AN INTEGRATED PEST MANAGEMENT STRATEGY FOR
Nylanderia fulva IN FLORIDA

By
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To KH
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<td>Brown crazy ant</td>
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<td>CCA</td>
<td>Caribbean crazy ant</td>
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<tr>
<td>IGR</td>
<td>Insect Growth Regulator</td>
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<tr>
<td>IPM</td>
<td>Integrated Pest Management</td>
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<td>Pest Management Professional</td>
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Abstract of Dissertation Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

AN INTEGRATED PEST MANAGEMENT STRATEGY FOR *Nylanderia fulva* IN FLORIDA

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Invasive species are a serious concern both economically and ecologically. *Nylanderia fulva* (Mayr) is an invasive ant that is a serious pest in the southern United States. Already established as an urban pest, this ant has the potential to be an agricultural, industrial and ecological pest, as well. Studies were undertaken to understand the biology and ecology of *N. fulva* with the goal of developing an Integrated Pest Management (IPM) strategy for Florida. The population density and dispersal of *N. fulva* was mapped at the Jacksonville Zoo for three years. *Nylanderia fulva* did not radiate naturally from points of infestation but rather spread to new parts of the zoo by human-mediated transport. *Nylanderia fulva* foraging activity decreased with decreasing temperature but precipitation volumes had little effect on foraging activity. Macronutrient studies were undertaken to determine seasonal food preference. *Nylanderia fulva* counts were higher on protein-based foods but carbohydrates were also consumed throughout the year. In no-choice bait acceptance and efficacy studies, it was shown that *N. fulva* had greater acceptance of ant baits composed of a protein and carbohydrate containing matrix. Additionally, insecticide active ingredients that
demonstrated efficacy against *N. fulva* were hydramethylnon (Amdro® Pro, Maxforce® Complete, Maxforce® Fine Granular and Maxforce®) and fipronil (Maxforce® Ant Killer Bait). Insecticide bioassays were performed to evaluate the repellency and efficacy of commercially available insecticides. *Nylanderia fulva* were not completely repelled by any insecticide tested although fewer ants crossed a surface treated with Temprid®. Few insecticides provided rapid control. Termidor® and Temprid® were the best performing with mean mortality of 100% in 13.4 and 19 days, respectively. Field studies were conducted using protein-based granular and carbohydrate-based liquid baits. Even when baits were applied prior to population peak, ant suppression was not sufficient. A program of insecticide application followed by baits was not conclusive but ant reduction at sites with lesser numbers of ants suggest that successful management is likely population-size dependent. These data provide valuable information to pest management professionals and homeowners in Florida to potentially manage this invasive pest, as well as, guidance in development of a sustainable Integrated Pest Management strategy.
CHAPTER 1
*Nylanderia fulva*, A NEW INVASIVE ANT SPECIES IN FLORIDA

**Introduction**

The tawny crazy ant, *Nylanderia fulva* (Mayr) is a serious pest ant in the southern United States. Until 2012, this ant was believed to be *N. pubens* (Forel), known by the unofficial common names “Caribbean crazy ant”, “brown crazy ant” and “hairy crazy ant”. However, using morphometric and molecular analyses in combination with type specimen comparisons, it was determined that ants from infestations in Gainesville, Florida, and Jacksonville, Florida, were actually *N. fulva* (Gotzek et al. 2012).

*Nylanderia fulva* has now been reported in 27 counties in Florida (Fig.1-1). Small populations of *N. fulva* were reported from Texas in 1938 (Trager 1984). In 2002, there were reports of large infestations from Houston, Texas and *N. fulva* currently infests 21 counties in Texas (Fig.1-1). *Nylanderia fulva* has also been reported from Mississippi (MacGown and Layton 2009) and Louisiana (Hooper-Bui et al. 2010).

Morphometric and phylogenetic analysis of the Texas populations did not provide conclusive evidence that the ant was either *N. pubens* or *N. fulva* (Meyers 2008a). Therefore, it was referred to in the literature as *Nylanderia* sp. nr. *pubens* or the Rasberry crazy ant, after Tom Rasberry the pest management professional who discovered it in Texas (Meyers 2008b). Molecular and morphological comparisons have since determined that the ants in Florida and Texas are the same species (Gotzek et al. 2012, Zhao et al. 2012).

**Taxonomy**

The genus *Nylanderia* is in the order Hymenoptera, family Formicidae, and subfamily Formicinae. *Nylanderia* is a large genus comprised of 130 extant and two
fossil species (LaPolla et al. 2011). It is an abundant and cosmopolitan genus with members in every geographical region, occupying habitats as varied as deserts and rainforests (LaPolla et al. 2011).

*Nylanderia fulva* is in the *Prenolepis* genus-group which includes the genera *Euprenolepis, Nylanderia, Paraparatrechina, Paratrechina, Prenolepis* and *Pseudolasius*. Emery established the genus *Paratrechina* in 1925 with the subgenera *Paratrechina* and *Nylanderia* (Trager 1984). In 1936, Wheeler elevated *Nylanderia* to genus level (Fernandez 2000). Few authors followed this taxonomic change and continued to use the proposal of Emery or simply treated *Paratrechina* and *Nylanderia* as synonyms (Trager 1984).

The taxonomy of this genus has been the subject of much confusion and few taxonomic revisions have been undertaken (LaPolla et al. 2010). *Nylanderia pubens* and *N. fulva*, until recently, were in the genus *Paratrechina*. In 2010, a taxonomic revision of the *Prenolepis* genus-group divided the genus *Paratrechina* into three clades. *Paratrechina* was redefined as a monotypic genus and the subgenera *Nylanderia* and *Paraparatrechina* were raised to generic status as names for the other two clades. All species except *Paratrechina longicornis* (Mayr) were transferred out of *Paratrechina* (Mayr) and most were transferred into *Nylanderia* (LaPolla et al. 2011).

*Nylanderia pubens* and *N. fulva* are similar enough to have been previously synonymized (Bolton et al. 2007, Creighton 1950). Trager (1984) revised the taxon and again separated the two species. As workers are virtually indistinguishable, species discrimination is more reliable by comparing male genitalia (Fig. 1-2). The paramere, an external genital lobe, of *N. pubens* is heavily sclerotized and has a fan-like fringe of
dense, light-colored macrochetae, whereas the paramere of *N. fulva* is not heavily sclerotized and with only a sparse fringe of macrochetae (Gotzek et al. 2012, Trager 1984).

**Description**

*Nylanderia fulva* workers (Fig. 1-3a) are small, monomorphic ants, ~ 2.0 to 2.5 mm in length. They are reddish-brown in color and the body is densely pubescent. The head is 0.77 to 0.85 mm in length and lacks ocelli. The antennae are 12-segmented. Abdominal segments bear rows of longitudinal setae. An acidopore is present at the tip of the gaster (Zenner-Polania 1990a). Reproductive females (Fig. 1-3b) are 4.0 mm or longer and otherwise similar in appearance to workers (Fernandez 2000, LaPolla et al. 2011). Males (Fig. 1-3c) are similar in color and range in size from 2.4 to 2.7 mm. The antennae are 13-segmented. The eyes of the males are much larger than those of the workers and there is a sparse pilosity surrounding the lightly sclerotized paramere (Zenner-Polania 1990a).

*Nylanderia fulva* eggs are round to oval shaped, approximately 0.39 mm long and 0.25 mm wide. Larvae range in mean length from 0.6 to 1.7 mm depending on the instar. The pupae are exarate and approximately 2.6 mm in length. As they develop, the pupae change color from white to light brown (Arcila et al. 2002a).

**Biology**

Arcila et al. (2002a) found that *N. fulva* colonies are comprised of multiple queens, workers and brood. In the late winter and early spring, alates may also be observed in the nests. Egg production increases dramatically in late winter and spring. Eggs are laid individually by the queen and are glued together by the workers’ saliva into masses. The eggs are incubated for 9 to 21 d. Worker ants undergo three instars, but male
alates require four. No molt occurs during the pre-pupal stage, but the meconium is expelled and feeding ceases. Under laboratory conditions, mean development times for egg, larvae and pupae are 16.2, 10.8 and 12.2 d, respectively. Development time from egg to adult is 23 to 50 d at 27°C and 80% RH.

**Characteristics of Invasive Species with Reference to Ants**

Introductions of exotic organisms are often the result of human-mediated transport (McGlynn 1999, Sakai et al. 2001). Organisms may be accidentally introduced in the cargo or ballast of ships, in contaminated seeds or the movement of infested soil in potted plants. Purposeful introductions include importation of ornamental plants for landscaping, exotic animals as pets or even organisms as biological control agents (Ewel et al. 1999). It is notable that the states currently infested with *N. fulva* are all states with one or more active seaports. One of the most severe infestations of *N. fulva* in Florida was at a military property in Jacksonville, Florida, a site adjacent to a major seaport. The location of the most recent incursion of *N. fulva* in Texas is 8 km from the port of Houston.

Exotic organisms are introduced into new habitats quite commonly, but of the few that survive and reproduce, only a very small percentage are invasive (Abbott et al. 2007, Pimentel et al. 2000, Liebhold and Tobin 2008). In some cases, initial invasion success is followed by a population collapse, or complete disappearance of the invasive ant (Haines and Haines 1978a, Simberloff and Gibbons 2004). For example, 40% of *Linepithema humile* (Mayr) populations in New Zealand had collapsed within 20 years of introduction (Cooling et al. 2012). Wetterer et al. (2014) observed a population of *N. fulva* on Saint Croix grow and divide into 3 populations from 2002-2006. In 2013, one
of the newer infestations had completely collapsed whereas the other two had continued to grow.

In other situations, an ecological equilibrium, whereby invasive ants are not displacing but rather coexisting with native species, is reached after a period of time. In 1971, one of several serious infestations of *N. fulva* was reported from Colombia. After 10 years, this single population is stable but small, and at this particular site, *N. fulva* is no longer considered a pest (Zenner-Polania 1990a).

The first step in successful colonization by an introduced organism is to survive in the new environment. Successful invasions are most often into environments that are similar in climate to the home range (Colautti et al. 2006, Holway et al. 2002a) with suitable food and nesting resources. *Nylanderia fulva* was introduced into the United States from South America as were two other invasive ant species, *L. humile* and *Solenopsis invicta* (Buren). *Solenopsis invicta* has become major pest ant species throughout Florida while *L. humile* is a localized pest. The success of these two species in Florida suggest that climatic conditions in Florida also may favor the survival of *N. fulva*.

Exotic species are often successful when introduced into depauperate environments such as some islands or disturbed areas (Holway 1999, Sakai et al. 2001, Yamaguchi 2004). Biodiversity may act as a defense against invasion by exotic species; however, environments disturbed by fire, agriculture or urbanization often undergo a temporary or permanent reduction in native biodiversity (Kennedy et al. 2002). Florida’s extensive urban development, fire-maintained ecosystems, and seasonal tropical storms and hurricanes could be defined as a “disturbed” environment.
Invaders also benefit by escaping natural enemies such as predators, parasites or diseases (Holway et al. 2002a). For example, *S. invicta* population densities in the United States are 5 to 10 times greater than in their native range (Porter et al. 1997), suggesting that *S. invicta* in its native range is kept in check by its natural enemies including decapitating flies in the family Phoridae (Morrison and Gilbert 1999, Porter 1998) and two known microsporidia: *Knealhazia (Thelohania) solenopsae* Knell, Allen and Hazard and *Vairimorpha invicta* Jouvanez and Ellis (Briano et al. 2002, Briano 2005, Moser et al. 2000).

Second, the introduced organism must produce progeny and establish a viable population (Rabitsch 2011) and fertilized or self-fertilizing invaders have an advantage as they can immediately begin to reproduce (Holway et al. 2002a). The number and size of propagules of the introduced organism, can also have an impact on the success of the invader (Sakai et al. 2001, Holway et al. 2002a, Colautti et al. 2006, Reaser et al. 2007, Liebhold and Tobin 2008). Increasing the number of propagules potentially increases genetic variation, especially if the propagules are from differing source populations (Sakai et al. 2001). Larger propagule sizes allow the organism to avoid Allee effects, a situation in which the population fails to survive below a certain threshold density (Keitt et al. 2001, Lewis and Kareiva 1993, Liebhold and Tobin 2008).

Finally, the colonizing population must grow in size and disperse to a new territory. To do this, successful invaders are often competitively superior to native species and can better cope with changing environmental conditions (Sakai et al. 2001). Even so, there is often lag time between the initial introduction and the period of growth and
territory expansion. It is proposed that this lag time is a result of the process of the introduced organism adapting to the new environment (Sakai et al. 2001).

Life history traits of invasive species are of particular interest as they may provide insight on characteristics that may predict the likelihood of an organism becoming invasive when introduced outside its native range (Sakai et al. 2001). Several proposed characteristics of invasive ants and mechanisms for their success in novel environments are described below along with examples from *N. fulva* and other invasive ant species.

**Opportunistic nesting behavior.** Colonies of *N. fulva* can cover large areas and possibly contain millions of individual ants, similar to colonies of *L. humile* (see subsection Unicoloniality). *Nylanderia fulva* nests have been found under leaf litter and trash, in potted plants, tree stumps, fallen branches, decomposing logs and in abandoned termite and ant mounds (Trager 1984). Occasionally, *N. fulva* may be found foraging and nesting inside structures (MacGown and Layton 2009). *Nylanderia fulva* has been observed nesting in discarded soda bottles and empty cans, in the bracts of palm trees and in the canopy of hardwood trees during periods of flooding (personal observation). When the nest is disturbed, *N. fulva* will quickly relocate, often within minutes.

In general, invasive ants take advantage of a wide variety of nesting substrates including agricultural commodities, nursery stock and other types of cargo, increasing the likelihood of human-mediated transport. Having flexible nesting habits also means that invasive ants can rapidly respond to physical disturbance, changing environmental conditions or food resource availability (Holway et al. 2002a).
**Food preferences.** Although published reports on the diet of *N. fulva* in its native range are lacking, the genus *Nylanderia* is generally described as omnivorous (LaPolla et al. 2011). Also an invasive ant species in Colombia, *N. fulva* feeds on honeydew and protein-based foods such as insect larvae (Zenner-Polania 1990b). This is consistent with observations from Florida (Sharma et al. 2013).

Many invasive ant species are omnivorous and this dietary flexibility may aid in their success in new environments (Hölldobler and Wilson 1990, Holway et al. 2002a, Sarty et al. 2006). The diets of introduced ant species can be quite different from conspecifics in the native source population. For example, in a long-term study comparing the diets of introduced *L. humile* with native *L. humile*, the introduced ant populations had shifted to a diet higher in sugars (e.g., honeydew) while native *L. humile* continued to prefer protein (Tillberg et al. 2007) and invasion of natural areas by *L. humile* in the introduced range is aided by an available carbohydrate supply (Rowles and Silverman 2009).

**Polygyny.** *Nylanderia fulva*, like many other invasive ant species, is polygynous, which is a colony structure that includes multiple reproductive queens (Holway 1999). The probability of invasion success by polygynous ant species is increased not only because the reproductive capacity of the colony is increased (Vargo and Fletcher 2002), but also because the colony can reproduce by budding. Budding, or reproduction by colony fragmentation, increases the opportunity for propagules to come into contact with humans and to be transported to new areas and that the propagule will have at least one reproductive member (Wilson 1971). In some species, even small colony fragments
can result in infestations of invasive ants. For example, Hee et al. (2000) demonstrated successful colony founding in *L. humile* with one queen and ten workers.

**Unicoloniality.** Infestations of *N. fulva* may be quite large and in some cases ant nests are dispersed over entire neighborhoods (Meyers 2008a). One reported infestation of *N. fulva* in Colombia was >10 ha in area (Zenner-Polania 1990b). Infestations of invasive ants often have high population densities and/or cover large geographic areas. For example, polygyne colonies of *S. invicta* may number 470 to 1,976 per ha (Macom and Porter 1996, Oi and Drees 2009). Supercolonies of *L. humile* in New Zealand and California span an estimated 700 and 900 km, respectively (Tsutsui et al. 2000, Tsutsui and Case 2001).

One characteristic of many invasive ants is unicoloniality, a lack of distinct boundaries between nests (Holway et al. 2002a). Invasive ant species are sometimes completely or seasonally unicolonial in their native range, but frequently exhibit this trait in their introduced range (Rowles and O'Dowd 2007). Unicoloniality results from reduced intraspecific aggression between conspecifics. Territorial boundary conflicts are costly to ant colonies (Thomas et al. 2006). Reduced intraspecific aggression allows invasive ant species to redirect resources that would be used for defense against conspecifics to colony growth (Holway et al. 2002a). This results in the formation of expansive supercolonies that allow ants to forage over large areas (Rowles and O'Dowd 2007). This was demonstrated by both laboratory and field studies where reduced interspecific aggression in *L. humile* was associated with increased colony size and density and improved competiveness at food baits (Holway and Suarez 2004, Holway et al. 1998a, Suarez et al. 1999).
**Adaptation.** Successful invasion may require adaptation to the stresses of the new environment (Lee 2002) and indeed changes in behavior, reproduction, physiology, morphology and food resource allocation often occur after species are introduced into new habitats (Suarez et al. 1999, Tsutsui and Suarez 2003, Gilchrist and Lee 2007). For example, *L. humile* workers from both introduced and native range colonies will display intraspecific aggression with genetically distinct conspecifics. Yet, *L. humile* populations in California appear to be comprised of only a few supercolonies, each with little intraspecific aggression even between worker ants collected 100 km apart (Holway et al. 1998a).

Adaptation may result from selective pressures of the new environment or from genetic drift or bottleneck effects. Because the introduced colony fragment is usually a subset of a larger population, a population bottleneck may occur with the introduced population having reduced genetic variation compared to the source population (Sakai et al. 2001, Tsutsui et al. 2001, Lambrinos 2004). In fact, by using microsatellite markers, it was revealed that introduced populations of *L. humile* in California have only 50% of the alleles of native populations and these populations are genetically homogenous within a 1,000 km radius (Suarez et al. 1999, Tsutsui et al. 2000, Tsutsui and Suarez 2003). Likewise, introduced populations of *S. invicta* also only possess half of the alleles of native populations (Kreiger and Keller 1999).

**Resource dominance.** Mechanisms by which some introduced ant species are able to out-compete native ants include exploitative competition and interference competition (Sarty et al. 2006). Ants with superior exploitative ability locate food resources faster, recruit more workers to the food resource (Holway 1998a, 1999,
Human and Gordon 1996, Rowles and O'Dowd 2007) and maintain recruitment for longer periods of time compared to competitors (Rowles and O'Dowd 2007). Exploitatively competitive ants also may forage a greater number of hours per day and, as in the case of L. humile, also forage over a wider range of habitats and temperatures (Carpintero et al. 2007).

Ants that demonstrate superior interference competition are able to effectively defend a food resource against competitors or displace competitors at a food resource that has already been discovered. For example, L. humile aggressively drives away native ants on already discovered food resources (Andersen 1992, Holway 1999, Rowles and O'Dowd 2007). Superior interference competition can result from numerical dominance, size differences between competitors or use of defensive compounds (Holway 1999). Nylanderia fulva uses the latter to out-compete S. invicta at contested food sources, winning 93% of the time (LeBrun et al. 2013). LeBrun et al. (2016) demonstrated that this resource dominance is partially due to the ability of N. fulva to detoxify the venom of S. invicta by use of secretions from the exocrine gland.

Often there is a trade-off between these two mechanisms, with an ant species excelling at one but not the other (Davidson 1998). However, with invasive species this trade-off may not exist. It has been proposed that the numerical advantage of many invasive ant species allows them to excel at both types of competition (Holway 1999, Holway et al. 2002a). For example, Holway (1999) found L. humile does indeed excel at both exploitative and interference competition against native ants in California. Studies by Heller (2004) concluded that the success in L. humile in dominating other ant species
in an introduced range was due to differences in competitive ability in those ants compared to heterospecifics in the native range.

However, invasive species do not always exhibit resource dominance against all native ant species in the introduced range. When native ants are able to effectively maintain dominance via these same mechanisms, they provide a biotic resistance to invasion (Roura-Pascual et al. 2010). Laboratory and field studies in Australia demonstrated that introduced *L. humile* could not displace *Iridomyrmex rufoniger* (Lowne) and only partially displaced *I. bicknelli* (Emery) (Rowles and O'Dowd 2007). *Pheidole megacephala* (F.) also slows down invasions of *L. humile* by recruiting to baits quickly and successfully defending them (Rowles and O'Dowd 2007). A 3 yr study of an *S. invicta* invasion in Texas reported that the ant expanded its range at a rate of only 35 m per year, suggesting biotic resistance by the native *S. geminata* (Buren) (Porter et al. 1988).

Although certain characteristics typically promote the success of invasive ant species, there are often distinctive differences in the behavior and ecology of invasive ant species in their native and introduced ranges (Tsutsui and Suarez 2003). Ecological comparisons of invasive ants in both native and introduced habitats may help identify the reasons for their success in introduced ranges.

**The Impact of Invasive Species**

With increased global travel and international cargo trade, the opportunity for organisms to be transported to new environments is greater than ever before (Mack et al. 2000) and invasions of exotic species are widespread. An estimated 50,000 or more non-native species have been introduced into the United States with about 500 having
become invasive (Pimentel et al. 2000). As highlighted below, invasive species may have significant impact on the environment and economy of the introduced range.

**Ecological Impact**

To be designated an invasive species, an organism must be: 1) non-native, 2) introduced into the wild, 3) become a self-sustaining population and 4) incur costs that are greater than benefits (Shogren and Tschirhart 2005). Invasive species are considered to be one of the leading contributors to biodiversity loss (Shogren and Tschirhart 2005) and ~42% of organisms on lists of threatened or endangered species are listed as a result of impacts of invasive species (Pimental et al. 2005). The dynamic equilibrium model states that an ecosystem is limited in the numbers of species it can support and that immigration of new species is balanced by extinction of others (Simberloff 1981). As a result of human activities, including human mediated transport of nonindigenous species to new ranges, extinction events are occurring at a more rapid rate (Chapin et al. 2000). Another impact of invasive species is the rapid evolution of native organisms, such as the increased length of *Leptocorus tagalicus* (Hahn) mouthparts in response to the introduction of non-native host plants (Carroll et al. 2005). Competitive exclusion by invasive species may result in displacement of native species (Mooney and Cleland 2001). Both *S. invicta* and *L. humile* are reported to displace native ants in their introduced range (Holway 1999, Human and Gordon 1996, Porter and Savignano 1990). In the southern United States, when occurring at high densities, *N. fulva* displaces most of the larger ant species including *S. invicta*. *Nylanderia fulva* also significantly lowers species richness and abundance of non-ant arthropods in the invaded area (LeBrun et al. 2013).
Introductions of exotic species into new environments do not always result in deleterious effects (Shogren and Tschirhart 2005, Townsend 1991) and many introductions of exotic species fail before they are noticed (Mack et al. 2000). Exotic species invasions are processes that occur over time and there is a high probability of failure at each step in the process (Leung et al. 2002, Mack et al. 2000). However, when successful, invasive organisms may have impact at the species, community and ecosystem levels as demonstrated by the introduction of the Asian clam (*Potamocorbula amurensis* (Schrenck)) (Grosholz 2002) and the Eurasian zebra mussel (*Dreissena polymorpha* (Pallas)) (Vitousek et al. 1996) into United States waters. Both bivalves interrupt algal production, impacting zooplankton and fish larvae, which in turn impacts the entire marine food web. In another example, the balsam wooly adelgid, introduced into the United States on nursery stock, has decimated the Fraser fir population in the Great Smoky Mountains National Park (Vitousek et al. 1996).

**Economic Impact**

Invasive species cause significant economic impact in introduced areas. Insects and other arthropods introduced into the United States cost an estimated $137 billion dollars per year (Pimentel et al. 2000). The costs associated with invasive species may be due to direct loss, damage, or control measures. For example, annual direct crop and forestry loss due to introduced insects in the United States is $15.9 and 2.1 billion, respectively (Pimental et al. 2001). Estimated costs to clear municipality water intake pipes of the Asian zebra mussel were $3.1 billion over a 10 yr period (Vitousek et al. 1996). When *S. invicta* was introduced into California, the financial impact on the state was estimated to be between $387 and $989 million dollars per year (Jetter et al. 2002).
Argentine ant control in New Zealand was predicted to cost up to $68 million per year (Anonymous, 2002).

**Considerations for Invasive Species in Florida**

Invasive species introductions are of special concern to Florida as invasions of exotic organisms occur more often in coastal areas (Grosholz 2002). With 2,170 km of coastline, 15 deep water seaports, international trade, imported agricultural commodities, urban development, tourism and a temperate to tropical climate, Florida provides favorable conditions for many potential invasive species. For example, at more than 50, Florida has the greatest number of introduced ant species in the United States, (Deyrup et al. 2000).

**Ecological Impact**

**Ecosystem impacts.** Because ants exert such a strong influence on the local ecosystem, changes in ant diversity or abundance as a result of the introduction of an invasive ant species can have a significant effect on the ecosystem (Crooks 2002). Ant food gathering activities and soil movement affect nutrient cycling, impacting many organisms in the ecosystem, from decomposers to herbivores and carnivores (Folgarait 1998, Hölldobler and Wilson 1990, Jones et al. 1997, Sanders and van Veen 2011).

The ecological impact of invasive ants is well documented. For example, *Anopolepis gracilipes* (Smith) had a devastating effect on the ecosystem of Christmas Island, where it killed the resident land crabs resulting in increased accumulated leaf litter and increased seedling growth (Abbott 2005). Simultaneously, the ant facilitated the invasion of a honeydew producing scale whose unchecked population resulted in the death of canopy trees (Abbott and Green 2007, O'Dowd et al. 2003). Invasive ants can compete with pollinators for honey (Buys 1987), displace seed-dispersing native
ants (Bond and Slingsby 1984, Howarth 1991) and interrupt ant-hemipteran mutualisms (Abbott and Green 2007).

LeBrun (2013) reports that introduced *N. fulva* may reach numerical abundance greater than 2 orders of magnitude over all other ants. Furthermore, species richness and abundance is also markedly reduced and where ranges overlap, *N. fulva* displaces *S. invicta* (LeBrun 2013) aided, in part, by *N. fulva*’s ability to detoxify *S. invicta* venom (Chen et al. 2013, LeBrun et al. 2016).

**Impact on native arthropods.** When introduced into Colombia as a biocontrol agent against snakes, *N. fulva* instead became a pest. Studies of ant diversity in areas with and without infestations of *N. fulva* show a marked reduction in ant species richness in infested areas (Zenner-Polania 1990b). Of 13 ant species collected in the study, only three occurred within the *N. fulva* infested area. Similarly, introduction of *N. fulva* into La Reserva Natural Laguna de Sonso in Colombia reduced native ant species richness by 74% (Aldana et al. 1995).

Invasive ants may have negative impact on other arthropods, as well (Cole et al. 1992, Wojcik et al. 2001). In Hawaii, *A. gracilipes* displaced native spiders (Gillespie and Reimer 1993) and when introduced into novel environments, *P. megacephala* may reduce native invertebrate populations by as much as 85% (Hoffman et al. 1999).

**Impact on vertebrates.** Vertebrates are also impacted by invasive ant infestations (Mount 1981, Wojcik et al. 2001). Bobwhite quail and other ground nesting birds (Allen et al. 1994, Allen et al. 1995), rats (Flickinger 1989), rabbits (Masser and Grant 1986), snakes, lizards (Bartlett 1997), deer (Allen et al. 1997a), toads (Freed and Neitman 1988), turtles (Montgomery 1996, Wetterer et al. 2007) and even hatchling alligators (Allen et al. 1997b) have been negatively affected by *S. invicta* infestations. *Anopolepis gracilipes* has been implicated in the disappearance of the Seychelles’ skink and in killing the chicks of ground nesting terns and thrushes (Feare 1999). It is possible that the massive infestations of *N. fulva* could pose a similar threat to wildlife by attacking ground nesting animals and birds.

**Economic Impact**

**Impact on residences and businesses.** Although there are few publications related to the pest status of *N. fulva* it is reasonable to suggest that they could have significant economic impact in the southern United States. Reports of *N. fulva* in residential areas describe massive infestations that are a nuisance to homeowners and domestic animals (Zenner-Polania 1990b). *Nylanderia fulva* has been reported to nest in electrical equipment and has damaged phone lines, pumps and computers (Meyers 2008a). In Texas, *N. fulva* is responsible for a lift-pump failure at a sewage facility and the shorting-out of a radiation scanner at the Port of Houston (Holden 2007). Concerned for critical systems at Johnson Space Center, the National Aeronautics and Space
Administration (NASA) contracted a pest management professional to monitor and treat for *N. fulva* (Meyers 2008a). In 2008, The Texas Department of Agriculture assembled a task force including university entomologists, pest management professionals, the Texas Forest Service, Texas Parks and Wildlife Department and NASA to address the problem. In 2015, the Southern Region IPM Center funded the formation of the Tawny Crazy Ant Working Group. One of the objectives of this group was to “reduce the spread of the tawny crazy ants and improve IPM tactics.” This group received additional funds in 2016.

While no economic impact figures currently exist for *N. fulva*, examples of the economic impact of other invasive ants include, *S. invicta* and *L. humile*. *Solenopsis invicta* is a serious urban pest, in the southern United States, infesting >150 million ha (Vander Meer et al. 2007). *Solenopsis invicta*’s painful sting and unsightly mounds make them unwelcome in the urban landscape. In a study of the economic impact to households for *S. invicta* control, the overall cost for just five metroplex areas in Texas was > $526 million in 1998 (Lard et al. 1999) with an average cost per household of $150.79 per year. Of those costs, insecticides, baits and other treatment measures comprised $279 million of the total, and repair costs were $126.43 million. In the same study, households reported that the estimated value of curtailed outdoor activities was $140 per household per year, almost as much as what was spent for control and repair. In addition to homeowner control costs against *S. invicta*, costs to repair damaged electrical and communications equipment totaled $111 million.

**Impact on agriculture.** In Colombia, *N. fulva* had a significant impact on the on the cattle and poultry industries resulting in blinded calves and asphyxiated poultry
(Zenner-Polania 1990b). The large numbers of ants were a nuisance to grazing animals. In addition, the ants contributed to the loss of rangeland by tending hemipterans whose sap feeding behavior dried out the grass.

In coffee, cacao, sugarcane and yucca plantations, *N. fulva* tended honeydew producing insects, increasing their numbers and causing damage to crops (Aldana et al. 1995). In addition to crop damage, the large numbers of ants were a nuisance to agricultural workers. Severe infestations of *N. fulva* caused some farms in Colombia to be abandoned (Zenner-Polania 1990b).

While there are currently no published records of *N. fulva* impacting agriculture in the U.S., there are numerous examples of other invasive ant species interfering with natural biological control of hemipterous insects, infesting and/or destroying agricultural equipment and damaging crops (Silverman and Brightwell 2008, Vega and Rust 2001). For example, *P. megacephala* tending cassava mealybug (*Phenacoccus manihoti* (Matile-Ferrero)) in cassava plantations in Africa resulted in a threefold reduction in predators of the mealybug (Cudjoe et al. 1993). When *P. megacephala* was excluded from coffee plants in Hawaii, scale insects were eliminated in 70 d. *Solenopsis invicta* has caused considerable damage to irrigation lines and pumping equipment (Jetter et al. 2002). *Solenopsis invicta* was also a serious pest of citrus by feeding on the bark, leaves and buds of young trees (Banks et al. 1991). Specialty crops such as fruits, vegetables and melons are also subject to damage by *S. invicta* that consume fruit, seeds and roots and may cause up to 50% yield loss (Adams 1983, Stewart and Vinson 1991). *Solenopsis invicta* in Texas cost the cattle industry up to $28 per acre in losses (Barr and Drees 1994). The ants infest hay and water causing malnutrition and
dehydration when cattle refuse infested food and water. Poultry may also suffer malnutrition and reduced egg laying when food is infested with ants (Jetter et al. 2002).

Beekeepers in Texas report *N. fulva* nesting in honeybee hives after driving away the adult bees and consuming the larvae (Harmon 2009). In addition to directly impacting bees in hives, nectar-robbing ants may interfere with flower pollination (Norment 1988) resulting in incomplete pollination and reduced seed production or fruit set (Wyatt 1980).

**Toward Developing an Integrated Pest Management Strategy**

There is a great need for information on management practices for this pest and to date there have been few studies on *N. fulva* control. Drees et al. (2010) investigated the acceptance of some commercially available insecticide baits. Advance® Carpenter Ant Bait® (abamectin, BASF, Research Triangle Park, NC) and Maxforce® Complete Ant Bait (hydramethylnon, BASF, Research Triangle Park, NC) were more accepted by *N. fulva* than Amdro® Ant Block (hydramethylnon, Central Garden and Pet, Atlanta, GA), ProBait® (hydramethylnon, Zoecon, Schaumburg, IL), Extinguish® Plus (hydramethylnon and methoprene, Wellmark International, Schaumburg, IL) and Esteem® (pyriproxyfen, Valent USA Corporation, Walnut Creek, CA). Studies of insecticide bait efficacy include a laboratory evaluation of dinotefuran in a gel matrix that resulted in 78% mortality of workers (Meyers and Gold 2007) but field studies with dinotefuran in a granular matrix were inconclusive (Meyers 2008a). Advance® Carpenter Ant Bait was tested in against *N. fulva* in a large field trial in Texas but it did not adequately suppress ant populations (McDonald 2012). Also in Texas, a field evaluation of one application of Esteem® Ant Bait was not effective against *N. fulva* as ants from nearby untreated areas re-infested the treated area within 14 d (Nester 2010). Oi (2014) reported that insect growth
regulating (IGR) baits significantly reduce brood in laboratory colonies but the IGRs pyriproxyfen and (S)-methoprene repel *N. fulva*. The use of Maxforce® Quantum ant bait (imidacloprid, Bayer Environmental Science, Research Triangle Park, NC) diluted in a sucrose solution has shown some promise. In laboratory studies it reduced brood 98% in 3 wk. In field trials, the diluted Maxforce® Quantum bait plus a stain was dispensed in large volume stations. The treatment did not provide sufficient suppression of ant populations but stained ants were collected over 30.5 m from where the bait was dispensed suggesting that liquid baits have the potential to be used to deliver bait to distant or difficult to access ant populations (Oi 2014). McDonald and Cook (2014) reported that baits formulated with the active ingredient spinosad are also highly effective against *N. fulva*.

Insecticides that have shown efficacy against *N. fulva* include Cobalt Advance (chlorpyrifos and lambda-cyhalothrin, Dow AgroSciences, LLC., Indianapolis, IN) and Warrior II (lambda-cyhalothrin, Syngenta Crop Protection, LLC., Greensboro, NC) when applied as a tree trunk spray plus broadcast orchard floor spray in a commercial pecan orchard (Ree et al. 2014). A single application of Arilon (indoxacarb, Syngenta Professional Products, Greensboro, NC) to the lawn and perimeter of a home, significantly reduced *N. fulva* foraging within one month (Graham et al. 2015). Field studies where multiple insecticides were used concurrently against *N. fulva* have been conducted. Phantom® (chlorfenapyr, BASF, Research Triangle Park, NC) and Termidor®, both non-repellent, slow acting insecticides used together and along with Advance® Carpenter Ant Bait (abamectin, BASF, Research Triangle Park, NC) demonstrated suppression of *N. fulva* for up to 12 wk compared to an untreated control.
(Meyers 2008a). However, field studies with combinations of Transport™ 50WP (acetamiprid and bifenthrin, FMC Corporation, Philadelphia, PA), Talstar® granules or Termidor® SC and TopChoice® (fipronil, Bayer Environmental Science, Research Triangle Park, NC) suppressed ants for only two weeks (Meyers 2008a).

The current construct of IPM is to use a holistic approach to pest management that begins with a thorough understanding of the target pest’s biology and ecology including life cycle, behavior, and interactions with other organisms. In IPM, the goal is pest control below a previously agreed upon threshold. In agriculture, this threshold is the economic injury level or level of crop injury or loss at which the cost of pest management is justified. In structural pest control, the threshold may be based on aesthetics or health and safety concerns. In the end, the IPM program must be sustainable both economically and environmentally.

There is little published information on the *N. fulva*’s biology, behavior and ecology, which are necessary components for IPM strategy development. In the studies described below, I have gathered data on the foraging density and distribution of *N. fulva* after a recent introduction to better understand the dispersal dynamics of this pest. The demographics of the colony, seasonal macronutrient preferences and acceptance of commercially available insecticidal baits were studied to improve our knowledge of bait selection and application timing. Laboratory evaluations of insecticides and field studies with combinations of insecticides and baits were conducted to demonstrate the utility of IPM based strategies to suppress *N. fulva* infestations. By combining best practices from successful IPM programs for other invasive ant species with the information gathered in these research studies, I have
prepared a set of IPM recommendations for *N. fulva* in Florida. These recommendations and a discussion of future research needs to prepare a complete IPM strategy for *N. fulva* are described in Chapter 6.
Figure 1-1. *Nylanderia fulva* infestations verified by a state or local expert and reported by the Tawny Crazy Ant Working Group (2017) to the Early Detection and Distribution Mapping System (EDDMapS 2017).
Figure 1-2. Morphological differences between *Nylanderia fulva* and *Nylanderia pubens* used to differentiate species. A) Paramere of *N. fulva* male. Note light sclerotization and sparse fringe of setae. B) Paramere of *N. pubens* male. Note heavy sclerotization and dense fringe of setae. Photographs from Gotzek et al. 2012 used under license from Creative Commons Attribution 2.0.
Figure 1-3. *Nylanderia fulva*: A) profile view of worker. Arrow points to rows of setae on abdominal segments. B) Profile view of queen. C) Profile view of male. Photo credits: Joe MacGown. Photos used with permission.
CHAPTER 2
POPULATION DENSITY AND DISPERsal OF AN INVASIVE ANT SPECIES, *Nylanderia fulva*: A THREE YEAR OBSERVATIONAL STUDY AT THE JACKSONVILLE ZOO AND GARDENS

Introduction

In 2005, an infestation of an invasive ant species, *Nylanderia fulva* (Mayr), was discovered at the Jacksonville Zoo and Gardens in Jacksonville, FL. Two years post-introduction, the ants infested several sites within the 56.7 ha property. At the zoo, the non-stinging *N. fulva* were a concern to patrons who often mistook them for the red imported fire ant, *Solenopsis invicta* (Buren). To zoo administrators, *N. fulva* was a serious pest that infested animal feed and forage, was a nuisance for building occupants, and caused short-circuits and service interruptions because of ants nesting in electrical switch boxes. The zoo’s revenue-generating train was halted due to the high density of *N. fulva* trailing on the railroad tracks making the wheels lose traction.

The initial infestation at the zoo was located in the plant nursery (Area 11) (Table 2-1, Fig. 2-1), an area on the southeast periphery of the zoo where potted plants and other landscaping materials were held in greenhouses or on large sheets of weed cloth until they were incorporated into an exhibit. It was suspected that *N. fulva* were transported from the nursery area to the administration building during landscape renovations in late 2005. The following year, *N. fulva* were transported to several sites within the zoo after infested pine straw stored in the nursery area was distributed throughout the zoo. In 2007, an infestation was discovered at a newly opened exhibit called Stingray Bay (Area 1) (Table 2-1, Fig. 2-1). Boxes of salt for the saltwater lagoon in Area 1 were stored on pallets in the nursery area until needed. *Nylanderia fulva* were
observed nesting inside the boxes and it was theorized that *N. fulva* found in Stingray Bay were transported with the salt.

Understanding how invasive species spread is important because the costs to manage the invasive pest increase with the area infested. Therefore, the rate of dispersal of invasive organisms can be an important component of a risk analysis (Neubert and Parker 2004). In addition, effective management strategies depend on an understanding of basic pest biology and its population dynamics (Dent 1997).

*Nylanderia fulva* was first introduced into Colombia in 1971 as a biological control agent against snakes but it became an agricultural pest that damaged crops, interfered with harvesting activities and killed small farm animals. New infestations in Colombia were attributed to transportation of infested soil and dung. In Colombia, *N. fulva* populations spread at a rate of approximately 1 km per yr (Zenner-Polania 1990b). *Nylanderia fulva* were reported from Texas since 1938 (Trager 1984) but did not become a significant pest problem. Since what is believed to be a subsequent introduction near Houston, Texas in 2002, *N. fulva* is now is present in 21 Texas counties. Meyers (2008a) reported that *Paratrechina* sp. nr. *pubens* (now identified as *N. fulva* (Gotzek et al. 2012)) populations in Texas spread at a rate of about 30 m per mo.

Infestations of *N. fulva* in Florida may be quite severe (Fig. 2-2). At the initiation of this study, little was known about the growth and dispersal of *N. fulva* populations in Florida. Therefore, the objectives of this study were: 1) To use the numbers of foraging ants to monitor the population density and dispersal of *N. fulva* in 12 sampling areas at the Jacksonville Zoo over a period of 3 yr: June 2008-May 2009 (year 1), June 2009-May 2010 (year 2) and June 2010-May 2011 (year 3) and 2) To determine if there
was a relationship between population density and abiotic factors (i.e. temperature and precipitation). Based on estimates by Meyers (2008a) and Zenner-Polania (1990b) of the spread of *N. fulva*, it was hypothesized that most of the 56.7 ha of the zoo would be infested within 5 yr. Based on observations of *N. fulva* in the laboratory and field, it was predicted that the ant populations would decrease with decreases in temperature and/or precipitation.

**Materials and Methods**

**Observations of *N. fulva* Population Densities and Dispersal**

Since *N. fulva* do not build distinct soil nests, mound counts could not be used to determine population density. Therefore, *N. fulva* foraging activity was used as an approximation of population density. Foraging activity was measured by placing a 5 mm thick slice of canned sausage (Armour®, Pinnacle Foods Group, LLC, Cherry Hill, NJ) directly onto the ground at each sampling point (Fig. 2-3). The numbers of ants on the visible surface of the sausage were counted in situ after a minimum of 15 min but no longer than 30 min.

To determine changes in *N. fulva* population density and to track ant dispersal from points of introduction, the zoo was divided into 12 sampling areas of unequal sizes (Table 2-1, Fig. 2-1) that delineated major attractions, the plant nursery and parking areas. A total of 136 sampling points were randomly selected within the 12 sampling areas (Table 2-1) and Global Positioning System (GPS) coordinates for 1-3 waypoints (Appendix) within each sampling area were recorded (Garmin model 60CSx). The number of sampling points per sampling site varied from 4 to 22. Sampling was conducted at the same points throughout the study between 0900 and 1300 every 4 to 12 wk, over 3 yr (2008-2011), depending on access to the zoo and weather events (i.e.,
rainfall, thunder and lightning, tropical storms, hurricanes, flooding, temperatures <10°C). Sampling was not conducted within animal exercise areas, inside animal nesting areas or housing, in areas where sampling would impact public viewing of animals, or where access was not possible due to heavy vegetation, standing water, or live electric fences.

**Data analysis.** The ant foraging count data per individual sampling point (sausage slice) were mostly zeros. In order to facilitate analysis, the data counts were averaged by sampling area and transformed using the formula log (y+1). A weighted mixed linear model was fitted to predict average counts (response variable) at each sampling time (explanatory variable) using a first order auto regressive structure (AR(1)) to account for the autocorrelation associated with repeated measures within the same sampling area (SAS Institute 2012). The mixed linear model is a variation of the linear model that allows data to exhibit correlation and heterogenous variability (SAS Institute 2012). In this analysis, the mixed linear model has a fixed effect associated with the explanatory variable sampling time and covariance associated with the repeated measure of sampling over time. Each sausage slice represented a replicate. The number of replicates within a sampling area was used as the weighting variable. Data were back transformed and presented in a line graph to demonstrate overall trend in mean ant foraging.

**Effect of Temperature and Precipitation on Mean Foraging Counts**

Hourly temperature and precipitation data were gathered from the Jacksonville International weather station (ID: JAX) located 14.5 km from the study site (NOAA 2012). Using hourly data, the mean minimum and maximum temperatures (°C) and mean precipitation (cm) for the 7 d preceding each sampling date were calculated
(n=168). The 7 d interval accounted for weather conditions that may have affected ant foraging counts but were not experienced on the day the counts were taken (i.e. a previous thunderstorm). Foraging ant counts for each sampling date (n=18) were calculated by summing ant counts from each sampling point within each sampling area.

**Data analysis.** To investigate the possible relationship between temperature and precipitation on foraging ant counts, the dependent variable, foraging ant counts was analyzed against the independent variables mean minimum temperature, mean maximum temperature and mean precipitation using a multiple linear regression (SAS Institute 2012). The independent variables mean maximum temperature and mean precipitation were found to be insignificant and were removed from the model. A simple linear regression was performed with the variable mean minimum temperature. This resulted in a regression equation with a negative intercept. The model was run again but with the regression through the origin (Eisenhauer 2003). The standard error, \(p\)-value and \(r^2\) value of the negative intercept and forced origin models were compared to determine the best fit. The data assumption of normal distribution was confirmed by a Shapiro-Wilk test. Lack of correlation between the independent variables was confirmed using a Pearson Correlation Coefficient test (SAS Institute 2012).

Levene’s tests rejected the assumption of homogeneity of variance; therefore, to test if the mean daily minimum temperature was significantly different any year from 2002 to 2012, the daily minimum temperature (dependent variable) and year (independent variable) were analyzed using a one-way Welch’s Analysis of Variance (ANOVA) (SAS Institute 2012). Data assumptions of normal distribution and independence were evaluated by Shapiro Wilk and Durbin-Watson tests, respectively. The data did not meet
the assumption normality; however, this test detects very small departures from normality with large sample sizes and are more likely to reject the null hypotheses as the sample size increases (SAS Institute 2012). Since the sample size was large (n=4,016) and a histogram of the data revealed a near normal distribution, it was decided to proceed with the Welch’s ANOVA (Fig. 2-6).

**Effect of Insecticide Applications on Mean Foraging Counts**

Pest management activities to suppress *N. fulva* were practiced at the zoo by an employee on an as-needed basis (Table 2-2). When *N. fulva* were reported inside buildings, spot applications (no greater than 0.61 x 0.61 m) (United States Environmental Protection Agency 1973) of Talstar® (bifenthrin, FMC, Philadelphia, PA) were made. Exterior infestations were treated with spot applications of Suspend® (deltamethrin, Bayer Environmental Science, Research Triangle Park, NC) or spot or perimeter applications according to the label (BASF 2012) of Termidor SC® (fipronil, BASF, Research Triangle Park, NC).

Detailed pest control records were limited; however, the name of the product, the date the product was applied and the area of the zoo to which it was applied were recorded by zoo personnel. Using these records, it could be determined if an insecticide application had been made within a sampling area in the 5 d before a sampling count was made. The volume of insecticide applied and the total area treated during the 3 yr study was estimated by assuming the highest concentration was used for spot and perimeter applications as defined by the product label.

Estimates of insecticide applied over 3 yr were calculated using the product label instructions. It was assumed that Talstar® was applied at the labeled dilution rate for liquid spot application of 29.6 ml/3.8 L of water at application rate of 3.8 L/92.9 m².
Suspend® labeled dilution rate for liquid spot application was assumed to be 22.2 ml/3.8 L of water at an application rate of 3.8 L/92.9 m². Termidor® labeled dilution rate for liquid spot application was assumed to be 23.7 ml/3.8 L of water at an application rate of 3.8 L/92.9 m². Termidor® labeled dilution rate for band application was assumed to be 23.7 ml/3.8 L of water at an application rate of 6.8 L/92.9 m². The assumptions for estimates are: 1. Talstar®, Suspend® and Termidor® were spot applied; 2. Pest control records indicate two applications of Termidor® were band applications. It is assumed the remaining applications were spot applications and; 3. The insecticides were applied to the maximum area allowed for the type of application.

Data analysis. To analyze the effect of insecticide applications on mean foraging ant counts, the foraging count data were coded “1” if a spray application was made within a sampling area in the 5 d before the sampling date or “0” if no insecticide application was made. Insecticide applications can provide immediate knockdown of N. fulva populations, but ants can return in as soon as 2 to 3 days (¹FMO, personal communication). Therefore it was not expected that applications of insecticides made greater than five days would impact ant foraging counts. The relationship between mean foraging ant counts (response variable) for all sampling areas and insecticide applications for all sampling areas (explanatory variable) were analyzed using a mixed linear model using a first order auto regressive structure (AR(1)) to account for the

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autocorrelation associated with repeated measures within the same sampling area (SAS Institute 2012). In this analysis, the mixed linear model has a fixed effect associated with the insecticide applications and covariance associated with the repeated measure of sampling over time.

Results

*Nylanderia fulva* Population Densities and Dispersal

The mean foraging ant counts for all sampling areas combined demonstrated a significant decline in ant foraging counts during the 3 yr of the study (F = 5.28, df = 22, 191, p < 0.001) (Fig. 2-4).

Effect of Temperature and Precipitation on Mean Foraging Counts

A simple linear regression of foraging ant counts (y) and mean minimum temperature (X) through the origin yielded significant model (y = 0 + 114.95X, r² = 0.7725, F = 57.75, df = 1, 17, p < 0.0001) and showed a positive linear relationship with mean minimum temperature (Fig. 2-5). Although the linear regression showed a decrease in ant foraging with lower mean minimum temperatures, there was no significant difference in the mean daily minimum temperature between 2002 and 2012 (F = 1.47, df = 10, 4006, p = 0.1441) (Fig. 2-6), suggesting that the decline in ant foraging over the three years of the study was not due to a decrease in temperature. The mean minimum temperature ranged from 13.0°C (2010) to 14.9°C (2002).

Effect of Insecticide Applications on Mean Foraging Counts

In the linear mixed model analysis, the effect of the explanatory variable insecticide application on the response variable mean ant foraging count was not significant (F = -1.00, df = 1, 187, p = 0.3195) (Table 2-3).
Discussion

The introduction of a novel species into an environment, whether intentional or accidental, often elicits concern regarding potential negative impacts on native species and ecosystem equilibrium. Indeed, many introduced species such as the red imported fire ant, *S. invicta*, and the Argentine ant, *Linepithema humile* (Mayr), have become significant pests in their introduced range (Holway 1998b, Oi and Drees 2009, Porter and Savignano 1990). Factors contributing to the success of introduced species include lack of competitors and natural enemies, as well as abiotic factors such as temperature and moisture (Holway et al. 2002b, Human et al. 1998). In addition, introduced species that attain pest status may have certain physical, morphological or behavioral advantages over native species (Gilchrist and Lee 2007, Ingram and Gordon 2003, Lee 2002).

**Population densities.** Mean foraging ant counts for *N. fulva* decreased over the 3 yr of the study; thus, my hypothesis that *N. fulva* would infest most of the zoo over time, is not supported by these data. In Colombia, where *N. fulva* has been established since the 1970s, it was observed that in continuously infested areas, *N. fulva* populations reached maximum density at 2 yr. After reaching maximum density the ant populations decreased until equilibrium, where introduced ants no longer outcompete native ants, was reached at 10 yr (Zenner-Polania 1990b). *Nylanderia fulva* have been reported from Jacksonville, FL, since the mid-1990s meaning that during this study infestations were nearing the 10 yr mark. If the trend in Jacksonville, FL, follows Colombia, it may mean that the population densities at the initial infestation sites of *N. fulva* in Jacksonville, FL, have peaked and are now on the decline.
It is not uncommon for introduced species of ants to experience a population boom followed by a collapse. Wetterer et al. (2014) observed a population of *N. fulva* on Saint Croix grow and divide into 3 populations from 2002-2006. In 2013, one of the newer infestations had completely collapsed whereas the other two had continued to grow. *Anoplolepis longipes* populations have diminished greatly in the 40 years since their introduction to the Seychelles (Haines and Haines 1978). In New Zealand, Cooling et al. (2012) showed that 40% of introduced *L. humile* populations collapsed between 10.5-17.8 yr depending on temperature and rainfall.

**Effect of temperature and precipitation.** The relationship between ant foraging counts and mean minimum temperature over the 7 d before the count was significant, but not the relationship between mean ant foraging and mean maximum temperature or mean precipitation. This suggested that lower temperatures may have a greater impact on *N. fulva* populations than higher temperatures or rainfall. In this study, ant foraging was used as a proxy for determining population densities and seasonal variation was expected. Reduced foraging can be the result of reduced metabolic activity due to cold temperatures, reduced requirement for food resources due to less brood production (Porter and Tschinkel 1987) or a behavior to maximize efficiency of foraging effort when resources may be reduced (Bernstein 1979). In this study, the numbers of foraging *N. fulva* declined as the mean minimum temperature declined (Fig. 2-5) suggesting that low temperatures negatively impacted *N. fulva* foraging. Sharma et al. (2013) also found reduced foraging in *N. fulva* at temperatures <19°C but in that study, the reduction was correlated with a reduction the availability of hemipteran prey at cold temperatures.
Brood production in *N. fulva* colonies is reduced in colder months (Chapter 3), another factor that would explain low foraging counts in the winter.

Additionally, the foraging activity of ants is constrained by several abiotic factors (Traniello 1989), including a critical thermal minimum below which ants do not forage (Bernstein 1976). Bentley et al. (2016) determined the critical thermal minimum for *N. fulva* to be 7.3°C and that *N. fulva* may thermoregulate by tunneling in the soil at temperatures as low as 15°C (Bentley et al. 2015). In this study, the mean minimum temperature ranged from 13.0-14.9°C, which is above the temperature thresholds in which this ant becomes completely inactive.

Invasive ant species introduced into temperate regions often demonstrate seasonal fluctuations in populations with greatly reduced numbers in winter months; however, ant populations return to normal levels in the warmer months (Holway 1998a). As predicted, *N. fulva* populations varied with season and these data agree with both LeBrun’s (2013) observation that *N. fulva* populations are denser in summer and fall, and Hill (2013) that *N. fulva* foraging is reduced in the winter; however, instead of populations returning to previous year’s numbers, the trend over the three years of this study was toward a smaller population each year. One possible explanation is that one or more years of the study experienced lower than normal temperatures that affected the overwintering survivability of *N. fulva*. Low temperatures have been shown to have an impact on the survivability of other invasive ant species (Porter and Tschinkel 1987, Heller and Gordon 2006). For example, *S. invicta* colonies in Georgia subjected to extremely cold winter temperatures suffered a 90% reduction in the number of mounds in the observed area (Morrill et al. 1978) and in Tennessee, areas with low mean winter
temperatures (-4.6°C) and consecutive days of temperatures below 0°C had a 87.5% 
reduction in S. invicta mounds (Callcott et al. 2000). Since there was no significant 
difference in the mean daily minimum temperature in Jacksonville from 2002 to 2012, 
other factors must account for why the population failed to recover to previous year’s 
numbers.

The ability of invasive ant species to reach high abundances is critical to their 
success (Lester and Gruber 2016). One factor that could lead to the decline of invasive 
ant populations over time is some loss of fitness as a result of a genetic bottleneck 
(Mattila and Seeley 2007, Tarpy 2003, Ugelvig et al. 2010). It is reported that the 
N. fulva population at the Jacksonville Zoo is from the introduction of a single propagule 
that spread throughout the zoo. If so, this may have led to a reduction in genetic 
diversity that had a deleterious effect on the population. However, this is not likely as 
N. fulva were also observed trailing into the zoo from the surrounding wooded area.

Alternatively, N. fulva may have been resource limited. In the most of the interior 
sampling sites at the zoo, populations declined to near zero by yr 3. It may be that these 
sites offered fewer food and nesting resources than the exterior perimeter of the zoo 
with access to nearby woods.

Introduced species tend to be successful under environmental conditions similar to 
their home range (Colautti et al. 2006, Walters and MacKay 2003). For example, 
L. humile in introduced ranges is limited to Mediterranean climates and to areas with 
adequate moisture such as riparian corridors and irrigated urban and agricultural areas 
(Heller and Gordon 2006, Holway 1998a, Holway et al. 2002b, Menke and Holway 
2006, Walters and MacKay 2003). Data from this study suggest the relationship
between mean precipitation and foraging ant counts is not significant. However, temperature and precipitation data gathered from a weather station 14.5 km from the zoo may not accurately reflect weather conditions at the zoo. Observations in the laboratory and in the field indicate that *N. fulva* do not survive in low moisture environments; however, because of their opportunistic nesting habits and ability to rapidly relocate nests, microclimate conditions may have a greater impact on *N. fulva* survivability than the volume of precipitation. For example, this study site is adjacent to a river and it is possible that the soil moisture is high due to capillary water rising from the underlying water table or lateral seepage from the river.

**Dispersal.** Since many sampling areas at the zoo were contiguous and of similar environmental conditions, it was hypothesized that *N. fulva* would disperse from points of introduction into adjacent areas and eventually inhabit most of the zoo. On the contrary, after 3 yr, Areas 1-3: Stingray Bay, Railroad Tracks and Rhino Overlook, respectively, were the only areas with relatively high ant foraging counts (Fig. 2-7). The Parking Perimeter had high populations yrs 1-2 but was destroyed in yr 3 as part of a landscape renovation project. Areas 7-10: Play Park, Range of the Jaguar, Giraffe Overlook and Wild Florida, respectively, had mean ant foraging counts of less than ten at the beginning of the study. Each area contained water features that provided adequate moisture, lush vegetation and landscaping for nesting and locations adjacent to infested areas. It was expected that *N. fulva* would disperse into these exhibits. At yr 3, however, the mean foraging ant counts for these areas had declined to zero or nearly zero.
The areas that had relatively high population densities throughout the three years of the study are located at the perimeter of the zoo adjacent to wooded areas that are infested with *N. fulva* (Fig. 2-7). It is likely the wooded area acted as refugia and was a source of continual infestation of the zoo perimeter. The most heavily infested area was Rhino Overlook, an area that is both on the perimeter of the zoo and that contains large bodies of water further supporting the observation that *N. fulva* prefer high moisture environments.

Range expansion by ant colonies that disperse via budding tends to occur at a slower rate than those that disperse via nuptial flights (Holway et al. 2002b, Roura-Pascual et al. 2010). Nonetheless, with budding only, *L. humile* may disperse 15 to 270 m per year (Suarez et al. 2001) and *S. invicta* 10 to 40 m per year (Porter et al. 1988). *Anoplolepis gracilipes* Emery supercolonies in the Seychelles dispersed at rates between 0.5 and 402 m per year (Abbott 2006, Gerlach 2004). In Texas, *N. fulva* was reported to disperse an average of 30 m per year (Meyers 2008a).

Alternatively, jump dispersal (Suarez et al. 2001) usually mediated by human transport, allows organisms to expand far beyond their current range. A good example of the relative rates of both methods of dispersal is given by studies of the biphasic territory expansion of *S. invicta*. The first phase is a rapid expansion phase immediately following a jump dispersal event followed by a slow expansion phase led by colony growth and budding (Porter et al. 1988).

Initial infestations at the zoo occurred in the Plant Nursery, Stingray Bay and the administrative building near the entrance to the zoo, areas that are not contiguous. Based on reports from zoo staff, introductions of *N. fulva* into these areas can be
attributed to human-mediated transport (SU, personal communication). Once *N. fulva* was introduced into an area, populations did not rapidly disperse into new areas as expected. Instead populations remained local and population densities remained stable. This suggests that *N. fulva* range expansion by colony growth and budding is a slow process. Expansion of *N. fulva* range in Florida is more likely caused by human-mediated jump dispersal.

**Effect of insecticide applications on mean foraging counts.** Because insecticides were applied as needed to control small infestations of *N. fulva* indoors or to exterior areas where ant activity was a nuisance to zoo patrons, it was important to test whether these applications were related to the decline in *N. fulva* populations during this study. Over the 3 yr of the study, insecticides were applied to suppress *N. fulva* 21 times (Table 2-2). It was estimated that a total of 2.11 L of insecticide dilution (insecticide concentrate diluted in water) was applied to a total area of 0.0037 ha and 19 applications were to an area no greater than 0.37 m$^2$ (Table 2-3). Because the exact locations of the insecticide treatments were not recorded, it is not known how close the ant foraging counts were to the treated area. Based on the calculated insecticide volume applied and the total applied area (from verbal and written estimates provided by the zoo which may be underestimated), the spot and band insecticide applications did not significantly impact the overall population density of *N. fulva* at the Jacksonville Zoo.

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These data also illustrate that spot applications and stand-alone perimeter applications of insecticides are not effective at providing sustained suppression. Stingray Bay, Rhino Overlook, and the railroad tracks each received five applications of insecticides during the study and the ant foraging counts in these areas remained consistent from year to year.

In summary, the population densities, as estimated from foraging ant counts, appear to be declining at the Jacksonville Zoo and Gardens. *N. fulva* does not appear to disperse rapidly from the point of introduction suggesting that the seemingly rapid range expansion of *N. fulva* within Florida is likely due to human-mediated jump dispersal. Low winter temperatures temporarily reduce populations which results in seasonal variation in *N. fulva* population densities. Low temperatures may impact the overwintering survivability of *N. fulva* as low temperature is correlated with reduced ant foraging counts. Spot applications and stand-alone perimeter applications of insecticides were not effective in suppressing *N. fulva* populations.
Table 2-1. List of sampling areas, number of sampling points in each area and description of each sampling area in a 3 yr study (2008-2011) on population density and dispersal of *Nylanderia fulva* at the Jacksonville Zoo and Gardens.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>No. of sampling points (n)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stingray Bay (SB)</td>
<td>8</td>
<td>A saltwater pool surrounded by a 1.2 m tall wooden fence adjacent to a covered concrete pad where the pumping and filtration equipment were housed. The entire exhibit was surrounded on three sides by water. The soil at Stingray Bay remained moist due to regular draining of excess salt water from the aquarium tanks and rinsing of debris from the concrete pad.</td>
</tr>
<tr>
<td>2</td>
<td>Railroad Tracks (RR)</td>
<td>11</td>
<td>The railroad tracks were along the northwest perimeter of the zoo, next to a wooded lot that separated the zoo and the highway.</td>
</tr>
<tr>
<td>3</td>
<td>Rhino Overlook (RO)</td>
<td>9</td>
<td>A raised wooden platform on the edge of a grassy field where large animals were on exhibit. Access to the platform was via two wooden walkways over a tidal pond. The main viewing area had a misting station at each point of entry onto the platform to keep zoo visitors cool as needed. The viewing platform, especially beneath the misters, was heavily infested with <em>N. fulva</em>, and there were few ants on the wooden walkways.</td>
</tr>
<tr>
<td>4</td>
<td>Trout River (TR)</td>
<td>8</td>
<td>A patch of grass lawn adjacent to the Trout River and the pier that accessed the river. In year two, four sampling points along the pier were deleted from the due to bird interference with data collection. The area was subject to daily tidal flooding and multiple <em>Solenopsis invicta</em> nests were within the sampling area.</td>
</tr>
<tr>
<td>5</td>
<td>Australia Outer Perimeter (AO)</td>
<td>5</td>
<td>This area was a grass lawn on the southwest perimeter of the zoo property between the Trout River and the Australia exhibit. Sampling was discontinued 10 April 2010 as the site was no longer accessible as a result of renovations.</td>
</tr>
<tr>
<td>No.</td>
<td>Name</td>
<td>No. of sampling points (n)</td>
<td>Description</td>
</tr>
<tr>
<td>-----</td>
<td>-------------------------------</td>
<td>----------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>6</td>
<td>Australia Inner Perimeter (AI)</td>
<td>9</td>
<td>The courtyard, gardens and fountain area inside the Australia exhibit and contained a restaurant and outdoor seating area. A misting station was also installed at the western edge of the exhibit.</td>
</tr>
<tr>
<td>7</td>
<td>Play Park (PP)</td>
<td>9</td>
<td>A children’s recreational area that consisted of a hedge maze, playground and splash park.</td>
</tr>
<tr>
<td>8</td>
<td>Range of the Jaguar (RJ)</td>
<td>15</td>
<td>Range of the Jaguar contained a large faux stone temple with gardens and a water feature that was the outdoor exhibit of the resident jaguars. It was surrounded by a courtyard with shops, a restaurant and an outdoor dining area.</td>
</tr>
<tr>
<td>9</td>
<td>Giraffe Overlook (GO)</td>
<td>9</td>
<td>Giraffe Overlook had a wooden boardwalk that guests walked onto to view the giraffe yard. The exhibit was fronted by extensive gardens and a small pond.</td>
</tr>
<tr>
<td>10</td>
<td>Wild Florida (WF)</td>
<td>16</td>
<td>Wild Florida consisted of a concrete path shaded by trees that passed through natural wetlands and several outdoor animal exhibits. An indoor snake exhibit and an alligator pond flanked the entrance.</td>
</tr>
<tr>
<td>11</td>
<td>Plant Nursery (PN)</td>
<td>8</td>
<td>Plants, mulch, pine straw and salt were stored in the nursery area until being delivered to other areas of the zoo. The area contained potted plants and trees placed on weed cloth and stacks of used plastic pots. The area was converted to an exhibit in May 2010 and became inaccessible as a sampling area.</td>
</tr>
<tr>
<td>12</td>
<td>Parking Perimeter (PK)</td>
<td>22</td>
<td>This area was separated from a wooded lot that was heavily infested with <em>N. fulva</em> by a grassy field that was used for overflow parking. In year two, construction began to convert the grassy field into a permanent parking area. In year three the sampling area was completely destroyed to make way for new landscaping and was therefore dropped from the study.</td>
</tr>
</tbody>
</table>
Table 2-2. Insecticide applications in each sampling area during a study from 2008-2011 on the population density and dispersal of *Nylanderia fulva* at the Jacksonville Zoo and Gardens.

<table>
<thead>
<tr>
<th>Sampling Area</th>
<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Total number of insecticide applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Stingray Bay</td>
<td>S TA, S TE (2), S SU</td>
<td>S TA</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>2 Rhino Overlook</td>
<td>B TE</td>
<td>S SU (2)</td>
<td>S SU (2)</td>
<td>5</td>
</tr>
<tr>
<td>3 Railroad Tracks</td>
<td>B TE</td>
<td>S TA (3)</td>
<td>S SU</td>
<td>5</td>
</tr>
<tr>
<td>4 Trout River</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>5 Australia Outer Perimeter</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>6 Australia Inner Perimeter</td>
<td>S TA</td>
<td>S TA</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>7 Play Park</td>
<td>S TA</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>8 Range of the Jaguar</td>
<td>S TA (3)</td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>9 Giraffe Overlook</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>10 Wild Florida</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>11 Plant Nursery</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>12 Parking Perimeter</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>All Areas</td>
<td></td>
<td></td>
<td></td>
<td>21</td>
</tr>
</tbody>
</table>

Key: S=Spot Application, B=Band Application, TE=Termidor, TA=Talstar, SU=Suspend. Band applications of Termidor are limited to an area 30.5 cm up and 30.5 cm out from where the ground meets the foundation (BASF 2012). Spot applications of insecticides are not to exceed 0.19 m² (US EPA 1973).
Table 2-3. Estimated total volume of insecticide applied and area treated from 2008-2011 during a study on the population density and dispersal of *Nylanderia fulva* at the Jacksonville Zoo and Gardens.

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Volume of Insecticide (L)</th>
<th>Total Treated Area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type</td>
<td>Area (m²)</td>
<td>Rate (L/92.9 m²)</td>
</tr>
<tr>
<td>Talstar</td>
<td>Spot</td>
<td>0.37</td>
<td>3.80</td>
</tr>
<tr>
<td>Suspend</td>
<td>Spot</td>
<td>0.37</td>
<td>3.80</td>
</tr>
<tr>
<td>Termidor</td>
<td>Spot</td>
<td>0.37</td>
<td>3.80</td>
</tr>
<tr>
<td>Termidor</td>
<td>Band</td>
<td>14.86</td>
<td>5.70</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>2.11</td>
<td>36.75</td>
</tr>
</tbody>
</table>

In a linear mixed model analysis, the effect of insecticide application on mean ant foraging count was not significant (F= -1.00; df=1,187; p=0.3195).
Figure 2-1. Schematic of Jacksonville Zoo and Gardens (2008). Numbers and marked areas indicate sampling areas.
Figure 2-2. The extreme intensity of *Nylanderia* fulva infestations at the Jacksonville Zoo and Gardens are exemplified by the outlined areas with piles of ant cadavers 1d after a single insecticide application to the perimeter of a structure.
Figure 2-3. Foraging counts of *Nylanderia fulva* were obtained from a slice of canned sausage 15-30 min after placement on the ground.
Figure 2-4. Mean number of *Nylanderia fulva* per sampling point (sausage slice) averaged over all sampling areas per sampling date, from 2008-2011, at the Jacksonville Zoo and Gardens. There was a significant decline in ant populations over time ($F=5.28; df=22,191; p<0.0001$).
Figure 2-5. The relationship between total ant foraging counts of *Nylanderia fulva* for each sampling time and the mean minimum temperature (°C) over the 7 d preceding each sampling time ($y = 0 + 114.95 \times$; $r^2 = 0.7725$; $F=57.75$, $df=1,17$, $p<0.0001$).
Figure 2-6. The mean minimum temperature (°C) each year from 2002-2012. There was no significant difference in the mean minimum temperature ($F=1.47$, $df=10$, $4006$, $p=0.1441$). *Nylanderia fulva* population and dispersal data were collected 2008-2011.
Figure 2-7. Mean foraging ant count by year for each sampling area during a 3 yr study (2008-2011) of population density and dispersal of *Nylanderia fulva* at the Jacksonville Zoo and Gardens. Throughout the study, the highest ant foraging counts were from sampling areas along the perimeter of the zoo. Legend: RR=Railroad Tracks, PN=Plant Nursery, PK=Parking Perimeter (sampled in years 1 & 2 only), TR=Trout River, SB=Stingray Bay, RO=Rhino Overlook, AO=Australia Outer Perimeter, AI=Australia Inner Perimeter, PP=Play Park, RJ=Range of Jaguar, GO=Giraffe Overlook, WF=Wild Florida (n=number of sampling points per sampling area).
CHAPTER 3
SEASONAL DEMOGRAPHICS AND MACRONUTRIENT PREFERENCES OF *Nylanderia fulva*

**Introduction**

*Nylanderia fulva* (Mayr) is an invasive ant introduced into the southern United States in the late 2000s (Warner and Scheffrahn 2004). Like other invasive ant species such as *Linepithema humile* (Mayr), *N. fulva* is polygynous, unicolonial, nests opportunistically and relocates nests when disturbed. Because of these characteristics and extensive infestations, often in sensitive environments, pest management professionals (PMPs) are challenged to maintain an acceptable level of suppression of *N. fulva* using pesticides according to the directions on the products’ labels. An Integrated Pest Management (IPM) approach that includes the use of toxic baits may aid in suppressing *N. fulva* in residential areas. However, bait selection and the timing of bait applications are critical components of an IPM protocol. Therefore, understanding colony composition and food preferences throughout the year will strengthen ant baiting within an IPM approach.

Ant colony composition data relevant to the development of an IPM program include colony size and the numbers of queens, workers, alates, pupae and larvae (Scharf et al. 2011). Census studies in which the ant colony composition is recorded multiple times over an annual cycle may identify opportunities for improved bait performance. For example, timing of bait applications may be optimized by understanding when brood production is increasing, which is associated with increased resource utilization by colonies. In addition to determining the most optimal time to apply toxic bait, the attractiveness of the bait to *N. fulva* must be considered. In general, food preferences of ants fall into three broad categories: proteins, carbohydrates or lipids.
Commercially available ant bait formulations include one or more of these broad categories of macronutrients. In reality, ant food preferences are more complex and may vary seasonally within a single colony depending upon resource availability and colony requirements. For example, ant colonies producing large quantities of brood require increased dietary protein (Abril et al. 2007, Sorensen and Vinson 1981).

Ants in the genera *Paratrechina* and *Nylanderia* have demonstrated a preference for carbohydrates and protein. Smith (1947) reported other *Paratrechina* (=*Nylanderia*) species’ workers feed on carbohydrates (e.g., fruits, vegetables and honeydew) in the spring and fall and protein (arthropods) in the summer. In a survey of ants in the experimental self-contained colony Biosphere 2, high numbers of the crazy ant, *Paratrechina longicornis* (L.), were associated with high numbers of hemipterans (Wetterer et al. 1999) and Sharma et al. (2013) showed a significant increase in *N. fulva* worker trailing into trees with high hemipteran densities, both studies suggesting a preference for honeydew. Also, choice tests with *Nylanderia* sp. nr. *pubens* showed a preference for carbohydrate foods (Wynalda 2008, Cook et al. 2012).

Observations of *N. fulva* in Colombia found that their diet is mainly composed of insect protein and honeydew (Zenner-Polania 1990b) and *Paratrechina* (=*Nylanderia*) *fulva* have been observed taking hemipteran nymphs into the nest in Brazil (de C. Campos-Farinha and Zorzenon). Aldana et al. (1995) successfully attracted *N. fulva* to traps with ground beef or tuna. In Texas, field studies of *N. fulva* demonstrated significant preference for protein-based food (hot dog) compared to a carbohydrate-based food (20% sucrose solution) (Meyers 2008a). Based on these studies and personal observations of ant foraging in the laboratory and field, I hypothesized that,
although omnivorous, *N. fulva* in Florida would prefer protein-based foods. I hypothesized further that the preference for protein-based foods would correspond with increased brood production within the colony. Therefore, the objectives of this study were to: 1) Determine *N. fulva* seasonal colony structure by censusing the developmental stages and castes of colony fragments collected over 12 months and 2) Compare colony structure to seasonal macronutrient preferences.

**Materials and Methods**

**Demographic Studies**

This study was a completely randomized design in which the proportions of each developmental stage and adult caste (dependent variable) within field collected *N. fulva* colony fragments (n=2) were compared each month (independent variable) for one year. *Nylanderia fulva* colony fragments were collected from the periphery of Basin Park (lat. 29.64588°, long. -82.332315°) in Gainesville, Florida. The park had an infestation of *N. fulva* that had been established approximately 2 yr before the start of the study. The collection site was a wooded lot between the park and residential areas. A creek ran along the outermost boundary of the collection area. In the study period, Gainesville received a total of 71.0 cm of precipitation. The average maximum temperature was 28.0° C and the average minimum temperature was 10.0° C (NOAA 2012).

In colony demographics studies of *Solenopsis invicta* (Buren), Tschinkel (1993) was able to locate and excavate discrete nests, thus capturing all colony members except those foraging away from the nest. *Nylanderia fulva* opportunistically nest in leaf litter and debris and relocate quickly when disturbed; therefore, colony boundaries cannot be visually determined and the groups of ants collected for this study are considered colony fragments. Colony fragments were selected by walking to the center
of the collection area, using a random number generator to select a cardinal direction and collecting the first colony fragment encountered regardless of size or location. This method was repeated until two colony fragments were located. *Nylanderia fulva* colony fragments were located in leaf litter, tree stumps, fallen tree limbs and debris at the park.

Fragments were collected by quickly scooping up ants and brood along with surrounding organic matter and placing everything into a plastic container with the inner walls coated with a thin layer of Insect-a-Slip® (BioQuip Products, Inc., Rancho Dominguez, CA), a slippery coating that prevents the ants from escaping the container. The container and contents were transported to the laboratory and placed uncovered for 2-3 d on the laboratory bench at ambient temperature and relative humidity to allow the organic matter to dry. Food and water were provided *ad libitum*. An artificial nest was also placed into the container. The artificial nest, or nest cell was constructed from a round polystyrene Petri dish (100 x 15 mm) filled ¾ with dental stone and with a small hole (3 mm diameter) melted above the level of the dental stone into the cover. As the organic matter dried, the ants relocated into the nest cell. The leaves, soil, and other debris, free of ants and brood, was gradually removed until only the nest cell with ants remained.

To census the colony fragment, an index card (7.62 x 12.7 cm) was placed into the container with the edge resting on the bottom of the container. Five to ten ants were allowed to climb onto the card. The number of workers, queens, male alates, and female alates on the card were counted. Workers were carefully examined to see if any brood was carried in their mandibles. If so, the brood was categorized as larva or pupa
and counted. The number of eggs, when carried in the mandibles of the workers could not be accurately determined and were not counted. Ants on the card were gently tapped into a new container. This was repeated until all the live ants were counted. After all the workers, queens and male and female alates were counted, any brood remaining in the nest cell was categorized and counted. The numbers of dead workers remaining in the container were also counted. Since the counting of each individual life stage and caste must be done in a timely manner to represent the condition of the colony at the time of collection, only 2 colony fragments per month were able to be censused.

**Data analysis.** Because the colony fragments were comprised mostly of workers, proportions of each life stage and caste (workers, queens, male alates, female alates, larvae and pupae) in each fragment to the total number of ants collected for the year yielded extremely low numbers that biased comparisons. For the purposes of data analysis, the numbers of individuals in each life stage or cast collected in one month was divided by the number of individuals in the same life stage collected during the year (proportion). The data were grouped by seasons: spring (March-May), summer (June-August), fall (September-November) and winter (December-February). Since the data were not normal even after transformation, the proportions of the all stages were rank-transformed and a one-way analysis of variance (ANOVA) by stage was conducted to see if the proportion of each stage changed through seasons (Conover and Iman 1981). If the ANOVA was significant, differences in proportions between seasons were separated using Tukey’s HSD for each life stage (SAS Institute 2012). Results are reported as proportions of life stages.
Macronutrient Studies

This study was a randomized complete block design, blocked by site, in which the proportion of *N. fulva* workers (dependent variable) foraging on each of six food choices was compared each month (independent variable) over one year at each of four sites (independent variable) in Florida. Four locations were selected for observation of macronutrient preference of *N. fulva*: Jacksonville (JAX) (lat. 30.403305°, long. -82.643353°), Sarasota (SAR) (lat. 28.151818°, long. -81.624234°) and Gainesville with two sites (GA1 and GA2) (lat. 29.64588°, long. -82.332315° and lat. 29.704045°, long. -82.338538°, respectively). For consistency, the same sites were used for all evaluations and all sites had a heavy infestation of *N. fulva* for approximately 2 yr before the start of the study. The sites were varied in their use patterns. The Jacksonville site was a zoo and gardens. The Sarasota site was a residential condominium complex. The Gainesville sites were an industrial park and a recreational park.

For evaluations of macronutrient foraging, ~ 250 mg each of protein, carbohydrate, or lipid-based food were placed in random order on a 101.6 mm diameter paper disk in a circular pattern (Fig. 3-1). Both a liquid/semi-solid and a solid form of each macronutrient were presented. Protein macronutrients were represented by canned tuna (StarKist® Chunk Light Tuna in Water) and pureed beef (Beechnut® Beef and Beef Broth); carbohydrate macronutrients were honey (Publix brand) and a cotton ball saturated with a 10% sucrose solution and lipid macronutrients were represented by a cotton ball saturated with peanut oil (Publix brand) and lard (Armour®). The disk, along with the macronutrient food choices, was placed on the ground near areas of *N. fulva* activity. After a minimum of 15 min but no more than 30 min the numbers of ants on each food were recorded. Since *N. fulva* are easily disturbed, ants were counted *in situ.*
Macronutrient foraging was evaluated at each site once per month for 12 mo except for JAX which was not evaluated in May and SAR which was not evaluated in May and June. At each evaluation, food choices on the disk were replicated ten times. Sampling was conducted between 0900 and 1200. Macronutrient foraging evaluations were conducted the same week as the colony fragment collections.

**Data analysis.** For each disk, the number of ants on each food choice were counted (dependent variable). Since the data were not normal even after transformation, the foraging ant counts on each macronutrient were assigned a rank-score between 1 to 5, where a rank score of “1” represented ant counts ranging from 1 to 20, “2” for counts ranging from 21 to 40, “3” for counts ranging from 41 to 60, “4” for counts ranging from 61 to 80, and “5” for counts ranging from 81 to 100. A one-way ANOVA was conducted on the rank-scores by season (Conover and Iman 1981, SAS Institute 2012). Since sites spanned a distance of ~443 km and each were of a different use pattern, it was expected that site would be a source of variation in the study. Therefore, site was used a blocking variable in the analysis. T-tests were performed on each pair of food choices to determine if they could be pooled for the analysis, but tuna and beef (t=-33.09, df=885, p=0.0020), and sucrose and honey (t=-5.99, df=887, p=0.0001) were significantly different and the data were not pooled. If an ANOVA was significant, rank-scores were separated using Tukey’s HSD to determine changes in foraging ant counts on each macronutrient by season (SAS Institute 2012). Results are reported as mean foraging ant counts.
Results and Discussion

Demographic Studies

Little is known about the colony demographics of tropical Nylanderia species and these data represent the first published effort of colony demographics for N. fulva. The data in this study indicate that except for male and female alates, all other life stages, including queens, are present in the colony all year. Only male alates (F=7.46, df=11, 12, p<0.0008) and pupae (F=2.85, df=11, 12, p<0.0427) proportions were significantly different between seasons. The numbers of individuals in the colony fragments ranged from 451 to 27,518 with a mean fragment size of 4294 (Table 3-1). On average, the colony fragments were comprised of 2833 workers (range 422-16,254), 602 larvae (range 0-2657), 802 pupae (range 19-8487), 35 male alates (range 0-122), 0 female alates (range 0-1) and 21 queens (range 2-120).

Seasonal fluctuations in colony fragment demographics were expected as many ants exhibit changes in foraging activity, population size and brood production reflecting varying environmental conditions and food resource availability (Abril et al. 2007, Callcott et al. 2000, Ichinose 1987a, Tripp 2000). In other tropical invasive ant species such as L. humile and S. invicta, colony demographics fluctuate annually. Markin (1970) showed that L. humile workers make up 88% of the colony in December and January. In the colony fragments collected for this study, workers were the predominant members comprising on average 66% of all ants collected. The proportion of workers collected each season compared to the total workers for the year was greatest in the fall (90%) and fewest in the spring (41%) (Fig. 3-2).

In studies of S. invicta colony demographics, reproductives were found in significantly greater numbers in spring (Tschinkels 1993). In contrast, male alates in this
study emerged in June colony fragment 2 and August in colony fragment 1. There were significantly fewer *N. fulva* male alates in the summer (n=71) compared to colony fragments collected in the winter (n=329) (Table 3-1). While female alates were found in the colony fragments only in August and September, queens were found year round. In other invasive ant species, the presence of reproductives provides a mechanism for improved survival of a transported propagules (Keller et al. 1996). In *L. humile*, for example, males and queens may develop from brood, mate within the nest, and therefore, provide a replacements for a queenless colony (Passera et al. 1988). Laboratory studies of field collected queen fecundity showed that *N. fulva* queens could lay as many as 0.25 eggs per hour and that the greater the number of queens the greater the egg laying capacity of each individual queen (McDonald 2012). This contrasts with queen fecundity studies from Colombia where multiple queens inhibited individual egg laying and the average queen fecundity was 0.88 eggs per hour (Arcila et al. 2002b). In this study, queens made up only 0.05% of all the ants collected but considering that the collected colony fragments represent only a small fraction of the population, it suggests that these ants have a very high reproductive capacity.

In *S. invicta*, the number of pupae in collected colonies was greatest from winter to midsummer (Tschinkel 1993). Ichinose (1987b) reports larvae and pupae significantly increase in *P. longicornis* colonies in May–July but are present in the colonies in low numbers all year. Arcila et al. (2002a) reported that *N. fulva* egg production increases dramatically in late winter and spring, meaning larvae and pupae should be present in the colony starting in spring. In this study, the larval counts in the colony fragments increased from 1343 to 7165 winter to spring. There was also significantly greater
number of pupae in spring (n=5,980) compared to fall (n=970) (F=2.85, df=11, 12, p<0.0427) (Fig. 3-2).

The colony fragment demographics of *N. fulva* collected in Gainesville, Florida can be used as the basis for planning an IPM program for suppression. However, it should be noted that these data are from only two colony fragments collected monthly from a single location and for only a one year period. Multiple colony fragments collected from multiple sites over a period of years should be done to confirm these demographics. Also, *N. fulva* respond to disturbance by rapidly escaping the immediate area which posed limitations to the study. When the adult members of the colony attempt to escape disturbance, they often pick up larvae and pupae in their mandibles. Therefore, it is also possible that the numbers of larvae and pupae in the collected colony fragments were underreported. Additionally, the collection method of scooping the ants into bins introduces possible experimental bias as the collection diameter and depth was arbitrary. These limited data suggest that along with polygyny, polydomy, omnivory and the ability to exploit a variety of nesting substrates, the ability of *N. fulva* to reproduce nearly year round may have significant implications on pest control programs.

**Macronutrient Studies**

Proteins were significantly more accepted by *N. fulva* during spring, summer and fall, while sucrose and honey were more accepted during the winter; lard and oil were the least accepted (Table 3-2, Fig 3-3). These data contrast with a previous study that concluded carbohydrates were more accepted (Cook et al. 2012). One reason for this difference may be because the ant collection in Cook et al. occurred monthly from December to May, and not the entire year. But more likely it was the food choices offered. Cook et al. offered casein and albumin as a dry, granular powders. The beef
and tuna in this study were also moisture sources and *N. fulva* are highly attracted to moist food sources (Chapter 4).

The tuna used in this study contained twice the protein as beef (tuna=22.00 g/100g and beef =11.27 g/100g) (USDA 2017). Both tuna and beef contained no carbohydrate and only minimal lipids (0.69g/100 g and 1.41 g/100 g, respectively) (USDA 2017). Since lipid macronutrients do not appear to be a driver for *N. fulva* foraging, the preference for tuna could be attributed to its higher protein content. A preference for proteins is seen in other invasive ants such as *Solenopsis xyloni* (McCook) and *L. humile*. Hooper and Rust (1997, 2000) tested the preference of *S. xyloni* and *L. humile* to several protein sources. Anchovy, with a protein content of 47% wt/wt, was highly accepted.

Seasonal fluctuations in macronutrient preference are expected owing to varying environmental conditions and food resource availability (Abril et al. 2007, Barbani 2003, Bernstein 1976, Cannon and Fell 2002). Additionally, macronutrient regulation studies have shown that many ants will actively manage macronutrient intake at the colony level, selecting nutrients that are required for colony growth and maintenance (Cassill and Tschinkel 1998). There are different nutritional requirements for workers versus the other colony members but since foraging is conducted by the workers, they must choose foods that meet their individual needs as well as those of the colony (Dussutour and Simpson 2008, Cook et al. 2009).

It is believed that protein is required for increased brood production (Sorensen and Vinson 1981). For example, it has been demonstrated in *S. invicta* that protein is preferentially distributed to larvae (Vinson 1968, Markin 1970). In *L. humile* and
*Camponotus pennsylvanicus* (De Geer) increased protein foraging corresponded to increased percentage of eggs and larvae in the nest (Cannon and Fell 2002, Rust et al. 2000). Dussutour and Simpson (2009) demonstrated that brood containing *Rhytidoponera impressa* (Mayr) colonies offered diets with varying protein: carbohydrate ratios would regulate nutrient intake to include a higher percentage of protein compared to colonies with no brood. The results of this study show an indirect relationship between protein foraging and brood production. A graphical representation of the mean ant foraging counts on protein combined with the proportions of larvae and pupae in the colony fragments show increased brood production in the spring but interestingly increased protein foraging is occurring in the season following (Fig. 3-4). It is important to note that this study was a field evaluation of macronutrient preference at four sites in Florida but that colony fragments were collected only from the GNV1 site on the same day as the macronutrient tests.

Aron et al. (2001) demonstrated experimentally that *L. humile* colonies whose diets were supplemented with protein produced a significantly greater number of reproductives (alates) but no increase in worker number. Conversely, workers culled a significant number of sexual brood in colonies without protein supplementation. *Nylanderia fulva* colony fragments in this study contained reproductives nearly year round. If protein deficiencies prompt *N. fulva* workers to cull resource intensive reproductives, it is possible that the availability of protein-based food year round supports the rearing to adulthood of alates in *N. fulva* colony fragments. This hypothesis requires further testing.
For invasive ants, colony founding is predicated by production of new workers. The resource preference hypothesis informs that this early colony growth (brood production) is supported by increased protein intake and that later the colony shifts to carbohydrate foraging to maintain colony workers (Sorensen and Vinson 1981). However, Shik and Silverman (2012) demonstrated that *L. humile* incipient colonies had more surviving workers and greater brood production when fueled by aphid honeydew rather than insect prey and Rowles and Silverman (2009) demonstrated experimentally that the availability of a carbohydrate source could improve success of *L. humile* in growth and range expansion. This may be because the incipient colonies had no brood to process solid food. But also because liquids are more quickly transferred between colony members and carbohydrate rich foods allow the few workers to forage at a higher rate (Shik and Silverman 2012). Cook et al. (2009) showed that *S. invicta* colonies presented with food containing equivalent protein to carbohydrate ratios collected significantly more food than colonies presented with food containing highly unbalanced ratios. However, colonies that were presented food with low carbohydrate content, collected the food, extracted the carbohydrates and stockpiled the remaining protein containing food, thus ensuring adequate carbohydrate intake to fuel foraging activities of workers. Further supporting the role of carbohydrates in brood maintenance, Grover et al. (2007) deprived *L humile* colonies carbohydrates resulting in a significant reduction in brood mass.

In this study, there was significantly greater mean ant foraging counts on sucrose in the winter ($F=4.68$, df=$5, 711$, $p=0.0003$) but there was some carbohydrate feeding all year. Because these data show that *N. fulva* accept both proteins and carbohydrates,
this may represent a transition from colony growth to colony maintenance. Alternatively, *N. fulva* colony fragments in this study had both brood and large numbers of workers to support the colony year round. If the already large colony is also growing, it is possible that both protein and carbohydrates are required for colony growth and maintenance that occur simultaneously. In addition to supporting colony level dietary needs, increased ant foraging to a specific food may reflect a resource limitation for that macronutrient. Kay (2004) demonstrated that *Dorymyrmex smithi* (Cole) colonies supplemented with carbohydrates foraged protein. In this study there was a higher proportion of ant foraging on tuna and beef possibly suggesting carbohydrates are readily available.

The proportion of foraging ants on soybean oil and lard was significantly less than protein or carbohydrate-based foods in all seasons. This is consistent with choice studies in which *Paratrechina longicornis* (L.) were not attracted to any of six oils offered (Cornelius et al. 1996). In Colombia, Zenner-Polania (1990b) observed that the diet of *N. fulva* is comprised of animal protein and honeydew but also reports that these ant forage on sugar and oily foods inside buildings. After testing a variety of food attractants, Zenner-Polania (1990b) formulated a bait matrix using fish meal, sugar solution and pork lard and claimed the lard provided protein and acted as a binding agent for the bait. The USDA Food Composition Database (USDA 2017) reports the protein content of lard as zero; therefore, it is unlikely that the lard as a protein source contributed to the attractiveness of the bait. In this study, lipid foraging is minimal throughout the year, suggesting this is not a food source that is attractive to *N. fulva* and
commercially available baits containing oil such as those formulated to attract *S. invicta* may not be highly accepted by *N. fulva*.

Presuming that these foraging patterns reflect the nutritional needs of the colony, these data support the hypothesis that *N. fulva* in Florida prefer protein-based foods in the spring, summer and fall. Carbohydrate acceptance is greater in the winter but overall foraging is reduced compared the rest of the year.

Insecticidal ant baits are an integral part of an IPM strategy and selecting acceptable and palatable baits for the target ant species increases the probability of success. Ant bait selection is challenging owing to the unique preferences of each species, as well as shifts in dietary requirements. Application timing is also critical to success when using toxicant baits.

PMPs should be cognizant of these demographic and dietary patterns when developing treatment plans for *N. fulva*. PMPs using insecticidal baits as part of an IPM strategy should consider early season baiting to take advantage of actively foraging populations in early spring (Chapter 2). In addition, the use of a toxic bait with a protein-based matrix or a combination of protein and carbohydrate may result in enhanced bait uptake by *N. fulva* during spring, summer and fall. Carbohydrate-based baits should be selected for winter baiting.
Table 3-1. Total numbers of queens, alates, workers, and brood in field collected colony fragments (n=2) of *Nylanderia fulva* collected May 2011 to April 2012 in Gainesville, FL.

<table>
<thead>
<tr>
<th>Colony Fragment 1</th>
<th>Queens</th>
<th>Male Alates</th>
<th>Female Alates</th>
<th>Workers</th>
<th>Pupae</th>
<th>Larvae</th>
<th>Total</th>
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<td>0</td>
<td>3903</td>
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85
Table 3-2. Mean rank-counts of foraging *Nylanderia fulva* on six foods. Summary of Tukey’s HSD ($\alpha=0.05$) results by season from four sites in Florida from May 2011 to April 2012 (n=120 replicates per season).

<table>
<thead>
<tr>
<th></th>
<th>Fall</th>
<th>Spring</th>
<th>Summer</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
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<td>bc</td>
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</tr>
<tr>
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<td>1.00</td>
<td>c</td>
<td>Oil</td>
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<th></th>
<th>Fall</th>
<th>Spring</th>
<th>Summer</th>
<th>Winter</th>
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</thead>
<tbody>
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<td>Beef</td>
<td>1.30</td>
<td>a</td>
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<td>1.70</td>
</tr>
<tr>
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<td>b</td>
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<th></th>
<th>Fall</th>
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</thead>
<tbody>
<tr>
<td>F=16.72</td>
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<td></td>
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<td>4.68</td>
</tr>
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<td>df=5, 580</td>
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<td>$p&lt;0.0001$</td>
<td>$p=0.0003$</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3-1. *Nylanderia fulva* macronutrient ant foraging assay set-up. Food choices were placed randomly on paper disk. The number of ants on each food was counted in situ 15-30 min after placing disk in area with ant activity. The food choices in this photograph (clockwise from 12:00) were tuna, peanut oil, lard, pureed beef, 10% sucrose solution and honey.
Figure 3-2. Proportion of each life stage and caste in field collected colony fragments (n=2) of *Nylanderia fulva* each season to the total numbers of individuals in the same life stage or caste collected in a year. There was a significant difference in the proportion of male alates (F=7.46, df=11, 12, p<0.0008) and pupae (F=2.85, df=11, 12, p<0.0427) between seasons. Proportions of each life stage between seasons with different letters are significantly different (Tukey's HSD α=0.05).
Figure 3-3. Mean ant counts of *Nylanderia fulva* on six foods from May 2011 to April 2012. Season means were combined from four sites (JAX, GA1, GA2 and SAR). Mean counts of ants feeding on each food within a season with different letters are significantly different (Tukey's HSD α=0.05) (spring: $F=17.15$, df=5, 580 $p<0.0001$; summer: $F=43.9$, df=5, 633, $p<0.0001$; fall: $F=16.72$, df=5, 711, $p<0.0001$; winter: $F=4.68$, df=5, 711, $p=0.0003$).
Figure 3-4. Mean ant counts of *Nylanderia fulva* on proteinaceous foods each season from May 2011 to April 2012 compared to the mean proportion of brood from field collected colony fragments.
CHAPTER 4
INSECTICIDE BAITING PROGRAM FOR SUPPRESSION OF *Nylanderia fulva*

**Introduction**

As demonstrated in Chapter 2, *N. fulva* is subject to human-mediated transport, can be a nuisance in sensitive areas that are difficult to treat and are unresponsive to the “spray-only” method of pest control, yet spraying a liquid insecticide continues to be a common approach to controlling *N. fulva*. Application methods include outdoor perimeter applications, and indoor crack and crevice applications to harborages and nesting sites. In a desperate attempt to control this ant, people have improperly used products in a way that could endanger the environment or themselves (1FMO, personal communication).

Management of *N. fulva* infestations in Florida may be improved by the implementation of an Integrated Pest Management (IPM) approach. Toxic baits are an integral part of an IPM strategy for ant control and may be used to control ants both indoors and outside. Compared to perimeter applications of liquid insecticides, the use of baits for ant control may offer the advantages of reduced insecticide application volume and reduced non-target exposure (Apperson et al. 1984, Barbani 2003). The use of insecticide baits to control invasive species may also have a positive impact on the abundance and diversity of native ants (Calixto et al. 2007). Another advantage is that baits do not have to be collected by all foraging ants to provide effective control as baits are returned to the nest and transferred via trophallaxis to nestmates within the

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colony (Vega and Rust 2003). This may improve toxicant transfer in an invasive ant species such as *N. fulva* that does not construct discrete nests and relocates nests frequently.

An effective IPM strategy for control of *N. fulva* may include the use of toxic bait; however, there are scarce data on *N. fulva* bait effectiveness to consult for bait selection. In order to be effective, a bait must first be accepted by ants. Therefore, the objectives of this study were to: 1) Evaluate the acceptance and efficacy of commercially available ant baits in the laboratory as a first step to identifying potential products for inclusion in an IPM strategy for suppression of *N. fulva* and 2) Evaluate the field efficacy of granular and liquid baits distributed in bait stations in suppressing infestations of *N. fulva*.

**Materials and Methods**

**Laboratory Bioassays of Bait Acceptance and Efficacy**

**Ant collection and colony maintenance**

Each month a *N. fulva* colony fragment was collected from one of two urban sites in Gainesville, Florida, each with an infestation that had been established for at least 2 yr prior to the study. Ant collection was achieved by locating a colony fragment in leaf litter and quickly scooping the leaf litter and a thin layer of the underlying soil into a plastic container. The interior walls of the plastic container were coated with Insect-a-Slip® (BioQuip Products, Inc., Rancho Dominguez, CA), a slippery coating that prevents ants from escaping the container. Care was taken to collect workers, brood and queens quickly as *N. fulva* will relocate immediately upon being disturbed. Containers with collected ants were transported to the laboratory where the ants were provided with an artificial nest constructed from a round polystyrene petri dish (100 x 15 mm) ¾ filled with
dental plaster and with a hole (3 mm) melted into the cover to allow entry (Fig. 4-1). As the leaf litter dried, the ants relocated into the artificial nest and the litter was gradually removed allowing for easy observation and harvesting of ants from the colony fragments. The ants were fed live termites and/or slices of canned sausage (Armour® brand, Pinnacle Foods Group, LLC, Cherry Hill, NJ) every other day. Water and 10% sucrose solution were provided *ad libitum* via test tubes stoppered with cotton.

In advance of conducting the bait tests, worker ants were removed from the laboratory colonies and placed in groups of twenty into test arenas consisting of round plastic containers (15.24 x 10.16 cm) containing moistened dental plaster poured to a height of 0.75 cm and inner walls coated with a thin layer of Insect-a-Slip. An overturned vial cap (11 x 4 mm), with a hole cut into the side was provided for harborage. Water was provided for the length of the study via a vial cap completely filled with cotton and saturated with deionized water. The ants were starved for 24 h prior to the start of the test.

To conduct the bioassays, ~250 mg of bait from an unopened container was measured into a vial cap. One bait-containing vial cap was placed into each arena. Control arenas received only cotton stuffed vial caps saturated with 10% sucrose solution. Test arenas were placed into a test chamber consisting of a polyvinyl chloride (PVC) frame (1.5 x 1.5 x 1.5 m) covered with plastic sheeting (Fig. 4-2). To maintain humidity levels inside the chamber, a water-filled tray was placed into the chamber. A plastic grid (1.5 x 1.5 m) was placed over the tray. The test arenas were then randomly placed atop this grid.
Acceptance and efficacy testing

Fifteen commercially available insecticide baits were tested (Table 4-1). Baits tested included solid (granular) and gel/liquid formulations. Each bait test was replicated 10 times in the spring (March-May), summer (June-August), and fall (September-November) for a total of 30 replicates per bait except Amdro Pro and Niban® Fine Granule which were not tested in the spring and Advion® Ant Gel which was not tested in the fall as these baits were not yet delivered at the time of testing. Bioassays were not conducted during the winter months as N. fulva foraging is typically reduced during colder weather (Chapter 2). All bait treatments were compared to a 10% sucrose solution as a control. Bioassays were conducted within 2 wk of N. fulva collection and all replications within a season are from a single colony.

To test acceptance of the baits, the numbers of ants on the surface of the bait were recorded at 10, 20, 30, 40, 50 and 60 min after placing baits into the test arenas. The numbers of ants on the bait for each time point were summed to create an acceptance count over all time points. Data normality, homoscedasticity and independence for acceptance counts were evaluated by Shapiro Wilk, Levene’s and Durbin-Watson tests, respectively. Since the data were not normally distributed and could not be normalized after transformation, the acceptance counts were assigned a rank-score of “1” was assigned if ant counts were between 0-20, “2” if counts were between 21-40, “3” if counts were between 41-60, “4” if counts were between 61-80 and “5” if counts were between 81-100. Rank-scores were analyzed for each season using a one-way analysis of variance (ANOVA) with the variable bait as the main factor (Conover and Iman 1981, SAS Institute 2012). When appropriate, means were
separated using Tukey’s HSD (α=0.05) test. Results are reported as mean acceptance scores.

After bait acceptance was evaluated, the same test arenas, still containing ants, water and bait were returned to the test chamber to determine bait efficacy and speed of mortality. The numbers of live ants in each test arena were recorded at 24 hr and then daily for 7d or until 100% mortality. If 100% mortality was not achieved in 7 days, mortality was recorded as 8+ days. Delayed toxicity allows optimal bait feeding, recruitment, and transfer of the toxicant to nestmates (Rust et al. 2004). Rust et al. (2004) found that for L. humile, 1-4 d of exposure to bait caused maximum foraging worker mortality; thus, 3 days after treatment (DAT) and days to 100% mortality were selected as the time intervals for data analyses. Normality, homoscedasticity and independence of percent mortality at 3 DAT and number of days until 100% mortality data were evaluated by Shapiro Wilk, Levene’s and Durbin-Watson tests, respectively. As data were not normally distributed and could not be normalized after transformation, the percent of dead ants at each evaluation were assigned a rank-score for the mean percent mortality at three days after treatment. A rank-score of “1” was assigned if percent mortality was between 0-20, “2” if percent mortality was between 21-40, “3” if percent mortality was between 41-60, “4” if percent mortality was between 61-80 and “5” if percent mortality was between 81-100. Rank-scores for mean number of days until 100% mortality were assigned the following: “5” = 0 to 1 days to 100% mortality, “4” = 2 to 3 days, “3” = 4 to 5 days, “2” = 6 to 7 days, and “1”=8+ days to 100% mortality. The rank-scores for percent mortality at 3 DAT and the number of days until 100% mortality, were each analyzed using a one-way ANOVA for each season with bait as the main
factor (Conover and Iman 1981, SAS Institute 2012). If significant, means were separated using Tukey’s HSD ($\alpha=0.05$) test. Results are reported as mean rank-scores.

**Field Evaluations of Bait Efficacy**

Field evaluations of the efficacy of granular and liquid baits delivered via bait stations placed along the perimeter of structures and lawn edges were conducted at three sites in Florida using a completely randomized design. Sites are described below.

**Bait station design**

**Liquid bait stations.** Two types of baits stations were used to dispense liquid bait. The first was a JT Eaton Top Loader Rodent Bait Station (Fig. 4-3) (J.T. Eaton & Co., Inc., Twinsburg, OH) modified so that the metal rod for holding rodent bait blocks in the upright tube was removed and replaced with an inverted plastic bottle (500 ml) containing liquid ant bait. A cotton plug was placed into the opening of the bottle to allow the contents to slowly drip onto a 2.5 cm thick disk of foam slightly smaller than the inside diameter of the tube that was placed at the base of the tube. The foam absorbed the bait and provided a feeding surface for the ants. This device worked well to dispense the liquid bait and ants were observed trailing to and from the inside of the bait station, however, the design was not tamper-proof. Raccoons were able to reach into the bottom of the station and remove the foam disk. Therefore, a more tamper-proof bait station was designed and constructed.

The second station designed to dispense liquid bait (Figure 4-3) was constructed of PVC (11.4 cm inside diameter, 25.4 cm long) pipe. Eight holes (0.3 cm diameter) were drilled equidistantly through the pipe 5.1 cm from one end. The end with the drill holes was capped with a plastic pipe cap and sealed with caulking. The pipe was placed upright on the sealed end and a 2.5 cm thick disk of foam slightly smaller than the inside
diameter of the pipe was placed into the pipe so that the foam rested on the sealed end. Liquid bait solutions were poured into plastic bottles (500 ml). A hole was melted into each of the bottle’s caps with a soldering iron and the hole was plugged with cotton. The caps were replaced onto the bottles and the bottles were then inverted and placed into the lengths of PVC pipe. A second plastic pipe cap was placed onto the top of the pipe but was not sealed.

Both types of bait stations were installed by placing the station flush to the ground and attaching the station to a tree, fence post or stake with plastic cable ties. The volume and quality of the liquid bait was observed at each evaluation interval to determine whether the liquid bait should be refilled or replaced.

Since worker ants require carbohydrates year round to fuel foraging activities (Carroll and Janzen 1973) and field choice tests (Chapter 3) have determined that _N. fulva_ will accept carbohydrate based foods throughout the year, a liquid carbohydrate-based bait was included in this study: a 1:10 dilution of Maxforce® Quantum Ant bait (imidacloprid, Bayer Environmental Science, Research Triangle Park, NC) in a 25% sucrose solution.

**Granular bait stations.** Two types of granular bait stations were used. The first consisted of a standard rodent bait box into which bait was placed (Fig. 4-4). The station lid was then closed and locked. The stations were placed along the perimeters of structures where they could be camouflaged by mulch or vegetation.

The second bait station was constructed of PVC pipe (11.4 cm inside diameter, 25.4 cm long). Eight holes (0.3 cm diameter) were drilled equidistantly through the pipe 10.2 cm from one end. The end with the drill holes was capped with a plastic pipe cap.
and sealed with caulk. The pipe was placed upright on the sealed end and granular bait placed inside the station. A second plastic pipe cap was placed in the top of the pipe but was not sealed. The PVC bait stations were placed in areas where the rodent bait boxes could not be secured or were aesthetically unappealing.

Rodent box bait stations were installed by securing the station to the ground with a metal spike. PVC pipe bait stations were installed as described above. Granular bait stations were not placed within 1 m of water or where tidal fluctuations may have caused the bait station to come into contact with water. Stations were opened and the condition of the bait noted at each evaluation interval. If bait was depleted or if clumped, wet, moldy or otherwise degraded it was removed from the bait station and replaced with fresh bait.

Laboratory bioassays have demonstrated that *N. fulva* accepted ant baits with matrices containing a mixture of protein and carbohydrate. Additionally, hydramethylnon has been demonstrated to be an effective active ingredient against *N. fulva* in no-choice, laboratory studies using worker ants (see acceptance and efficacy section). Therefore, the granular bait used in this study was Maxforce® Complete granular ant bait (Bayer Environmental Science, Research Triangle Park NC) comprised of a protein and carbohydrate-based matrix and containing the active ingredient hydramethylnon.

**Study sites**

**MTD1.** This site was a newly constructed subdivided community in Mt. Dora, FL (lat. 28°47'35.01"N, long. -81°35'44.58"W) (Fig. 4-5). The community consisted of single family and multifamily units. *Nylanderia fulva* infestation occurred in many residences along the perimeter of the community where it is adjacent to a hardwood forest preserve. The preserve is heavily infested with *N. fulva* and serves as a source of
continuous re-infestation. Ants were actively trailing along the exterior perimeter of the structures and trails were observed leading from the preserve into and around the retaining wall that separates the structures from the preserve.

Pest control methods included a quarterly exterior perimeter application of insecticides and crack and crevice application of insecticides on the interior of the structure as needed to address homeowner complaints. However, not all residences on the affected streets were under contract for professional pest control services. Lawn care and landscape management were provided by the homeowner or any number of local companies. Only six homes in the community with *N. fulva* infestation were under contract with the pest control company cooperating on this study; therefore, these were the buildings selected for the study. Buildings were randomly assigned as treated (n=4) or untreated (n=2).

Six sampling points per home were selected and marked with flags: three along the rear perimeter of the home and three along the interface between the lawn and the wooded area. Sampling points were approximately 3 m apart. To ascertain the baseline ant foraging at the site, sampling pre-counts were taken by placing a 5 mm thick slice of canned sausage on the ground at the marked sampling points. Then liquid bait stations (n= 4) containing 500 ml of bait and granular bait stations (n=3) containing 113.4 g of bait were installed along the interface between the lawn and wooded area. Bait stations were placed approximately 1.5 m apart and alternated between granular and liquid. Rodent bait stations (n=3) containing 113.4 g granular ant bait were also installed along the rear perimeter of each of the treated buildings approximately 3 m apart.

Supplementing the available food resources with the addition of baits may result in
increased foraging at the treated structures; therefore, the control buildings received liquid bait stations \( n=7 \) containing 500 ml of 10% sucrose solution, installed at the interface between the lawn and wooded area at 1.5 m intervals. Ant foraging counts were taken 3, 5 and 6 weeks after treatment (WAT).

**SAR1.** This site was a newly constructed condominium complex in Sarasota, FL (lat. 27°23’55.16”N, long. -82°27’35.93”W) (Fig. 4-6). The complex consisted of 14 buildings with 4-8 units in each bounded on two sides by additional condominium neighborhoods and a road on the third. The remainder of the property was surrounded by a preserve with open water and seasonally marshy areas. The preserve was heavily infested with *N. fulva* and served as a source of continuous re-infestation of the residences. The condominium complex had a heavy infestation of *N. fulva* around the buildings directly adjacent to the preserve. Ants were actively trailing along the exterior perimeter of the structure and trails were observed leading from the preserve into the lawn. Small trees in the yards were infested at the bases with nests of ants and the ants were observed trailing into the tree canopies. Foundation landscaping was infested with scales, aphids and whiteflies and ants were observed tending these. Sanitation was excellent with regular trash service and no excessive trash or yard debris on the exterior of the buildings.

Pest control methods included monthly exterior perimeter applications and as-needed interior crack and crevice applications of Temprid® (a.i. imidacloprid and Beta-cyfluthrin, Bayer Environmental Science, Research Triangle Park, NC). Homeowners used a variety of over the counter insecticides and “home remedies” to prevent insects from entering the condominiums. Lawn care and pest management
were provided by two separate companies. The lawn care company practiced an IPM approach whereby Arena 0.25G (Valent U.S.A. Corporation, Walnut Creek, CA) was spot applied to the lawn only as necessary for insect control.

Six buildings along the perimeter of the property and adjacent to the preserve were infested with *N. fulva*. Since the buildings were not far enough apart from one another to provide a buffer between treatments, the first four buildings were designated as treatment and the remaining buildings were assigned as controls. Sampling points were selected, ant foraging pre-counts were taken and baits stations were installed as described above. Ant foraging counts were taken 1, 2, 3, 4 and 6 WAT.

**SAR2.** This site was a condominium community in Sarasota, FL (lat. 27°23'55.16"N, long. -82°27'35.93"W) (Fig. 4-7). The community consisted of 20 multi-story buildings situated along a road that passes through the length of the community. The perimeter of each building was surrounded by a hardwood mulched bed containing ornamental foliage typical of southern Florida. The mulched bed extended approximately 3 m from the building. At the rear of each property in buildings 1 to 15, there was a grass lawn that extends from the mulched flower bed to the edge of a man-made lake. In the front of each property there was a grassy area that extends 1 m from the mulched bed to the edge of the road. Across the road there was ~ 3 m of grassy area that slopes gradually to a marsh. Trees, approximately 3 m apart and 1 m from the road, line the length of the road. Two to five trees were directly across from each building. The marsh extended away from the community and drained into a creek that runs parallel to the road. Buildings 15 through 19 are situated in a *cul de sac* such that the grass lawn at the rear of each building extends to wooded area and the front of each
building leads to a parking lot rather than the marsh. Buildings to receive the bait treatment were randomly assigned.

Sampling points were selected and ant foraging pre-counts were taken as described above. One liquid bait station containing 500 ml of bait and one granular bait station containing 113.4 g of bait were installed adjacent to each tree (n=2-5) at the interface of the road and marsh. Trees at the control buildings received only liquid bait stations containing 500 ml of 10% sucrose solution. At this site, the front of the buildings faced the infested area and for aesthetic reasons, bait stations were not installed at the building’s perimeter.

To install the bait stations, wooden grade stakes (20.3 cm long) were inserted approximately 10 cm into the ground as close as possible to the base of each tree. The bait stations were secured to the stakes with plastic cable ties. Ant foraging counts were taken at 4, 5 and 6 WAT.

It was not acceptable to the management or residents of the community to eliminate pest control services. During this study, the site received an application of 0.15% Temprid® SC (imidacloprid and beta-cyfluthrin, Bayer Environmental Science, Research Triangle Park, NC) applied to the exterior perimeter of each building as per manufacturer’s label directions.

**Data analyses.** Due to the variability of each site, the data from each site was analyzed separately. Data assumptions of normal distribution, homoscedasticity and independence for ant foraging count data were evaluated by Shapiro Wilk, Levene’s and Durbin-Watson tests, respectively. As the data were not normally distributed and could not be normalized after transformation, the foraging counts were rank-scored. A
rank-score of “1” was assigned if ant counts were between 0-20, “2” if counts were between 21-40, “3” if counts were between 41-60, “4” if counts were between 61-80 and “5” if counts were between 81-100. To determine if there were significant differences between the mean ant foraging counts at the perimeter of the structure and the interface between the lawn and the wooded area and between baited and untreated structures at each site, rank-scores were analyzed using a 3-way ANOVA with main factors weeks after treatment, sampling location and treatment (Conover and Iman 1981, SAS Institute 2012). If significant, means separation for weeks after treatment was conducted with Tukey’s HSD ($\alpha=0.05$). Results are reported as foraging ant counts.

Results

Laboratory Bioassays of Bait Acceptance and Efficacy

Acceptance

Advion® Ant Gel in the spring and InTice™ Smart Ant Gel in the summer and fall had significantly greater acceptance by N. fulva compared to the other baits (Table 4-2). Ant acceptance of Advion® Ant Gel in the spring was not significantly different from the sucrose control and the sucrose control was not significantly different from InTice™ Smart Ant Gel, Advance® 375A Select, and Maxforce® Fine Granular baits. Advion® Ant Gel was not tested during the fall.

In the summer, acceptance of InTice™ granular bait and Advion® Ant Gel were not significantly different but InTice™ was significantly more accepted than the sucrose control. In the fall, acceptance of InTice™ granular bait and InTice™ Smart Ant Gel were not significantly different but InTice™ Smart Ant Gel had significantly greater acceptance compared to the sucrose control. The sucrose control proved to be more acceptable than the majority of the baits tested.
Efficacy

Ant baits that demonstrated significantly greater mortality at 3 DAT also provided 100% mortality in the fewest number of days (Tables 4-3 and 4-4). Assuming quick knock-down of the foraging ant population is the objective, Amdro® Pro, Maxforce® Complete Insect Bait and Maxforce® Ant Killer Bait Gel were the best performing baits when performance criteria acceptance, percent mortality at 3 DAT, and number of days until 100% mortality were considered together (Figs. 4-8 and 4-9). Ant baits with the active ingredient boric acid did not achieve 100% mortality within the 7 d of the study.

Field Evaluations of Bait Efficacy

The overall ANOVA at SAR1 was not significant (F=1.56, df=15,128, p=0.0927) (Fig. 4-10). At this site, ant foraging counts around the perimeter of the baited and unbaited structures were zero throughout the study. Even though pre-counts were zero, the study was continued to determine if the perimeter baiting could act as a barrier to ant infestation. Ant foraging counts at the interface between the lawn and wooded areas increased over time in both baited and unbaited structures. However, the ant foraging counts ant the perimeter of all structures remained zero throughout study.

The only significant factor for the SAR2 site was sampling location (F=19.50, df=1, 192, p<0.0001). Figure 4-11 shows that there were no ants foraging around the perimeter of the SAR2 structures. However, at the interface between the lawn and wooded area in the unbaited structures, the ant foraging counts continued to increase, while the ant foraging counts in the baited structures decreased during the 6 week period. Perhaps if sampling continued, we would have seen a statistically significant outcome.
At MTD1, there was a significant 3-way interaction (WAT*Location*Treatment) which can be attributed to the large number of ants around the perimeter of baited homes at the start of the experiment which decreased and remained at relatively low levels for the duration of the test (Fig. 4-12). There was a significant difference in ant foraging counts between the perimeter of the structure and the interface between the lawn and the wooded area at the MTD1 site (F=53.22, df=1, 185, p<0.0001). There was also a significant difference in ant foraging counts between the baited and unbaited structures. While ANOVA indicates that the treatments were significantly different (F=15.03, df=1, 185, p=0.0001), in this case the ant foraging counts at the interface between the lawn and wooded area at the unbaited structures remained relatively stable with a slight decrease, while the ant foraging counts in the baited interface increased, which is not the desired bait treatment outcome, but not unexpected given that the numbers of ants at MTD1 were between ~3 to 10 times greater than the highest ant counts taken at a similar position at the other two sites.

Discussion

Laboratory Bioassays of Bait Acceptance and Efficacy

Effective ant baits require a feeding matrix that is acceptable to the target species and long-lasting enough to deliver the active ingredient (Stringer et al 1964, Warner 2003). An acceptable feeding matrix is critical, for without which, the ant will not consume the bait rendering even the most toxic active ingredient ineffective. If the workers do not carry the bait back to the colony, it also will be ineffective. Thus, we targeted workers for the acceptance study and found that baits that were most readily accepted were not necessarily the ones that resulted in the greatest mortality in a no-choice test.
In addition to an acceptable feeding matrix, effective ant baits must have an active ingredient that is non-repellent, slow-acting, transferrable, and have low mammalian toxicity (Stringer 1964). Delayed toxicity is important because the bait must not kill the foraging ant before it is able to return to the nest, transfer the toxicant to nest mates and recruit additional foragers (Rust et al. 2004, Williams et al. 2001, Warner 2003).

Little was known about the acceptance of various bait formulations to N. fulva. In a review of the acceptance and efficacy of ant baits by Stanley (2004), protein-based baits were recommended for Nylanderia spp. and protein containing baits have been used to control N. fulva in Colombia (Zenner-Polania 1990b). In a previous field assay choice test of bait acceptance, Advance® Carpenter Ant Bait® (abamectin, BASF, Research Triangle Park, NC) and Maxforce® Complete Ant Bait (hydramethylnon, BASF, Research Triangle Park, NC) were more accepted by N. fulva than Amdro® Ant Block (hydramethylnon, Central Garden and Pet, Atlanta, GA), ProBait® (hydramethylnon, Zoecon, Schaumburg, IL), Extinguish® Plus® (hydramethylnon and methoprene, Wellmark International, Schaumburg, IL) and Esteem® (pyriproxyfen, Valent USA Corporation, Walnut Creek, CA) (Drees et al. 2010). Oi (2014) reports that insect growth regulating (IGR) baits significantly reduce brood in laboratory colonies but the both pyriproxyfen and (S)-methoprene repel N. fulva.

In this study, the sucrose control proved to be more acceptable than almost all other baits except Advion® Ant Gel and InTice™ Smart Ant Gel. Although InTice™ Smart Ant Gel had the highest acceptance score, it did not induce significant mortality. This is not surprising as the active ingredient, borax (=sodium tetraborate decahydrate) is known to be slow acting (Klotz et al. 1997). It was surprising that 381 B Advance®, a
liquid formulation, was not highly accepted. The active ingredient 1.3% borax is also the active ingredient in InTice™ Smart Ant Gel (5% borax), a bait that was highly accepted. While the inert ingredients are proprietary and unknown, the advertising for InTice™ Smart Ant Gel claims that it is “super sweet,” suggesting it may have a higher concentration of sugar, and therefore, be more acceptable. In general, gel baits were more accepted by *N. fulva* in this study. However, their utility in an IPM program is limited. The volume of gel bait required to impact a *N. fulva* infestation would be costly and aesthetically unappealing. The recent label amendment to MaxForce Quantum (imidacloprid, Bayer Environmental Science, Research Triangle Park, NC) allows the gel bait to be mixed into a 25% sucrose solution without compromising efficacy in an effort to satisfy the need for large quantities of bait.

Most granular baits were less accepted than the sucrose control by *N. fulva*. Advance® 375A (spring, fall) and Advance® Carpenter Ant Bait (summer) had some of the highest acceptance scores for granular baits. These baits contain both protein and carbohydrate constituents. The preference for baits with both protein and carbohydrate content is consistent with data presented in Chapter 3 in which *N. fulva* accept both protein-based and carbohydrate based foods throughout the year. Advance® 375A and Advance® Carpenter Ant Bait both contain the active ingredient abamectin and were not significantly different from each other in acceptance, yet the Advance® Carpenter Ant Bait provided significantly greater percent mortality than Advance® 375A at 3 DAT. All of the hydramethylnon-containing granular baits had >75% mortality by 3 DAT. Granular baits containing the active ingredient boric acid were the poorest performing baits based on the performance criteria defined in this study which was high percent mortality by 3
DAT and 100% mortality in 7d. However, boric acid baits did provide approximately 50% mortality by 3 DAT and if the study were conducted for a longer period of time may have eventually reached 100% mortality. Therefore, boric acid-containing baits may have utility in IPM programs in sensitive environments where the use of other classes of chemical insecticides is limited.

The oil-containing baits, developed to attract red imported fire ants, were the least accepted by *N. fulva*. This finding is consistent with the data presented in Chapter 3 in which *N. fulva* were not attracted to lipid-based foods in a choice assay and with data from Stanley and Robinson (2007) showing that *N. fulva* were not attracted to oil containing baits. This contrasts the efficacy data that show Amdro® Pro as having the highest percent mortality at 3 DAT (Table 4-3). In the small arena, no-choice assay, ants were unable to avoid the bait. Under field conditions where ants have dietary choices, an unacceptable bait will not provide this level of efficacy. The efficacy of Amdro® Pro does highlight the need for an appropriate active ingredient. InTice™ Smart Ant Gel and Advance® Ant Bait were the most accepted baits in the study yet they were the least efficacious (Fig. 4-9).

Considering bait acceptance, delayed mortality and efficacy together allows the direct comparison of commercially available products included in this study (Figs. 4-8 and 4-9). These data suggest that Maxforce® Ant Killer Bait Gel and the granular baits Amdro® Pro and Maxforce® Complete may be effective bait products for the suppression of *N. fulva* and further field testing is suggested. While this study concentrated on commercially available baits as a way to provide timely information to pest management professionals, these data also suggest that the active ingredients
hydramethylnon and fipronil could be very effective against *N. fulva*, especially when combined with a matrix optimized for attractiveness and palatability.

**Field Evaluations of Bait Efficacy**

In this study the application of liquid and granular bait alone around the home and at the interface of the lawn and nearby natural area did not significantly reduce the overall mean ant foraging counts with the number of stations used in this experiment. Bait efficacy studies with *L. humile* showed that suppression could be achieved in six weeks to several months (Daane et al. 2006, Klotz et al.1997). Perhaps conducting the study for a longer period of time and adding more stations with bait would demonstrate overall suppression of *N. fulva* populations. Increasing the number of baits stations has been shown to suppress ant populations (Daane et al. 2006) but in an urban environment there are additional considerations of aesthetics and safety (children and pets). Adding baits stations also increases costs and takes more time to install and maintain (Klotz et al. 2002).

Few field studies have been conducted on bait efficacy against *N. fulva*. A laboratory evaluation of dinotefuran in a gel matrix resulted in 78% mortality of workers and suggested it may be an effective bait active ingredient against *N. fulva* (Meyers and Gold 2007); however, further field studies with dinotefuran in a granular matrix were inconclusive (Meyers 2008a). Advance® Carpenter Ant Bait (abamectin, BASF, Research Triangle Park, NC) was tested against *N. fulva* in field studies but was used in combination with Phantom® (chlorfenapyr, BASF, Research Triangle Park, NC) and Termidor® (fipronil, BASF, Research Triangle Park, NC); therefore, the efficacy of the ant bait alone is not known (Meyers 2008a). Advance® Carpenter Ant Bait also was tested in against *N. fulva* in a large field trial in Texas but it did not adequately suppress
ant populations (McDonald 2012). Also in Texas, a field evaluation of one application of Esteem® Ant Bait was not effective against *N. fulva* as ants from nearby untreated areas re-infested the treated area within 14 d (Nester 2010). The use of Maxforce® Quantum ant bait (imidacloprid, Bayer Environmental Science, Research Triangle Park, NC) diluted in a sucrose solution reduced brood 98% in 3 wk. In field trials, the diluted Maxforce® Quantum bait plus a stain was dispensed in large volume stations. The treatment did not provide sufficient suppression of ant populations but stained ants were collected over 30.5 m from where the bait was dispensed suggesting that liquid baits have the potential to be used to deliver bait to distant or difficult to access ant populations (Oi 2014). McDonald and Cook (2004) reported that baits formulated with the active ingredient spinosad are also highly effective against *N. fulva*.

In many residential *N. fulva* infestations in Florida, ants are migrating from a nearby refugia to the lawn and home. In previous studies of perimeter baiting to suppress *L. humile*, the baits were placed around the perimeter of the structure only (Blachly and Forschler 1996, Forschler and Evans 1994, Klotz et al. 2002). The baits effectively reduced the numbers of ants entering the structures and resident complaints. *Nylanderia fulva* infest in such large numbers that baits applied only to the perimeter of the structure would do little to reduce the numbers of ants entering the home. At two of the study sites, baits were placed around the perimeter of the structure but also along the edge of the lawn to intercept foragers migrating from the refugia. It was expected that the mean ant foraging counts near the edges of the lawns would be significantly greater than those along the perimeters of the structures. But the mean foraging ant counts at the interface of the lawn and natural area were not reduced enough to claim
suppression of the population. In this study, the first evaluation was 7 d after the
application of the baits which may too long an interval to capture any short-term
suppression.

Pest management professionals in Florida have had difficulty suppressing *N. fulva*
infestations that have peaked in late spring and remain problematic through fall. In this
study, the bait stations were placed at the interface of the refugia and the lawn (or
exhibit areas) with the intent of intercepting foraging ants before they reached the
structures. These results did not support the hypothesis, therefore, further studies of
perimeter baiting at sites with heavy infestation levels of *N. fulva* are warranted.
Table 4-1. Insecticide baits tested for acceptance and efficacy against *Nylanderia fulva* in laboratory bioassays.

<table>
<thead>
<tr>
<th>Product</th>
<th>Active Ingredient</th>
<th>Formulation Type</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>InTice™ Granular Ant Bait</td>
<td>Orthoboric acid</td>
<td>Granular</td>
<td>Rockwell Labs, Ltd., North Kansas City, MO</td>
</tr>
<tr>
<td>381B Advance® Liquid Bait</td>
<td>Borax</td>
<td>Liquid</td>
<td>BASF, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Advance® 375A Select</td>
<td>Abamectin</td>
<td>Granular</td>
<td>BASF, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Advance® Carpenter Ant Bait</td>
<td>Abamectin</td>
<td>Granular</td>
<td>BASF, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Advion® Ant Gel</td>
<td>Indoxacarb</td>
<td>Gel</td>
<td>Dupont, Wilmington, DE</td>
</tr>
<tr>
<td>Amdro® Pro</td>
<td>Hydramethylnon</td>
<td>Granular</td>
<td>BASF, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Esteem® Ant Bait</td>
<td>Pyriproxifen</td>
<td>Granular</td>
<td>Valent USA Corporation, Walnut Creek, CA</td>
</tr>
<tr>
<td>Extinguish® Professional</td>
<td>Methoprene</td>
<td>Granular</td>
<td>Wellmark International, Schaumberg, IL</td>
</tr>
<tr>
<td>InTice™ Smart Ant Gel</td>
<td>Borax</td>
<td>Gel</td>
<td>Rockwell Labs, Ltd., North Kansas City, MO</td>
</tr>
<tr>
<td>Maxforce® Ant Killer Bait Gel</td>
<td>Fipronil</td>
<td>Gel</td>
<td>Bayer Environmental Science, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Maxforce® Complete Bait</td>
<td>Hydramethylnon</td>
<td>Granular</td>
<td>Bayer Environmental Science, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Maxforce® Fine Granular Bait</td>
<td>Hydramethylnon</td>
<td>Granular</td>
<td>Bayer Environmental Science, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Maxforce® Granular Bait</td>
<td>Hydramethylnon</td>
<td>Granular</td>
<td>Bayer Environmental Science, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Niban® Fine Granular Bait</td>
<td>Orthoboric acid</td>
<td>Granular</td>
<td>Nisus Corporation, Rockford, TN</td>
</tr>
<tr>
<td>Optigard® Ant Gel Bait</td>
<td>Thiamethoxam</td>
<td>Gel</td>
<td>Syngenta Crop Protection, Greensboro, NC</td>
</tr>
</tbody>
</table>
Table 4-2. *Nylanderia fulva* acceptance of 15 commercially available baits in no-choice laboratory assays over three seasons (2009-2010) (n=10 replicates of each bait per season).

<table>
<thead>
<tr>
<th>Bait</th>
<th>Spring</th>
<th>Bait</th>
<th>Summer</th>
<th>Bait</th>
<th>Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advion (g)</td>
<td>19.9 a</td>
<td>Intice (g)</td>
<td>7.7 a</td>
<td>Intice (g)</td>
<td>24.8 a</td>
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<td>Control (l)</td>
<td>9.1 abc</td>
<td>Advion (g)</td>
<td>5.7 ab</td>
<td>Intice (gr)</td>
<td>4.1 abc</td>
</tr>
<tr>
<td>Intice (g)</td>
<td>7.7 bc</td>
<td>Advance Carpenter Ant (gr)</td>
<td>3.9 bc</td>
<td>Control (l)</td>
<td>3.9 b</td>
</tr>
<tr>
<td>Advance 375A Select (g)</td>
<td>4.1 cd</td>
<td>Optigard (g)</td>
<td>2.8 cd</td>
<td>Advance 375A Select (g)</td>
<td>2.7 bcd</td>
</tr>
<tr>
<td>Maxforce Fine Granular (g)</td>
<td>3.3 cd</td>
<td>Maxforce Complete (gr)</td>
<td>2.2 cd</td>
<td>Niban FG (gr)</td>
<td>1.9 bcd</td>
</tr>
<tr>
<td>Maxforce Ant Killer (g)</td>
<td>2.5 de</td>
<td>Intice (gr)</td>
<td>1.9 cd</td>
<td>Maxforce Fine Granular (g)</td>
<td>2.8 cd</td>
</tr>
<tr>
<td>Optigard (g)</td>
<td>1.4 de</td>
<td>Control (l)</td>
<td>1.8 cd</td>
<td>Advance Carpenter Ant (gr)</td>
<td>2.3 cd</td>
</tr>
<tr>
<td>Maxforce (gr)</td>
<td>0.5 de</td>
<td>Niban FG (gr)</td>
<td>1.3 cd</td>
<td>Optigard (g)</td>
<td>2.2 cde</td>
</tr>
<tr>
<td>381B Advance (l)</td>
<td>0.4 dc</td>
<td>Esteem (gr)</td>
<td>0.7 cd</td>
<td>Maxforce (gr)</td>
<td>1.2 cde</td>
</tr>
<tr>
<td>Advance Carpenter Ant (gr)</td>
<td>0.4 de</td>
<td>Amdro Pro (gr)</td>
<td>0.5 cd</td>
<td>Maxforce Complete (gr)</td>
<td>1.1 cde</td>
</tr>
<tr>
<td>Intice (gr)</td>
<td>0.1 e</td>
<td>Extinguish Pro (gr)</td>
<td>0.4 cd</td>
<td>381B Advance (l)</td>
<td>1.0 cde</td>
</tr>
<tr>
<td>Maxforce Complete (gr)</td>
<td>0.0 e</td>
<td>Maxforce Fine Granular (g)</td>
<td>0.3 cd</td>
<td>Amdro Pro (gr)</td>
<td>0.6 cde</td>
</tr>
<tr>
<td>Extinguish Pro (gr)</td>
<td>0.0 e</td>
<td>Advance 375A Select (g)</td>
<td>1.1 d</td>
<td>Maxforce Ant Killer (g)</td>
<td>0.5 de</td>
</tr>
<tr>
<td>Esteem (gr)</td>
<td>0.0 e</td>
<td>Maxforce Ant Killer (g)</td>
<td>0.9 d</td>
<td>Esteem (gr)</td>
<td>0.5 de</td>
</tr>
<tr>
<td>Niban FG (gr)</td>
<td>NT</td>
<td>381B Advance (l)</td>
<td>0.3 d</td>
<td>Extinguish Pro (gr)</td>
<td>0.0 e</td>
</tr>
<tr>
<td>Amdro Pro (gr)</td>
<td>NT</td>
<td>Maxforce (gr)</td>
<td>0.3 e</td>
<td>Advion (g)</td>
<td>NT</td>
</tr>
</tbody>
</table>

Mean acceptance counts in the same season followed by the same letter are not significantly different (Tukey’s HSD α=0.5). Key: g=gel, l=liquid, gr=granul
Table 4-3. Mean ranked percent mortality of *Nylanderia fulva* at 3 days after continuous exposure to 15 commercially available baits in no-choice laboratory assays over three seasons (2009-2010) (n=10 replicates of each bait per season).

<table>
<thead>
<tr>
<th>Bait</th>
<th>Spring</th>
<th>Bait</th>
<th>Summer</th>
<th>Bait</th>
<th>Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxforce Fine Granular (g)</td>
<td>4.9 a</td>
<td>Maxforce Complete (gr)</td>
<td>5.0 a</td>
<td>Maxforce Ant Killer (g)</td>
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<tr>
<td>Maxforce Ant Killer (g)</td>
<td>4.9 a</td>
<td>Amdro Pro (gr)</td>
<td>5.0 a</td>
<td>Maxforce Complete (gr)</td>
<td>5.0 a</td>
</tr>
<tr>
<td>Maxforce (gr)</td>
<td>4.9 a</td>
<td>Niban FG (gr)</td>
<td>4.8 ab</td>
<td>Amdro Pro (gr)</td>
<td>5.0 a</td>
</tr>
<tr>
<td>Extinguish Pro (gr)</td>
<td>4.8 a</td>
<td>Maxforce Ant Killer (g)</td>
<td>4.7 abc</td>
<td>Maxforce Fine Granular (g)</td>
<td>4.9 a</td>
</tr>
<tr>
<td>Advion (g)</td>
<td>4.6 a</td>
<td>Maxforce (gr)</td>
<td>4.7 abc</td>
<td>Niban FG (gr)</td>
<td>4.7 ab</td>
</tr>
<tr>
<td>Maxforce Complete (gr)</td>
<td>4.5 ab</td>
<td>Optigard (g)</td>
<td>4.6 abc</td>
<td>Advance Carpenter Ant (gr)</td>
<td>4.3 abc</td>
</tr>
<tr>
<td>Advance Carpenter Ant (gr)</td>
<td>4.4 ab</td>
<td>Advance Carpenter Ant (gr)</td>
<td>4.6 abc</td>
<td>Optigard (g)</td>
<td>4.2 abcd</td>
</tr>
<tr>
<td>Intice (g)</td>
<td>4.0 abc</td>
<td>Advance 375A Select (g)</td>
<td>4.5 abcd</td>
<td>Extinguish Pro (gr)</td>
<td>4.1 abcde</td>
</tr>
<tr>
<td>Esteem (gr)</td>
<td>3.8 abcde</td>
<td>Intice (g)</td>
<td>3.9 abcd</td>
<td>381B Advance (l)</td>
<td>3.9 abcde</td>
</tr>
<tr>
<td>Optigard (g)</td>
<td>3.3 de</td>
<td>Advion (g)</td>
<td>3.7 abcde</td>
<td>Intice (g)</td>
<td>3.1 bcdef</td>
</tr>
<tr>
<td>Advance 375A Select (g)</td>
<td>1.4 d</td>
<td>Extinguish Pro (gr)</td>
<td>3.5 abcde</td>
<td>Maxforce (g)</td>
<td>2.7 cdef</td>
</tr>
<tr>
<td>381B Advance (l)</td>
<td>1.2 d</td>
<td>Maxforce Fine Granular (g)</td>
<td>3.2 bcde</td>
<td>Advance 375A Select (g)</td>
<td>2.6 cdef</td>
</tr>
<tr>
<td>Control (l)</td>
<td>1.1 d</td>
<td>Intice (gr)</td>
<td>3.1 cde</td>
<td>Intice (gr)</td>
<td>2.5 def</td>
</tr>
<tr>
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<td>Esteem (gr)</td>
<td>2.9 de</td>
<td>Control (l)</td>
<td>2.4 ef</td>
</tr>
<tr>
<td>Niban FG (gr)</td>
<td>NT</td>
<td>381B Advance (l)</td>
<td>2.2 ef</td>
<td>Esteem (gr)</td>
<td>1.8 f</td>
</tr>
<tr>
<td>Amdro Pro (gr)</td>
<td>NT</td>
<td>Control (l)</td>
<td>1.0 f</td>
<td>Advion (g)</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT=not tested                  | F=33.31| F=11.39                      | F=10.20|                  |
|                               | df=13, 126 | df=15, 144                    | df=14, 135|                |
|                               | p<0.0001    | p<0.0001                     | p<0.0001|                |

Means in the same season followed by the same letter are not significantly different (Tukey’s HSD α=0.5). Key: g=gel, l=liquid, gr=granular.
Table 4-4. Mean ranked days until 100% mortality of *Nylanderia fulva* after continuous exposure to 15 commercially available baits in no-choice laboratory assays over three seasons (2009-2010) (n=10 replicates of each bait per season.)

<table>
<thead>
<tr>
<th>Bait</th>
<th>Spring</th>
<th>Bait</th>
<th>Summer</th>
<th>Bait</th>
<th>Fall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxforce Ant Killer (g)</td>
<td>4.2 a</td>
<td>Maxforce Complete (gr)</td>
<td>4.0 a</td>
<td>Maxforce Complete (gr)</td>
<td>4.2 a</td>
</tr>
<tr>
<td>Advance 375A Select (g)</td>
<td>4.1 a</td>
<td>Amdro Pro (gr)</td>
<td>3.8 ab</td>
<td>Maxforce Ant Killer (g)</td>
<td>4.0 ab</td>
</tr>
<tr>
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<td>Optigard (g)</td>
<td>3.6 ab</td>
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<td>4.0 ab</td>
</tr>
<tr>
<td>Maxforce (gr)</td>
<td>3.5 ab</td>
<td>Advion (g)</td>
<td>3.3 abc</td>
<td>Maxforce Fine Granular (g)</td>
<td>3.3 ab</td>
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<td>Maxforce Ant Killer (g)</td>
<td>3.0 abcd</td>
<td>Niban FG (gr)</td>
<td>3.0 bcd</td>
</tr>
<tr>
<td>Advion (g)</td>
<td>3.0 bc</td>
<td>Maxforce Fine Granular (g)</td>
<td>2.9 abcd</td>
<td>Extinguish Pro (gr)</td>
<td>2.6 cde</td>
</tr>
<tr>
<td>Optigard (g)</td>
<td>3.0 bc</td>
<td>Extinguish Pro (gr)</td>
<td>2.6 abcde</td>
<td>Advance Carpenter Ant (gr)</td>
<td>2.5 cdef</td>
</tr>
<tr>
<td>Maxforce Fine Granular (g)</td>
<td>2.9 bc</td>
<td>Niban FG (gr)</td>
<td>2.6 abcd</td>
<td>Maxforce (gr)</td>
<td>2.2 def</td>
</tr>
<tr>
<td>Intice (g)</td>
<td>2.6 bc</td>
<td>Advance 375A Select (g)</td>
<td>2.4 bcdef</td>
<td>Optigard (g)</td>
<td>2.1 def</td>
</tr>
<tr>
<td>Advance Carpenter Ant (gr)</td>
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<td>Advance 375A Select (g)</td>
<td>1.9 cdef</td>
<td>381B Advance (l)</td>
<td>2.1 def</td>
</tr>
<tr>
<td>Esteem (gr)</td>
<td>2.3 cd</td>
<td>Esteem (gr)</td>
<td>1.7 def</td>
<td>Intice (g)</td>
<td>2.0 defg</td>
</tr>
<tr>
<td>Control (l)</td>
<td>1.0 d</td>
<td>Intice (g)</td>
<td>1.4 ef</td>
<td>Intice (gr)</td>
<td>1.8 efg</td>
</tr>
<tr>
<td>381B Advance (l)</td>
<td>1.0 d</td>
<td>Intice (gr)</td>
<td>1.2 ef</td>
<td>Esteem (gr)</td>
<td>1.5 fg</td>
</tr>
<tr>
<td>Intice (gr)</td>
<td>1.0 d</td>
<td>Control (l)</td>
<td>1.0 f</td>
<td>Control (l)</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Niban FG (gr)</td>
<td>NT</td>
<td>Maxforce (gr)</td>
<td>0.3 e</td>
<td>Advance 375A Select (g)</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Amdro Pro (gr)</td>
<td>NT</td>
<td>381B Advance (l)</td>
<td>0.3 e</td>
<td>Advion (g)</td>
<td>NT</td>
</tr>
</tbody>
</table>

Means in the same season followed by the same letter are not significantly different (Tukey’s HSD α=0.5). Key: g=gel, l=liquid, gr=granular.
Figure 4-1. Artificial nest for laboratory maintained *Nylanderia fulva* colony fragments. The artificial nest consists of a plastic Petri dish (100 x 15 mm) filled ¾ with dental plaster. A hole (a) melted into the petri dish cover allowed ants to access to the interior of the nest.
Figure 4-2. Humidified test chamber for conducting *Nylanderia fulva* bait bioassays. The chamber consists of a PVC frame (1.5 x 1.5 x 1.5 m) covered with plastic sheeting. A data logger records temperature and humidity within the chamber. The bottom of the chamber contains a water filled pan that is covered by a plastic grid. The individual bioassay arenas are placed atop the grid.
Figure 4-3. Liquid bait stations. A) A rodent bait station modified for dispensing liquid ant bait and B) A liquid bait station crafted from PVC pipe.
Figure 4-4. Rodent bait station for dispensing granular ant bait.
Figure 4-5. Diagram of MTD1 site. Granular bait stations (n=3) were placed along the rear perimeter of each structure adjacent to sampling points. Liquid (n=4) and granular (n=3) bait stations were placed along the interface between the lawn and the wooded area. Bait stations containing only 10% sucrose solution (n=7) were placed at structures 2 and 6 (controls). Sampling points are marked with a white dot.
Figure 4-6. Diagram of SAR1 site. Granular bait stations (n=3) were placed along the rear perimeter of each structure adjacent to sampling points. Liquid (n=4) and granular (n=3) bait stations were placed every along the interface between the lawn and the natural area. Bait stations containing only 10% sucrose solution (n=7) were placed at structures 5 and 6 (controls). Sampling points are marked with a white dot.
Figure 4-7. An individual building at the SAR2 site. Liquid and granular bait stations (one each) were placed adjacent to each tree across the road from the building. Diagram depicts sampling points (white dots) and locations of treatment or control bait stations (yellow dots).
Figure 4-8. Laboratory assay results for bait performance criteria of acceptance, days until 100% mortality and percent mortality at 3 DAT for commercially available granular ant baits combined into a single graph to allow direct comparisons. Baits indicated by bubbles in lower, right quadrant and with largest diameter are those with highest mortality at 3 DAT, fewest days to 100% mortality and highest acceptance.
Figure 4-9. Laboratory assay results for bait performance criteria of acceptance, days until 100% mortality and percent mortality at 3 DAT for commercially available liquid or gel ant baits combined into a single graph to allow direct comparisons. Baits indicated by bubbles in lower, right quadrant and with largest diameter are those with highest mortality at 3 DAT, fewest days to 100% mortality and highest acceptance.
Figure 4-10. Total ant counts for all sampling points of *Nylanderia fulva* foraging on sliced canned sausage at site SAR1 for each treatment combination (perimeter-control, perimeter-treatment, interface-control, interface-treatment). Four structures were treated with both liquid and granular baits. Two were untreated controls. A 3-way ANOVA on factors weeks after treatment, sampling location and treatment was not significant (F=1.56, df=15, 128, p=0.0927). Key: Perimeter=perimeter of structure, Interface=interface between lawn and wooded area, CTL=unbaited, TRT=baited.
Figure 4-11. Total ant counts for all sampling points of *Nylanderia fulva* foraging on sliced canned sausage at site SAR2 for each treatment combination (perimeter-control, perimeter-treatment, interface-control, interface-treatment). Four structures were treated with both liquid and granular baits. Two were untreated controls. Results of a 3-way ANOVA on factors weeks after treatment, sampling location and treatment showed significant differences in ant counts between the perimeter of the structure and the interface between the lawn and wooded area (F=19.50, df=1, 192, p<0.0001). Key: Perimeter=perimeter of structure, Interface=interface between lawn and wooded area, CTL=unbaited, TRT=baited.
Figure 4-12. Total ant counts for all sampling points of *Nylanderia fulva* foraging on sliced canned sausage at site MTD1 for each treatment combination (perimeter-control, perimeter-treatment, interface-control, interface-treatment). Four structures were treated with both liquid and granular baits. Two were untreated controls. Results of a 3-way ANOVA on factors weeks after treatment, sampling location and treatment showed a significant difference in ant counts between the perimeter of the structure and the interface between the lawn and the wooded area (F=53.22, df=1, 185, p<0.0001) and between the baited and unbaited structures (F=15.03, df=1, 185, p=0.0001). Key: Perimeter=perimeter of structure, Interface=interface between lawn and wooded area, CTL=unbaited, TRT=baited.
CHAPTER 5
COMBINATION INSECTICIDE AND BAIT PROGRAM FOR SUPPRESSION OF
\textit{Nylanderia fulva}

Introduction

The common practice of pest management companies to control \textit{N. fulva} populations is an insecticide barrier and crack and crevice treatment. This use pattern can provide immediate knockdown of \textit{N. fulva} populations, but ants can return in as soon as 2 to 3 days (\textsuperscript{1}FMO, personal communication). Barrier treatments are applications of liquid or granular insecticides around the exterior surfaces of structures to prevent or restrict ants from entering the structure (Rust et al. 1996, Scharf et al. 2004, Silverman and Brightwell 2008, Vega and Rust 2003). Crack and crevice treatments are indoor applications of liquid insecticides to areas that provide harborages or nesting sites for ants. These structural voids may also be treated with a desiccant dust or a foam formulation of a liquid insecticide.

Applications of insecticides to the exterior perimeter of a structure are effective at killing thousands of \textit{N. fulva} (Meyers 2008a). However, if the cadavers are not removed, live ants simply use them as a bridge over the treated area. Homeowners often use water to wash away the cadavers, effectively removing the insecticidal barrier. The immense numbers of ants in a typical infestation, homeowner actions to remove dead ants, intense solar radiation, high humidity and seasonal daily rainfall present challenges to \textit{N. fulva} control in Florida. When infestations are heavy, the currently used

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insecticides applied outdoors for control of *N. fulva* in Florida are effective for only one to two weeks (personal observation). As described in Chapter 4, toxic baits may also be applied indoors and outdoors for ant control. Because of the number of ants to be controlled, an effective Integrated Pest Management (IPM) strategy for *N. fulva* may require the concurrent application of liquid insecticides and toxic baits in different areas.

To develop an integrated approach to *N. fulva* in Florida the optimal combinations of liquid insecticides and toxic baits and the methods of delivery of each must be determined. Therefore, the objectives of this study were to: 1) Evaluate the repellency and efficacy of commercially available professional liquid insecticide products against *N. fulva* in laboratory assays as a first step to identifying potential products for inclusion in an IPM strategy. 2) Evaluate the efficacy of a two-step program by which the infestation level of *N. fulva* is reduced by the use of contact insecticides followed by an application of liquid and granular baits to the perimeter of the property.

**Materials and Methods**

**Laboratory Bioassays of Insecticide Repellency and Efficacy**

*Nylanderia fulva* were collected and maintained as described in Chapter 4. Test arenas (Fig. 5-1) were prepared by coating the inside walls of rectangular aluminum pans (31x21x5 cm) with Insect-a-Slip (BioQuip Products, Inc., Rancho Dominguez, CA), a slippery coating that prevent ants from escaping the container. Panels of plywood (0.64 cm thick) were cut to fit the inside bottom of each tray. The plywood panels were painted with Behr Premium Plus (Masco Corporation; Taylor, MI) water-based white paint and allowed to dry for 24 h. Nine commercially available insecticides (Table 5-1) were diluted in water according to each product's manufacturer label directions at the highest rate allowed for exterior application for ants and tested in two experiments.
Termidor®, Temprid® and Suspend® and an untreated control were tested first, then Arilon®, Ortho®, Optigard®, Phantom® SC, Phantom® pressurized aerosol, and Talstar® with an untreated control. All treatments were replicated 5 times. The first set of contact insecticides were ones that were commonly used by pest control companies at the time of testing. The second set of insecticides were ones that emerged as additional products being used by the pest control industry. The insecticide dilutions were agitated thoroughly and decanted into disposable plastic spray bottles. The insecticide dilutions or deionized water (control) were applied to the plywood panels simulating an outdoor perimeter application. The panels were allowed to dry for 24 h. Once dry, the panels were attached to the inside bottom of the aluminum pans with foam tape. To prevent ants from going under the panels, the edges were sealed to the aluminum pans with caulking. Caulking was allowed to cure for 24 h.

Large plastic trays were prepared as secondary containment vessels by coating the inside walls with Insect-a-Slip. One aluminum pan with an insecticide treated panel (treated surface) was placed inside the plastic tray along with one aluminum pan with Insect-a-Slip coated interior walls only (untreated surface).

An index card was placed on end into a container with field collected N. fulva. Ants were allowed to climb onto the card and the card was gently tapped with the fingers to drop the ants into a new container. In this manner, approximately two-thousand N. fulva workers and 15 queens were collected from the same colony and placed into each untreated surface pan. Brood was not included in the bioassay. The ants were provisioned with a nest cell and water as described in Chapter 4 and starved for 24 h before the test began. Additionally, a black plastic container (15.2x7.6x1.6 cm) was
notched on one end and placed upside down over the nest cell to provide additional harborage and to maintain a humid microclimate. After 24 h, the water tube was removed from the untreated surface pan and placed into the treated surface pan along with live termites and a test tube containing 10% sucrose solution stoppered with cotton. A bridge constructed of wire fabric wrapped with aluminum tape was used to connect the two pans. The food and water was placed so that foraging ants had to cross the entire distance of the treated panel to obtain food or water.

**Experimental design**

In a completely randomized design to assess the relative repellency of the insecticides, the number of *N. fulva* crossing a fixed point on the treated surface to reach the food was counted for a 60 second interval every 30 min for the first 2 hours and summed to arrive at a foraging count. Each of the two sets of insecticides was analyzed separately. Repellency data met the assumptions of normality, homoscedasticity and independence as evaluated by Shapiro Wilk, Levene’s and Durbin-Watson tests, respectively. For each experiment, differences in foraging counts among the insecticides were analyzed with a one-way analysis of variance (ANOVA) with the main factor treatment. If significant, means were separated using Tukey’s HSD (\(\alpha=0.05\)) (SAS Institute 2012).

To evaluate the efficacy of each insecticide, the percent mortality for each colony fragment was recorded daily for 30 d. Percent mortality was calculated as the number of dead ants divided by 2000, the approximate number of ants placed into the container at the beginning of the study. The data for each experiment at 10 and 30 days did not meet the assumptions of ANOVA and were then rank-scored. Ranks, “3”=90-100% mortality, “2”=50-89% mortality and “1”=0-49% mortality, were selected to indicate high,
medium and low mortality, respectively. Ten days was selected to allow some of the slower acting products time to impact the ants; 30 days was selected because 1) this is the point where control mortality was no longer acceptable and 2) it is the common pest control service interval (i.e., monthly service). Rank-scores were compared among treatments with a one-way ANOVA for each experiment at 10 or 30 DAT. If significant, means were separated using Tukey’s HSD (α=0.05) (SAS Institute 2012).

Field Evaluations of Efficacy of Insecticide and Bait Combinations

Study sites

A completely randomized design to test the efficacy of a combination of baits and insecticides against *N. fulva* was conducted sites MTD1, a newly constructed subdivision adjacent to a wooded preserve (Fig. 4-5) and SAR1, a condominium complex also adjacent to a preserve (Fig. 4-6). Sites and sampling point selection are as described in Chapter 4 with the exception of MTD1 structure number four where there was no access to the three sampling points along the perimeter of the home. Assignment to treatments was as described in Chapter 4 with the exception of MDT1. To avoid insecticide application to fruit trees in the rear lawn of home number two, it was assigned as a control. The study was conducted six weeks after the completion of the bait application study. As there was no significant difference between the baited and untreated structures in the previous study and an additional 6 weeks without bait treatment had passed, it was assumed the previous bait applications did not influence the ant foraging counts during this study.

After pre-counts were taken as described in Chapter 4, insecticides were applied to treated structures as described in Table 5-2. Each treated structure received a single application of each of the insecticides listed and all insecticides were applied on the
same day. Arena® granular insecticide was applied to the lawn to kill ants migrating from the wooded area to the home. Although hemipteran counts were not included in this study, hemipterans may be food source for *N. fulva*; therefore, trees and shrubs in the lawn were treated with a drench application of the systemic insecticide Merit®. (Sharma et al 2013). A band application the contact insecticide Temprid® was made to each tree trunk to prevent ants from foraging in tree canopies. No other pesticides were applied by the pest control company. Homeowner applications of pesticides are not known. After insecticide applications were made, liquid bait stations containing 500 ml of Maxforce® Quantum Ant Bait diluted in a 25% sucrose solution (*n*=2) and granular bait stations (*n*=2) containing 60g of Maxforce® Complete Ant Bait were installed at each structure along the interface between the property and the wooded area. Ant foraging counts as described in Chapter 4 were taken at 1, 2, 3, 4 and 6 WAT for MDT1 and 3, 5 and 6 weeks WAT for SAR1.

Due to the variability of each site, the data from each site was analyzed separately. Data assumptions of normal distribution, homoscedasticity and independence for ant foraging counts were evaluated by Shapiro Wilk, Levene’s and Durbin-Watson tests, respectively. Since the data were not normally distributed and could not be normalized through transformation, the foraging counts were ranked-scored using the same 1-5 scale as described in Chapter 4. To determine if, after treatment, there were significant differences between the mean foraging ant counts at the perimeter of the structure and the interface between the lawn and the wooded area, between treated and untreated structures at each site, and between weeks after treatment, rank-scores were analyzed using a 3-way ANOVA with main factors weeks.
after treatment, location, and treatment (Conover and Iman 1981, SAS Institute 2012). If significant, means for weeks after treatment were separated using Tukey’s HSD ($\alpha=0.05$). Results are reported as foraging counts.

**Results**

**Laboratory Bioassays of Insecticide Repellency and Efficacy**

**Repellency.** The ANOVA for main factor repellency was significant for Experiment 1 ($F=3.55$, df=3, 16, $p=0.0385$) and Experiment 2 ($F=2.69$, df=6, 28, $p=0.0342$). In the repellency bioassays, no product completely repelled *N. fulva*, as some ants in each treatment traversed the treated surface to obtain food and water (Fig. 5-2). However, over the initial 2 h of the Experiment 1, significantly fewer ants crossed surfaces treated with Temprid® compared to the control but the numbers of ants crossing Temprid® were not significantly different from Termidor® or Suspend® (Fig. 5-2). In Experiment 2, only Ortho® was significantly more repellent than the control (Fig. 5-2).

**Efficacy.** The ANOVA for main factor mortality was significant for Experiment 1 ($F=34.8$, df=3, 15, $p<0.0001$). Suspend®, Termidor® and Temprid® provided 90-94% worker mortality in 10 DAT compared to 16.3% mortality in the control (Table 5-3, Fig. 5-3). In the second experiment, the ANOVA for main factor mortality was not significant at 10 DAT, ($F=0.83$, df=6, 28, $p=0.5545$), but was significant at 30 DAT ($F=3.72$, df=6, 28, $p=0.0007$). Optigard® and Ortho® insecticides had significantly higher mortality compared to the control; however, all of the insecticides were not significantly different from each other (Table 5-3).

An unexpected observation was that in the Termidor® and Temprid® treatments queens lived ~2 days after all workers had died (Fig.5-4). Thus, any transference that
of active ingredients associated with these contact insecticide applications appears to have minimal impact on queens which may help explain why “spraying only” is the least effective method of control for *N. fulva*. In the Suspend® treatment, mortality did not reach 100% by the end of the study but the number of workers was reduced to only a few individuals and queens were able to survive at least 30 days with very few workers to tend them.

**Field Evaluations of Efficacy of Insecticide and Bait Combinations**

At the MDT1 site, there was a significant difference in ant foraging counts between the perimeter of the structure and the interface between the lawn and wooded area (F=44.89, df=1, 167, p<0.0001), between treatments (F=10.03, df=1, 167, p=0.0018) and between the weeks after treatment (F=7.03, df=5, 167, p<0.0001) (Fig. 5-5). Figure 5-5 shows that ant foraging counts at the perimeter of the treated structures decreased sharply and remained lower than at the untreated structures for the entire study. There was also a decrease in ant foraging counts at interface of the lawn and wooded area of the treated structures but ant counts at the interface of the untreated structures were zero for the entire 6 wk.

The ANOVA for the SAR1 site was not significant (F=1.56, df=15, 128, p=0.0927). At this site, ant foraging counts at all sampling locations were very low at the beginning of the study and remained low at the perimeter of the structures but increased slightly at the interface between the lawn and wooded area (Fig. 5-6).

**Discussion**

**Laboratory Bioassays of Insecticide Repellency and Efficacy**

A common pest control practice for this ant includes the use of liquid insecticides applied to the exterior perimeter of a structure. Usual service intervals for pest control
companies is monthly or quarterly. In Florida, pest management professionals (PMPs) who only sprayed for the control of *N. fulva* were not achieving satisfactory results and as a result were applying insecticides as often as every few days. Some companies refunded fees because they were unable to satisfactorily manage *N. fulva* (1FMO, personal communication). Because of this, additional material and labor costs to the PMP are often the consequence of a pest control account with *N. fulva* infestation.

In this study, we examined the repellency and efficacy of commonly used insecticide products in Florida under experimental conditions that are similar to the way in which *N. fulva* would interact with the treated exterior perimeter of a structure. It was not unexpected that products such as Termidor®, Phantom® and Phantom® Pressurized would not repel *N. fulva* from traversing a treated surface to forage as these products contain known non-repellent active ingredients (Ibrahim et al. 2003, Rust and Saran 2006). However, none of the products tested were truly repellent and only Temprid® resulted in significantly fewer ants compared to the untreated control. In this closed-system bioassay, ants were required to cross an insecticide treated surface to receive food and water. It was expected that foraging workers would be exposed to insecticide and die requiring additional workers to take on the duty of foraging. Thus, eventually all workers would succumb to the effects of the insecticide. Without workers to tend them, queens would eventually die of starvation (Silverman and Brightwell 2008).

Alternatively, with non-repellent, slow-acting insecticides such as Termidor® and Phantom®, the foraging ants would contact the insecticide and transfer the toxicant to non-foraging nestmates resulting in worker and queen mortality (Klotz et al. 2004, Choe and Rust 2008).
Most of the insecticides tested did not provide 100% mortality by the end of the study even when worker ants were forced to cross the treated substrate for food and water. The wood panels used in the study, even though painted and treated with the highest rate of insecticide allowed by the label, may not have had sufficient active ingredient bioavailability. Substrate effects on pesticide efficacy have been demonstrated previously. Wagner and Strawn (1980) found that knockdown of *L. humile* was less than 90% one day after treating concrete with chlorpyrifos compared to six months on other substrates. A comparison of substrate effect on the efficacy of *Termidor*, *Phantom*, and *Talstar* showed that worker mortality of *Monomorium pharaonis* (L.) was less on concrete than hardwood mulch (Buczkowski et al. 2005). Structures in Florida are constructed of a variety of building materials and future studies should include additional commonly used construction materials. However, even if efficacy is improved by choice of a suitable substrate, laboratory bioassays do not account for environmental conditions such as overspray from irrigation, intense UV radiation and high temperatures that may degrade the active ingredient.

Furthermore, this bioassay did not account for insect behaviors that may impact the effectiveness of contact insecticides for *N. fulva* control. In this study, at least some foraging ants contacted the insecticide treated surface daily to obtain food and water. Under natural conditions, *N. fulva* would likely have many food resources to choose from meaning that exposure to the insecticide on the exterior perimeter of a structure is not guaranteed. In addition, the *N. fulva* queens in this study survived without workers for ~2 d (Fig. 5-4). As a polydomous tramp ant species lacking intraspecific aggression, an *N. fulva* queen without workers in the field may simply relocate to another nest. The
results of this study suggest further work can be conducted on the success of queen adoption by new nests. Additional studies may also investigate mechanisms that may confer reduced susceptibility of *N. fulva* to insecticides on treated surfaces.

**Field Evaluations of Efficacy of Insecticide and Bait Combinations**

The decrease in ant foraging counts at the MTD1 site at the treated structures at the interface between the lawn and wooded area suggested that the treatments may have had an effect. Further studies are warranted to determine if maintaining the combination of insecticides followed by baiting protocol over a longer period of time would be successful in suppressing *N. fulva* populations.

Combining multiple insecticide application types has been shown to be more effective than a single application type. Rust et al. (1996) showed that a combination of chlorpyrifos spray and granules provided 80% control of *N. fulva* for 60 days, twice as long as when chlorpyrifos is only sprayed. Perimeter applications of Termidor® (fipronil, BASF, Research Triangle Park, NC) reduced *L. humile* activity near and away from structures for fewer weeks than Termidor® plus a lawn application of Talstar® (bifenthrin, FMC Corporation, Philadelphia, PA) granules or Termidor® plus a liquid borate bait (Klotz et al. 2007).

Field studies where multiple insecticides were used concurrently against *N. fulva* have also shown some promise. Phantom® (chlorfenapyr, BASF, Research Triangle Park, NC) and Termidor®, both non-repellent, slow acting insecticides used together and along with Advance® Carpenter Ant Bait (abamectin, BASF, Research Triangle Park, NC) demonstrated suppression of *N. fulva* for up to 12 wk (Meyers 2008a) compared to an untreated control. However, field studies with combinations of Transport™ 50WP (acetamiprid and bifenthrin, FMC Corporation, Philadelphia, PA),
Talstar® granules or Termidor® SC and TopChoice® (fipronil, Bayer Environmental Science, Research Triangle Park, NC) suppressed ants for only two weeks (Meyers 2008a).

In an urban environment where the objective is to reduce ant foraging in and around the structure, the selection and application of contact insecticides and baits must be optimized so that they work in a compatible manner. In Meyers' (2008a study), insecticides were only applied to the perimeter of the structure compared to the entire lawn in this study. In addition, Termidor® and Phantom® are non-repellent insecticides which would not keep ants out of the home. In Meyers (2000b) other study, either repellents were used on both the structure and in lawn or vice versa. Perhaps if, the repellent were used on the structure and the non-repellent in the lawn suppression may have improved.

This study provides preliminary data on an IPM approach to tawny crazy ant management. The widely varying numbers of ants at each site suggests that successful management is likely population-size dependent and further studies are recommended to confirm the efficacy of this protocol under differing residential community types.
Table 5-1. Nine commercially available contact insecticides tested for repellency and efficacy against *Nylanderia fulva* in laboratory bioassays.

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Active Ingredient</th>
<th>Marketed as Repellent or Non-repellent</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arilon®</td>
<td>Indoxacarb</td>
<td>Non-repellent</td>
<td>Syngenta, Greensboro, NC</td>
</tr>
<tr>
<td>Optigard®</td>
<td>Thiamethoxam</td>
<td>Non-repellent</td>
<td>Syngenta, Greensboro, NC</td>
</tr>
<tr>
<td>Ortho®</td>
<td>Acephate</td>
<td>Repellent</td>
<td>Monsanto, San Ramon, CA</td>
</tr>
<tr>
<td>Phantom®</td>
<td>Chlorfenapyr</td>
<td>Non-repellent</td>
<td>BASF, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Pressurized</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phantom® SC</td>
<td>Chlorfenapyr</td>
<td>Non-repellent</td>
<td>BASF, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Suspend®</td>
<td>Deltamethrin</td>
<td>Repellent</td>
<td>Bayer, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Talstar®</td>
<td>Bifenthrin</td>
<td>Repellent</td>
<td>FMC, Philadelphia, PA</td>
</tr>
<tr>
<td>Temprid®</td>
<td>Imidaclorid+ Beta cyfluthrin</td>
<td>Non-repellent and repellent active ingredients</td>
<td>Bayer, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Termidor®</td>
<td>Fipronil</td>
<td>Non-repellent</td>
<td>BASF, Research Triangle Park, NC</td>
</tr>
</tbody>
</table>
Table 5-2. Contact insecticides and baits used in a multi-product approach for suppression of *Nylanderia fulva* in field studies. Each insecticide was applied one time. All insecticides were applied on the same day.

<table>
<thead>
<tr>
<th>Product</th>
<th>Active Ingredient</th>
<th>Manufacturer</th>
<th>Application Rate</th>
<th>Application Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arena® 0.25G</td>
<td>Clothianidin</td>
<td>Valent U.S.A. Corporation, Walnut Creek, CA</td>
<td>120 lbs/acre</td>
<td>Broadcast application with handheld spreader.</td>
</tr>
<tr>
<td>Merit® 75 WP</td>
<td>Imidacloprid</td>
<td>Bayer Environmental Science, Research Triangle Park, NC</td>
<td>0.7 tsp/in of trunk diameter</td>
<td>Drench application. Each tree or shrub received 1 gallon of dilution.</td>
</tr>
<tr>
<td>Temprid®</td>
<td>Imidacloprid and Beta-cyfluthrin</td>
<td>Bayer Environmental Science, Research Triangle Park, NC</td>
<td>16 mls/gal</td>
<td>Each tree received ~0.25 gallons of dilution. Applied as a spray application using a handheld sprayer with fan tip on tree trunk from base to a height of 1 m.</td>
</tr>
<tr>
<td>Maxforce® Quantum Ant Bait</td>
<td>Imidacloprid</td>
<td>Bayer Environmental Science, Research Triangle Park, NC</td>
<td>500 ml dilution/station</td>
<td>Liquid bait station with 500 ml of 1:10 dilution of Quantum in 25% sucrose solution.</td>
</tr>
<tr>
<td>Maxforce® Complete Granular Bait</td>
<td>Hydramethylnon</td>
<td>Bayer Environmental Science, Research Triangle Park, NC</td>
<td>60 g/station</td>
<td>Granular bait station with 60 g of granular bait.</td>
</tr>
</tbody>
</table>
Table 5-3. Mean percent *Nylanderia fulva* mortality at 10 and 30 DAT in a bioassay requiring ants to cross a painted wood panel treated with insecticide or water (control). Insecticides were tested in 2 groups (Experiment 1 and Experiment 2) (n=5 replicates for each insecticide).

<table>
<thead>
<tr>
<th></th>
<th>Percent Mortality</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 DAT</td>
<td>30 DAT</td>
</tr>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temprid</td>
<td>94.0 a</td>
<td>100.0 b</td>
</tr>
<tr>
<td>Termidor</td>
<td>92.8 a</td>
<td>100.0 b</td>
</tr>
<tr>
<td>Suspend</td>
<td>90.0 a</td>
<td>98.8 b</td>
</tr>
<tr>
<td>Control</td>
<td>16.3 b</td>
<td>62.3 a</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Optigard</td>
<td>27.0</td>
<td>95.0 a</td>
</tr>
<tr>
<td>Ortho</td>
<td>21.0</td>
<td>89.9 a</td>
</tr>
<tr>
<td>Phantom P</td>
<td>19.0</td>
<td>84.0 ab</td>
</tr>
<tr>
<td>Arilon</td>
<td>19.0</td>
<td>62.0 ab</td>
</tr>
<tr>
<td>Talstar</td>
<td>18.0</td>
<td>53.0 ab</td>
</tr>
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<td>Phantom SC</td>
<td>9.0</td>
<td>53.0 ab</td>
</tr>
<tr>
<td>Control</td>
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<td>26.0 b</td>
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Insecticides within the same experiment followed by the same letter are not significantly different (Tukey’s HSD, α=0.05). Experiment 1 10 DAT: F=34.8, df=3, 15, p<0.0001; 30 DAT: F=7.89, df=3, 15, p=0.0002. Experiment 2 10 DAT: F=0.83, df=6, 28 p=0.5545; 30 DAT: F=3.72, df=6, 28, p=0.0007.
Figure 5-1. Experimental set-up of laboratory bioassay evaluating repellency and efficacy of commercially available insecticides against *Nylanderia fulva*. The interior dimensions of each arena were 31x21x5 cm. Insecticides were applied to a painted wood panel that was then attached to the bottom of the treated arena (shaded area). An artificial nest with 2000 workers and 15 queens (no brood) was placed in the untreated arena. Worker ants were required to cross the length of the insecticide treated arena for food and water.
Figure 5-2. Mean number of *Nylanderia fulva* crossing a predetermined point on a painted wood panel treated with insecticide or water (control) (n=5 replicates). Counts were taken for 1 min intervals every 30 min for 2 h. Means in the same experiment followed by the same letter are not significantly different (Tukey’s HSD, α=0.05) (Experiment 1: F=3.55, df=3, 16, p=0.0385, Experiment 2: F=2.69, df=6, 28, p=0.0342).
Figure 5-3. Daily percent mortality of *Nylanderia fulva* workers over 30 days in a bioassay requiring ants to cross a painted wood panel treated with insecticide or water (control) (n=5 replicates).
Figure 5-4. Mean number of days until 100% mortality of *Nylanderia fulva* workers and queens in a bioassay requiring ants to cross a painted wood panel treated with insecticide or water (control).
Figure 5-5. Mean ant counts of *Nylanderia fulva* foraging on sliced canned sausage at site MDT1 for each treatment combination (perimeter-control, perimeter-treatment, interface-control, interface-treatment) over all sampling areas. Four structures were treated with contact insecticides and liquid and granular baits. Two were untreated controls. Results of a 3-way ANOVA on factors weeks after treatment, sampling location and treatment showed a significant difference in ant counts between the perimeter of the structure and the interface between the lawn and wooded area ($F=44.89$, $df=1$, 167, $p<0.0001$), the treatments ($F=10.03$, $df=1$, 167, $p=0.0018$), and between the weeks after treatment ($F=7.03$, $df=5$, 167, $p<0.0001$). Mean ant counts with the same letter are not statistically different (Tukey’s HSD $\alpha=0.05$). Key: Perimeter=perimeter of structure; Interface=interface between lawn and wooded area; CTL=untreated structure; TRT= treated structure.
Figure 5-6. Mean ant counts of *Nylanderia fulva* on sliced canned sausage at site SAR1 for each treatment combination (perimeter-control, perimeter-treatment, interface-control, interface-treatment) over all sampling areas. Four structures were treated with contact insecticides and liquid and granular baits. Two were untreated controls. A 3-way ANOVA on factors weeks after treatment, sampling location and treatment was not significant (F=1.56, df=15, 128, p=0.0927). Key: Perimeter=perimeter of structure; Interface=interface between lawn and natural area; CTL=untreated structure; TRT= treated structure.
Integrated Pest Management

Integrated Pest Management (IPM) is an approach to pest control that focuses on sustainably mitigating existing pest problems and taking measures to prevent further infestations by the use of monitoring, preventive actions, combinations of control measures and economic thresholds. Effectively managing infestations of the invasive ant species, *N. fulva*, will require an IPM approach. Reliance on contact insecticide applications to the exterior perimeter of structures for control of *N. fulva* have been demonstrated to be ineffective. In addition, it is not environmentally sustainable or economically feasible to apply insecticides on a weekly basis. It is unrealistic to expect that invasive ants like *N. fulva* will be eradicated from already heavily infested areas (Tsutsui and Suarez 2003) but the goal of an IPM approach is to reduce infestation to levels acceptable to the pest management professional (PMP) and the homeowner.

**IPM Tactics for Management of *Nylanderia fulva***

IPM does not solely rely on application of insecticides for remedial or preventive control. Rather, an IPM strategy is created from a menu that may include biological, cultural, physical or chemical control options. Biological control includes the use of natural enemies of the target pest to control their populations. Natural enemies may include predators, parasites and pathogens. Cultural control involves modification of a pest’s environment to make it less favorable for survival. Physical control includes methods to exclude pests from the environment and chemical control is the rational use of pesticides to manage the target pest. In IPM, pesticides are selected and applied to minimize negative impact on non-target organisms and the environment. By integrating
well established IPM strategies for urban pest control with the data collected from the research studies in Chapters 2-5 of this dissertation, a synthesis of recommended IPM tactics for *N. fulva* management is given below.

**Identification.** Identification of the pest species and a thorough understanding of lifecycle, behavior, and reproduction and development are critical aspects required for development of an effective IPM strategy (Oi and Drees 2009). As there has already been much confusion in Florida as to the identification of this invasive pest, PMPs are encouraged to seek confirmation of identification as a first step toward implementing a control strategy. Accurate identification can be obtained by sending a sample of the insect in question to the nearest county extension office.

**Monitoring and inspection.** IPM monitoring for ants typically involves placing an attractive food source near an area of suspected activity and observing any ants that forage on it. The immense infestations of *N. fulva* are obvious and this step may not be necessary during an initial inspection unless the infestation is still localized and small. Monitoring may be needed to locate entry points if *N. fulva* is entering a structure and monitoring is necessary to track changes in infestation level to determine if an insecticide application is warranted. Monitoring for *N. fulva* may be done with a thin slice of canned sausage or a small container of sucrose solution. *Nylanderia fulva*, if present, should locate the foods within 15-30 min. However, as with all sampling methods, there are limitations. Ant foraging is impacted by several factors including nutritional needs of the colony, temperature extremes and precipitation. Using both proteinaceous and carbohydrates food lures and avoiding placing bait lures during very high or low temperatures increases the opportunity to locate actively foraging *N. fulva*.
Inspections for *N. fulva* should be in three dimensions. *Nylanderia fulva* are often found in leaf litter, yard debris, potted plants, and in other items on the ground. However, *N. fulva* can also be found in trees, shrubs, fallen branches and mulch, foraging on honeydew producing insects. Occasionally, colony fragments of *N. fulva* have been found nesting in loose roof shingles and in attics.

**Exclusion and sanitation.** While *N. fulva* is predominately an outdoor pest, workers do occasionally enter structures seeking food and harborage. To prevent ants from entering structures, seal cracks and crevices around foundations, doors, windows and utility penetrations. *Nylanderia fulva* have been observed entering through windows and patios on the second and even third level of a structure, therefore, do not limit exclusion to ground level. *Nylanderia fulva* are small enough to enter structures though the openings in mesh screens.

Sanitation indoors includes eliminating food and moisture sources. Store food and pet food in sealed containers. Food debris and spills should be cleaned promptly. Trash should be removed regularly and the trash bin cleaned inside and out. Rinse food containers before placing them in the recycling bin. Outdoors, keep trash areas and bins clean and have trash removed on a frequent basis.

**Cultural control.** Landscape management practices that reduce the number of outdoor nesting sites for *N. fulva* are recommended. Remove items such as leaf litter, fallen branches and other lawn debris from the property. Trim trees and shrubs away from the structure to prevent ants from using branches to bypass insecticide treated areas and entering the structure. To prevent *N. fulva* access to honeydew producing insects in tree canopies, tree trunks may be sprayed with a liquid insecticide as a band.
application to target ants as they trail on the tree (Ree et al. 2014). *Nylanderia fulva* often form nests at the bases of trees. If this occurs, trees and shrubs may be treated with a liquid insecticide as a soil drench or soil injection. Also, *N. fulva* are often found in moist areas (personal observation). Therefore, eliminating moisture sources such as leaking faucets and hoses, puddles around sprinkler heads and standing water may make the lawn less hospitable.

**Chemical control.** This research has demonstrated that in laboratory studies, baits with a mixture of protein and carbohydrates are accepted by *N. fulva* in spring summer and fall. In winter, carbohydrates were preferred (Chapter 2). The active ingredients hydramethylnon and fipronil were highly efficacious against *N. fulva* (Chapters 4 and 5). While this study did not include baits containing insect growth regulators (IGRs), IGRs may be a good choice for control of *N. fulva* as reduced intraspecific aggression may allow the IGR to spread more rapidly through the population (Oi and Drees 2009). In the situation where the infestation has reached high numbers, an IPM strategy may include a knock-down application of insecticides followed by application of the baits but this strategy needs to be tested further.

**Future Research Needs**

These data provided some basic information related to the biology and ecology of *N. fulva* intended to be useful for PMPs and aid in the development of an IPM strategy. With some tactical approaches to *N. fulva* management now available, the focus must now shift to the development of a comprehensive IPM strategy for this pest. However, to accomplish this, there are some additional research gaps to be addressed. Following are some areas of research that need attention in order to develop a complete IPM strategy for *N. fulva.*
There are three phases of invasion of exotic species where management techniques may be applied. The first is arrival, when a species is transported to a new range (Liebhold and Tobin 2008, Rabitsch 2011). With 15 deep-water seaports, Florida bears significant risk of introduction of exotic species via cargo. Inspecting all incoming goods is not economically feasible; therefore, research is needed to identify high-risk commodities or ports of origin to help narrow the focus of an inspection program. Range expansion models like those developed by Kumar et al. (2015) may predict areas at greater risk for *N. fulva* invasion and allow for mitigation strategies and quarantine plans to be put into place prior to *N. fulva* introduction.

The second phase of invasion is establishment, the population grows and becomes self-sustaining. *Nylanderia fulva*, now found in 27 counties, clearly has established in Florida. Because of the large area of infestation, eradication of this ant is not likely. Therefore, IPM programs targeted at population suppression must be developed. In Chapter 3, I showed that some commercially available ant baits are acceptable and efficacious for *N. fulva*. However none of these baits were developed specifically for this ant. For granular baits, further research needs to be done on acceptable particle size, optimal protein:carbohydrate ratios, and effect of weathering. For liquid baits, the sugar concentration for maximum uptake of the bait as well as the effective concentration of active ingredient for intracolony transfer need to be determined. For all baits, baseline oral toxicities of insecticide active ingredients need to be determined. From these data, a *N. fulva* specific bait may be formulated.

Once an acceptable and efficacious bait is identified, there is the further need for an effective, inexpensive and easy to service bait station or novel bait delivery device.
Current research on the use of low-toxicant sucrose liquid baits distributed via polyacrylamide crystals has shown significant efficacy against *Linepithema humile* (Mayr) in the laboratory and field (Boser et al. 2014, Buczkowski et al. 2014a, Buczkowski at al. 2014b, Rust et al. 2015). Bait delivery to *N. fulva* by this method should also be tested. Baiting success also may be improved by determining the foraging distance of *N. fulva* workers and efficiency of toxicant transfer between workers and from workers to queens. This will aid in determining optimal bait station density and placement (Song et al. 2015).

Some contact insecticides have been shown to be efficacious against *N. fulva* (Chapter 4). However, more work needs to be done to determine their effectiveness on various substrates and under conditions of a Florida urban environment. The use of baits combined with insecticides may reduce *N. fulva* populations (Chapter 5). Further research to be done in this area to arrive at the best combinations of products and application methodologies.

Classical biological control can be an important part of a comprehensive IPM strategy. Possible *N. fulva* biological control agents have been recently identified: *Pseudacteon convexicauda* Borgmeier, a phorid fly (Brown et al. 2011), *Myrmecomorba nylanderiae* gen. et sp. nov., a microsporidian (Plowes et al. 2015) and *Nylanderia fulva* virus 1 (Valles et al 2012, Valles et al. 2016). Here, future research should be directed toward confirming efficacy, feasibility, and safety (Mack et al. 2000, Rabitsch 2011, Simberloff et al. 2005).

Spread is the third phase of invasion by exotic species and the establishment of a quarantine program is a management approach that may be applied to reducing the
dispersal of the *N. fulva* (Liebhold and Tobin 2008). In Chapter 2, it was demonstrated that *N. fulva* do not disperse a great distance from the point of introduction, implicating human-mediated jump dispersal in the long-distance spread of *N. fulva* throughout Florida. Therefore, research must be conducted to determine the most common ways in which *N. fulva* are being transported throughout the state, allowing the development of appropriate quarantine programs.

As an emerging invasive ant species in Florida, *N. fulva* provides a wide array of research opportunities including studies in invasion biology and ecology, biological control, dispersal dynamics and modeling. While there is still much to learn, these data represent some initial steps toward understanding the biology, ecology and behavior of this ant and the development of an IPM strategy for Florida stakeholders.
APPENDIX
GPS COORDINATES OF WAYPOINTS WITHIN EACH SAMPLING AREA

<table>
<thead>
<tr>
<th>Sampling Area</th>
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<th>Waypoint</th>
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BIOGRAPHICAL SKETCH

Dawn Calibeo received a Doctor of Philosophy degree in Urban Entomology from the University of Florida in Gainesville, Florida in 2017. She is currently employed at Gowan Company as Global Pre-Commercial Development Manager.

Dawn attended the University of Florida where she majored in Biology/Pre-Med and minored in Entomology. After obtaining her Bachelor of Arts degree, Dawn earned a Master of Science degree in Medical and Veterinary Entomology from North Carolina State University. The title of her M.S. thesis was “Role and Mitigation of Two Vectors of Turkey Coronavirus, Musca domestica L. and Alphitobius diaperinus (Panzer)”.

Dawn was formerly a Technical Training Specialist and Entomologist for Home Paramount Pest Control responsible for developing and implementing state approved Pest Control CEU Training Programs. She then joined BASF as a Biology Project Manager for specialty products including Pest Control, Turf and Ornamentals, Forestry and Public Health. Later she was promoted to Global Marketing Manager for insecticides. In 2010, Dawn returned to University of Florida to earn her Ph.D. Dawn’s research on a new pest ant species, Nylanderia fulva, focuses on understanding the biology and ecology of this invasive pest with the goal of developing an Integrated Pest Management strategy for Florida. Dawn is a Licensed Pesticide Applicator, a Dow Sentricon Apprentice and a graduate of the Broward Community College School of Structural Fumigation.

Awards Dawn has been granted include the Entomological Society of America, 2002 President’s Prize - Student Paper Competition (M.S.), Entomological Society of America, Southeastern Branch, 2003 Kirby Hays Award for Outstanding Masters Student, Entomological Society of America, 2012 President’s Prize - Student Paper
Competition (Ph.D.) and the 2012 Shripat Kamble Urban Entomology Graduate Student Award for Innovative Research. Dawn has received multiple BASF Recognition Awards for work associated with the development of the metaflumizone-based “Siesta” insect bait line, including the identification of the trademark and the development of training tools.

Professional memberships include the Entomological Society of America, the North Carolina Entomological Society, the Florida Entomological Society and Sigma Delta Honor Society. Professional activities include organizing and moderating symposia at the National Conference on Urban Entomology, serving as a representative on Southeastern Branch, Student Affairs Committee, serving as Vice-President of the NC State Graduate Student Association, Captain of the NC State Linnaean Games Team (National Champions) and a BugFest volunteer for many years. Dawn is the author of nine publications (4 peer reviewed) and has given over 16 presentations at scientific conferences.