraging of *N. pubens*, and of their foraging strategy optimizing the chance that the bait will be located and foraged upon. The potential for broadcast application of granular baits makes this formulation ideal for large-scale ant control.

In some cases there is little distinction between liquid and solid baits, as in the instance of popular fire ant baits. Fire ant baits consist of oil placed on a carrier (de-fatted corn cob) (Loggren et al. 1963, Stanley 2004). This method of baiting targets foraging ants, which can only feed from liquid-based foods. With fire ant baits, workers forage on and remove the oil off the bait, so this bait looks like a solid granular bait, but behaves like a liquid. This behavior does not force the foraging ants to return to the nest with the bait particle, so the active ingredient will enter the colony as a liquid. On the other hand, the experimental components that I tested were solid foods, which would have to be taken back to the colony in order for them to be broken down by the larvae before they could be utilized by the adult ants. Solid bait matrices I chose were based

on the foraging strategies of *N. pubens* and previous research on ideal components of solid baits.

Components of baits consist of an attractant, carrier and active ingredient (Stanely 2004). Traditional baits for *S. invicta*, consist of oil (attractant) on de-fatted corn grit matrix (carrier) and multiple active ingredients have been applied to these components (Loggren et al. 1963, Stanley 2004). This method of control is ideal for *S. invicta* because, it capitalizes on the ant's recruitment foraging strategy, and on the food preferences this ant species has toward lipids (Stanley 2004). *N. pubens* was not determined to be lipid feeders, nor are they mass-recruitment ants, as described previously, so traditional baits that work for *S. invicta*, do not work for *N. pubens*. On the other hand, baits that contain proteins and carbohydrates are very attractive to species such as *L. humile* and *Paratrechina* spp. that are not attracted to the lipid-based fire ant baits (Stanley 2004). In order to enhance a carrier, it is important to know the ant species food preferences, based on field observations, laboratory experiments, and by studying the literature on other similar ant species.

I have observed *N. pubens* to be feeding from insect tissue, and to be attracted to honey in field and laboratory environments. In other observations, *N. pubens* foraged on honey dew from aphids, plant nectaries and insect tissue (Creighton 1950). *N. pubens* has been blamed for crop destruction from tending to aphids and mealy bugs (Wetterer et al. 2008). *N. pubens* prefers carbohydrate powders 2:1 to protein powders (Cook et al. 2012). In similar ant species, such as *Rhytidoponera metallica* (Smith), Dussutour et al. (2009) found that the ants also preferred carbohydrates to proteins in a 2:1 ratio. Petralia et al. (1980) suggests, that a solid proteinaceous food which has to be digested

by the brood would be ideal bait. With the findings of Cook (2012), and Dussutour (2009), the observations of Wetterer (2008), Creighton (1950), in conjunction with *N. pubens* foraging theories and strategies, and my own personal observations, I chose to test a number of animal foods based on their protein and carbohydrate/minerals as possible granular bait matrices along with additive for possible enhancement to the carriers.

Because *N. pubens* foraging follows the optimal foraging theory, field colonies of ants displayed a preference to dog treats, the highest protein matrix choice. In the laboratory, given the limited number of ants and brood per satellite colony, along with the close proximity of the nest cell, ants foraged on most food matrix choices evenly. The laboratory results also point to the optimal foraging theory as the predominant force in *N. pubens* foraging. *N. pubens* actively foraged on all foods available thus increasing the amount of calories returned to the nest cell. The relative composition of essential elements in the bait matrices may be important in maximizing foraging by specific ant species (Stanley 2004). Although dog treat was more readily foraged upon in the field experiments, dog food had the closest carbohydrate to protein ratio (1.5: 1), as prescribed by Cook et al. (2012), compared with other materials used. Dog food was an optimal carrier component, not only because of its carbohydrate/ protein composition and spread ability to take advantage of *N. pubens* foraging patterns, but also because it was readily accepted by the ants and could be easily enhanced by addition of other materials.

The enhancements made to the granular bait were based on a compilation of knowledge obtained from experiments done on food preferences of: *S. invicta* (Vogt et

al. 2002), *Anoplolepis gracilipes* (Smith) (Harris et al. 2012), and *Paractrechina longicornis* (Latreille) (Kenne et al. 2005). *S. invicta* and *P. longicornis* have different foraging characteristics but are similar to *N. pubens* in at least one aspect of their nutritional needs, whereas *A. gracilipes* has both a similar foraging strategy and diet to *N. pubens*.

S. invicta is an omnivore which actively mass recruits and forages on a broad range of liquid materials, seeds, and arthropods, including: plant sap, plant nectars, and honeydew from hemipteran tending (Vogt et al. 2002). *A. gracilipes* has a broad diet and displays scatter-pattern foraging. *A. gracilipes* is described as a scavenging predator that preys on a variety of insects, isopods and their diet can include larger animals such as birds and reptiles (Harris et al. 2012). They also actively forage on carbohydrate rich foods including: honey-dew from aphids, plant exudates, and fruit particles (Haines et al. 2008). *Paratrechina longicornis* are opportunistic omnivores, which employ the foraging strategy of mass recruitment and group hunting (Kenne et al. 2005). They thrive on live and dead insects, honeydew, fruits, plant exudates, and foods from around human dwellings (Pagad 2010). *N. pubens* is somewhat similar to these three ant species, based on observed dietary habits such as aphid tending, scavenging and foraging on plant nectars. The information learned from these ant species led me to try oils, sugars, and insects applied to the dog food carrier, as possible optimal enhancements.

The oil and sugar bait enhancements I chose were based on other ant preferences and baits used in their control, but they did not significantly increase foraging by *N. pubens*. However, the addition of live cricket tissue, resulted in increased foraging, as

was the case in a study by Williams et al. (1990), who used live fly pupae as a carrier for bait. Although the use of live insects as bait worked in experiments done by Williams et al. (1990), live insects are currently impractical as commercial bait. However, insects can be incorporated into baits as macerated tissue added to the baits. In my experiments *N. pubens* actively foraged well on the dog food matrix with added cricket tissue, but field colonies did not differentiate between plain dog food and dog food with the cricket additive.

The active ingredients used in my experiments were chosen for their relatively fast action, despite being slow enough to allow transfer through the colony by social grooming and trophallaxis. Slow-acting ant toxicants are preferred because, if the toxicant ants too quickly, for instance by paralyzing the mouthparts of the ants or killing the foraging ants; that can prevent the toxicant from reaching the entire colony, such as paralyzing the mouth parts of the ants or killing the foraging ant. Because of the limited foraging of the baits with the commercially available label rates, the active ingredients were diluted by 50% which caused an increase in foraging. In my experiments, baits with all three active ingredients were foraged upon evenly, indicating that the active ingredients applied to the bait matrix did not discourage foraging.

In my laboratory experiments, fipronil displayed faster mortality and higher rate of mortality than the other tested active ingredients. Fipronil has been effective in control of many ant species mentioned previously: *A. gracilipes*, *L. humile* (Stanley 2004, Wiltz et al. 2010b), and *S. invicta* (Wiltz et al. 2010a). The reason for the success of fipronil in my laboratory experiments was probably its high rate of horizontal transfer within a colony besides transfer by trophallaxis. The horizontal transfer can be from social

grooming or the removal of dead ants that are contaminated with fipronil, as seen in experiments by Soeprono et al. (2004), Choe et al. (2008), Wiltz et al. (2009), and Wiltz et al. (2010a,b). Choe et al. (2008) suggest that the high rate of horizontal transfer seen is due to the chemical nature of fipronil that give this active ingredient a high affinity for the lipids found on the wax layer of insect cuticles. Although fipronil outperformed the indoxacarb and imidacloprid in the laboratory experiments, further research should be conducted to explore the performance of all of these insecticidal active ingredients in a field environment. My observations and those of other scientist (Vogt et al. 2003, Challet et al. 2005 and Wiltz et al. 2010b) point to noticeable differences in the preference and behaviors between laboratory and field insects.

Differences between controlled environment in the laboratory and more variable environment in the field lead to differences in foraging behaviors preference to bait components (Traniello et al. 1983, Vogt et al. 2003, Challet et al. 2005). Differences in the presence and proportion of different developmental life stages in the colony (Traniello 1989) can also be important factors in determining difference between laboratory and field results. Temperature also can play an important role in the foraging activities of *N. pubens*. Calibeo-Hayes et al. (2010) described limited foraging by *N. pubens* when the temperature was below 15.6˚C. Temperature also plays important role in the shape and structure of ant nesting areas (Challet et al. 2005. In my experiments, the difference in food choices between the laboratory and field colonies could have resulted from to the varying ranges of temperatures (23.9-26.6˚C) and microclimates in the field location used (Vogt et al. 2003, Challet et al. 2005 and Wiltz et al. 2010b).

Another reason for the differences seen between results from laboratory and field environments could be attributed to the developmental differences between the colonies. Selection of food sources is done at a colony level, and can be affected by age of foragers, their life expectancy and their foraging ability (Traniello 1989). The average life of ant foragers in the field is wrought with dangers from predators, and other factors, but in the laboratory, the controlled conditions allow for a longer life span. Foragers of *Cataglyphis bicolor* (Fabricius) have a life expectancy of 6.1 days under field conditions, whereas in a laboratory setting they can live up to months (Traniello 1989).

Life stages in ant colonies also play an important role in the foraging preferences and the fitness of the colony. The amount of brood in the colonies, in the laboratory and field, could be responsible for the foraging preference differences observed. Fourth instar larvae do the most of the digestion of the protein digestion in the ant colony's (Petralia et al. 1980, Weeks et al. 2004), and their presence in a colony can determine the colonies foraging on proteinaceous materials. The development of bait that may work at different times of the year, when numbers of the 4^{th} instars may be at very different levels, may require use of formulation that is not affected by the ant population composition (Weeks et al. 2004). Bait matrices choices in the laboratory could have been caused by the absence of 4^{th} instar larvae in the colonies (Petralia et al. 1980). In my laboratory colonies, choices of components removed could have been a reflection of their inability to digest materials with high protein content. This was not the case in the field colonies, which probably had relatively more brood than in the laboratory colonies, given the time of year the experiments were conducted and the high ant populations

observed. Temperature and colony developmental stages, I believe, are the reasons for the foraging and preference differences that I observed. Even with the differences between in the laboratory and field experiments, my observations can help In the design of future strategic control of *N. pubens*.

My experimental results provide a better understanding of potential components for a granular bait to be used with *N. pubens*. Future experiments should include other active ingredients beyond those I applied to the formulated bait matrix. Other future experiments should include longer laboratory studies on the active ingredients I tested, as explained in chapter 4, to explore the possibility of horizontal transfers of active ingredients. My results can serve as a basis for future development of baits for *Nylanderia pubens*.

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BIOGRAPHICAL SKETCH

Jodi Michelle Scott, daughter of Patricia and Robert Bower, was born in Orlando, Florida. She was raised in Orlando, Florida, with her older sister, Nichole Bower. She graduated from Colonial high school in 1998. She attended Valencia community college, east campus and graduated with an Associate of Arts degree in 2001. She then joined the United States National Guard from June 2001 to June 2007. While in the Guard she attended the University of Central Florida, earning the degree of Bachelor of Science in 2008. She then became a laboratory technician in the physiological department at the University of Florida. She entered the graduate program in the Department of Entomology and Nematology at the University of Florida specializing in the urban entomology under Dr. Philip Koehler in 2011.