

FORAGING STRATEGIES AND AGGRESSION PATTERNS OF *NYLANDERIA FULVA*  
(Mayr) (HYMENOPTERA: FORMICIDAE) IN NORTH CENTRAL FLORIDA

By

STEPHANIE KAY HILL

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To my family and friends that shared in this journey with me

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Abstract of Dissertation Presented to the Graduate School  
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Stephanie Kay Hill

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Native ecosystems are constantly at threat from invasive species. Better control and management techniques can be designed with more information about an invasive species in its native and introduced range. The first step in this process is proper identification. Until 2012, an ant infesting the southern United States was misidentified as *Nylanderia pubens* when it really was *Nylanderia fulva*. This misidentification led to inefficient control techniques. The clarification of the species identification allowed research to be refocused on proper control strategies.

The seasonal foraging activity of *N. fulva* was examined in north central Florida. The foraging densities of *N. fulva* were at their lowest in the winter and early spring, when *N. fulva* foraged in small discrete locations most likely close to permanent nesting sites. Through the late spring and summer, the density and distribution of the foraging increased, later decreasing in the fall and winter. At certain times of the year, *N. fulva* foraged in discrete locations in north central Florida, thus control measures need to be applied to discrete locations, because small populations are being targeted and not as much toxicant is needed, reducing the economic and environmental impact.

Laboratory and field investigations of the aggressive behavior of *N. fulva* towards *S. invicta* allowed identification of the behavioral mechanisms responsible for resource competition. In laboratory one-on-one bioassays, *N. fulva* and *S. invicta* avoided conflict, most likely to decrease the risk of injury or death, and neither species experienced success in small group interactions. However, in large groups, *N. fulva* cooperated in defending nestmates. Field investigations showed that this defensive behavior helped dictate the dominance of *N. fulva* over *S. invicta*. These results explain the behavioral mechanisms responsible for *N. fulva*'s competitive ability.

Food preference and seasonal food change was also documented. Results showed that *N. fulva* preferred protein as a food source year-round; indicating that baits incorporating protein should be effective year-round.

This research combined with current integrated pest management plans for *N. fulva* should help in managing this invasive species. Further research is needed in assessing the ecological effect of *N. fulva* and in designing effective baits.

## CHAPTER 1 PRELUDE

Ever since humans have been able to cross mountains, rivers, and oceans they have been transporting domestic animals, plants, and goods in order to survive. Unknowingly, non-domestic species have travelled or been traded; whether it be on humans, plants, or on the transportation device (Lockwood et al. 2007). Some of these exotic species have been able to colonize the new areas and have caused a great deal of problems. Exotic species are one of the greatest threats worldwide to native ecosystems and biodiversity (Vitousek et al. 1996, McGlynn 1999, Abbott 2006, Lockwood et al. 2007). Exotic species are also expensive, causing the United States alone approximately \$137 billion dollars in damages each year (Leung et al. 2002). Human travel and trade is on the increase (Hu and Reuscher 2004), thus the science of invasion ecology has an ever growing number of species in its repertoire.

Invasive ants pose ecological and economical concerns especially in human-developed ecosystems. The majority of invasive ants have a super colony structure (Heller 2005). Super colonies are polygynous, polydomous, and the workers do not show any aggression between nests (Hölldobler and Wilson 1990). The high densities and lack of aggression between colonies affect the abundance and diversity of native ant species, other invertebrates, and some vertebrate species by becoming competitors for food resources and territory (Porter and Savignano 1990, Human and Gordon 1996, Holway and Case 2001, Abbott 2005). They also become economically expensive to manage and control (Abbott 2006, Silverman and Brightwell 2008).

*Nylanderia fulva* are exotic ants that were introduced into Florida, Texas, and along the Gulf States (MacGown 2012). *Nylanderia fulva* form large super colonies and

take over vast expanses of land (Zenner-Polania 1990a, Fowler et al. 1994, Meyers 2008, Valles et al. 2012). Like other invasive ants, *N. fulva* causes disruption in natural ecosystems, affecting native ants, other arthropods, and vertebrates (Zenner-Polania 1990a, Zenner-Polania 1994, Aldana et al. 1995, LeBrun et al. 2012). These ants have also been observed tending aphids, damaging electrical equipment, and causing homeowners problems in their lawns (Zenner-Polania 1990a, 1990b, MacGown and Layton 2010, Calibeo and Oi 2010, 2011). The economic impact of *N. fulva* can only be speculated at this time, but it is expected to be substantial.

*Nylanderia fulva* have posed a great challenge to the pest control industry (Zenner-Polania 1990b, Zenner-Polania 1994, Pereira and Koehler 2007). Given the social behaviors of ants such as trophallaxis, shared resources, and grooming, active ingredients in pesticides should be passed around an ant colony. Other super colony ants, *Linepithema humile* (Mayr) and *Anoplolepis gracilipes* (Jerdon) have been successfully controlled, despite their high densities, by taking advantage of their social behaviors (Kruchelnycky et al. 2004, Abbott and Green 2007). To manage high densities of *N. fulva*, a broad-spectrum bait is normally applied to the entire treatable location (Culbert 2007, Pereira and Koehler 2007) with the idea that workers carry the bait back to the nest, passing the toxicant to the other ants. Bait applications have been unsuccessful in controlling *N. fulva* for long periods of time. Insecticidal sprays used for treatment on foraging trails and nesting areas also have been ineffective because *N. fulva* uses the accumulation of dead ants to bridge over the treatment (Drees et al. 2009, Nester and Raspberry 2010).

This research was undertaken in an effort to answer questions about the invasion ecology *N. fulva*. Spatial distribution and density studies were conducted on urban lots in North Central Florida. A thorough understanding of this ant's foraging behavior can help us understand it spreads and how to effectively control its populations. The aggressive behavior between *N. fulva* and *S. invicta* was assessed to better understand the behaviors responsible for *N. fulva*'s competitive ability. Finally, seasonal food preference changes were determined to aid in bait attractant selection for a given time of year. Besides adding to the information we already have about this species, this research will help in designing better control strategies and propel future research.

## CHAPTER 2 LITERATURE REVIEW

### Classification

*Nylanderia fulva* (Mayr) is an ant species in the family Formicidae, subfamily Formicinae, and tribe Lasiini. The classification of this ant in the United States has been a source of constant confusion.

On record, large populations of these ants were found in Florida in the 1990s (Klotz et al. 1995). Originally *N. fulva* were identified as *Paratrechina pubens* (Forel) because of reports of *P. pubens* in south Florida in the 1950s (Trager 1984). Large populations of these ants were discovered in Texas in 2002 (Meyers 2008). The ants were classified as *Paratrechina* species near *pubens* because of minor morphological variations from *P. pubens* that were more similar to characteristics of *Paratrechina fulva* (Forel) (Meyers 2008). The morphological similarities between *P. pubens* and *P. fulva* led Creighton (1950) and Bolton et al. (2007) to synonymize the two species. Meyers (2008) tried to determine the ants' true identity using genetic and morphological studies, but the research was inconclusive. In 2009, these ants were discovered in Mississippi and then, in 2010, in Louisiana and referred to as *P. sp. nr. pubens* (Hooper-Bui et al. 2010, MacGown and Layton 2010).

In 2010, LaPolla et al. did a taxonomic revision, using molecular data, to the *Prenolepis* genus group in which *P. pubens* belonged. In the revision, *Nylanderia* was elevated from a subgenus to a genus, and 133 species, including *P. pubens*, were moved into the genus *Nylanderia* from the genus *Paratrechina* (LaPolla et al. 2010).

Zhao et al. (2012) compared molecular samples of *Nylanderia pubens* and *Nylanderia sp. nr. pubens* from Florida and Texas respectively. They determined that

the two groups were the same species. Unfortunately, Zhao et al.'s information was derived from few samples. With many areas offering abundant samples of this ant species, more accurate species identification and a better understanding of the species distribution could have been obtained. Later in 2012, Gotzek et al. (2012) published a paper identifying *N. sp. nr. pubens* as *Nylanderia fulva* using morphometric and molecular data. The researchers had a representative sample of ants from the Caribbean Islands, Florida, Texas, Mississippi, and Louisiana (Gotzek et al. 2012). In this research, they also showed that *N. pubens* is restricted to the Caribbean islands, thus the ant populations introduced in the 2000s in Florida were most likely misidentified and the actual ant is *N. fulva* (Gotzek et al. 2012).

### **Distribution**

*Nylanderia fulva* originates from Brazil (Zenner-Polania 1990a, Zenner-Polania 1994). In the late 1960s and early 1970s, *N. fulva* was introduced into Colombia as a biological control agent against leaf-cutting ants (*Atta* sp.) and poisonous snakes based on research done in Brazil from the 1930s and 1940s (Zenner-Polania 1990a, Zenner-Polania 1994). As of 2006, *N. fulva* can be found in all the regions of Colombia (Germàn et al. 2006, Zenner-Polania 1990a). *Nylanderia pubens* has been documented from Anguilla, Argentina, Bermuda, Brazil, Colombia, Cuba, Guadeloupe, Martinique, Mexico, Panama, Puerto Rico, St. Croix, Lesser Antilles, and US Virgin Islands (Trager 1984, Wetterer and Keularts 2008, Pagad 2011, MacGown 2012). Given possible misidentifications and the genetic evidence put forth, there is a possibility that some of the ants from those countries are actually *N. pubens* while others are *N. fulva* (Gotzek et al. 2012).

In the United States, *N. fulva* has been documented in Florida, Texas, Mississippi, and Louisiana (McGlynn 1999, Meyers 2008, MacGown and Layton 2010, Warner and Scheffrehn 2010). Because of misidentification, it is not known how long *N. fulva* has been in the United States. In Florida, the first record of *N. pubens* was in Miami in 1953 (Trager 1984). The next documentation of this species was in 1990 in and around a hospital and several buildings in Miami (Klotz et al. 1995). Beginning in 2000, *N. pubens* were reported in Everglades National Park, Fort Lauderdale, Port St. Lucie, Jacksonville, and Sarasota (Warner and Scheffrehn 2010). Most of these infestations are most likely *N. fulva*. Currently *N. fulva* can be found in 24 counties in Florida (Alachua, Brevard, Broward, Clay, Collier, DeSoto, Duval, Hardee, Hillsborough, Indian River, Lee, Manatee, Marion, Martin, Miami-Dade, Orange, Palm Beach, Pasco, Pinellas, Polk, Saint Johns, Saint Lucie, and Sarasota) (MacGown 2012).

The first observations of *N. fulva* in Texas were in Harris County in 2002 (Meyers 2008). Gold (2012) listed 24 counties with confirmed *N. fulva* (Bexar, Brazoria, Brazos, Comal, Cameron, Fort Bend, Chambers, Galveston, Hardin, Harris, Hidalgo, Jefferson, Jim Hogg, Liberty, Matagorda, Montgomery, Nueces, Polk, Orange, Travis, Victoria, Walker, Wharton and Williamson). Along the Gulf Coast, there are three counties in Mississippi (Hancock, Harrison, and Jackson) and two parishes in Louisiana (Calcasieu and West Baton Rouge) with confirmed established colonies of *N. fulva* (Hooper-Bui et al. 2010, Morgan 2011, MacGown 2012).

### **Description**

*Nylanderia fulva* is a one-node, reddish brown ant species. The workers are monomorphic and can range from 2.0-2.4 mm in length. The males and queens are

slightly larger measuring 2.4-2.7 mm and 4.0 mm or larger respectively (LaPolla et al. 2011, Gotzek et al. 2012, MacGown 2012).

The head appears darker and shiny due to the lack of pubescence. Characteristic to *Nylanderia* species, *N. fulva* has a 12 segmented antenna with no club. The scape is long, measuring two times the width of the head. The ants have no ocelli. The thorax and abdomen are smooth and shiny except for areas of dense yellow pubescence. The gaster, after feeding, is lighter in color and appears striped because it is stretched. The appendages are also covered with a yellow pubescence. An acidopore is present in the females (Trager 1984, LaPolla et al. 2011, Gotzek et al. 2012, MacGown 2012). In males, the defining characteristic from other species is a strongly triangular (in profile) paramere that is lightly sclerotized and has few macrosetae originating from the margin of the paramere, as opposed to the heavily sclerotized paramere in *N. pubens* (Trager 1984, Gotzek et al. 2012).

### **Colony Structure**

Most ant species live in colonies of cooperating specific individuals. *Nylanderia fulva* is a polygyne and polydomous; therefore, they are considered a super colony ant species (Zenner-Polania 1990a, Meyers 2008, Valles et al. 2012). Polygyne is defined as the presence of more than one queen in a nest and polydomy is defined as the use of two or more spatially separated nests by one ant colony (Hölldobler and Wilson 1990). New nests are formed in super colonies by budding; one or more queens will leave the original nest and form a separate nest (Hölldobler and Wilson 1990). Super colonies are then very large communities with multiple queens and many nests that allow workers to mix freely across the community. The lack of aggression allows resource allocation so resources can be used to expand the colony rather than be spent

on intraspecific aggression (Tsutsui et al. 2003). There is a complete loss of colony borders and thus, super colonies have the ability to take over a large expanse of land (Hölldobler and Wilson 1990).

Zenner-Polania (1990a) characterized the two types of nests that *N. fulva* constructs depending on the abiotic conditions. The first is a permanent nest, which is always occupied by the colony and is found in protected areas. Permanent nests have been found occupying one square meter and containing immatures up to 40 cm below the ground surface. In the dry seasons or when the weather conditions are not favorable, only permanent nests are built. In the rainy season, or when conditions are more favorable, temporary nests are utilized. Temporary nests are found under leaf debris, in or under waste material, in soil crevices, and in abandoned arthropod habitats. When temporary nests are disturbed, the ants will move to another nest. Since temporary nesting sites do not buffer *N. fulva* from temperature and moisture variations, the ants can alter their conditions by moving nest locations or changing the nest size, which in turn affects the metabolic heat generated by the group (Seeley and Heinrich 1981).

The number and type of caste and developmental forms differ between each nest type. In 80 permanent nests sampled in Columbia, the caste and developmental form number varied with 1- 14 queens, 366- 2302 workers, 214- 1034 eggs, 815- 1314 larvae, and 565- 4539 pupae (Zenner-Polania 1990a). Temporary nests are much smaller. The average number, caste, and developmental forms found in 556 seasonal nests were 46 workers, no reproductives, 21 eggs, 84 larvae, and 49 pupae (Zenner-Polania 1990a).

The number of permanent and temporary nests at a location vary depending on how infested the location is with *N. fulva*. In areas that are completely infested, permanent nests are more common than temporary nests. In areas that *N. fulva* have not completely infested, the number of temporary nests outnumber the permanent nests by 88% (Zenner-Polania 1990a). Zenner-Polania (1990a) hypothesized that temporary nests are used to new areas. At a coffee plantation in Colombia, *N. fulva* was recorded advancing their territory at a rate of 100 m per month (Zenner-Polania 1990a).

### **Environmental Factors**

Environmental factors can encourage or suppress the local spread of invasive ants because of the ants' vulnerability to desiccation and temperature stress (Hölldobler and Wilson 1990). The foraging workers are severely vulnerable to these two factors because they travel away from the nest (Krushelnycky et al. 2010). In Colombia, *N. fulva* have been found between 150 and 2600 m above sea level (Zenner-Polania 1990a). In Puerto Boyaca, Colombia, the elevation is 150 m above sea level and the temperatures can reach up to 40°C (Zenner-Polania 1990a). At this elevation and temperature, the infestation of *N. fulva* was low as compared with higher elevations and lower temperatures (Zenner-Polania 1990a). *Nylanderia fulva* can develop within a wide range of average temperatures, ranging from 13°C to 29°C (Zenner-Polania 1990a). In Colombia, *N. fulva* develops best at temperatures averaging 22°C and at an altitude of 1500 m above sea level (Zenner-Polania 1990a).

### **Recognition**

The ability to distinguish between nestmates and non-nestmates is critical in social insect societies (d'Ettorre and Lenoir 2010). The ability to distinguish between nestmates and non-nestmates prevents robbery of brood, parasitism, and colony

takeover (d’Ettorre and Lenoir 2010). With the exchange of queens, brood, and workers, the ability to distinguish nestmates is even more important in super colonies.

The way insects discriminate nestmates and non-nestmates is much debated (Chen and Nonacs 2000, d’Ettorre and Lenoir 2010). The three most generally accepted ways of insect recognition are prior association, phenotype matching, and recognition alleles (d’Ettorre and Lenoir 2010). Prior association is where individuals learn cues and become familiar with other individuals they encounter and treat them as nestmates (d’Ettorre and Lenoir 2010). Only those individuals encountered previously can be recognized as nestmates. Similar to prior association, in phenotypic matching, individuals have an internal template that allows them to recognize other individuals of the same nest and/or species (d’Ettorre and Lenoir 2010). Recognition alleles do not require learning cues; they are solely based on the recognition of alleles (d’Ettorre and Lenoir 2010).

There are other factors, such as diet, that have proven to play a role in insect recognition (Chen and Nonacs 2000, Say-Piau and Chow-Yang 2003, Buczkowski and Silverman 2005). Colonies of *Linepithema humile* (Mayr), the Argentine ant, originating from the same polygyne colony, fed on two different protein diets, exhibited aggressive behavior when introduced to a common arena (Buczkowski and Silverman 2005). There was a 100% attack rate between the two groups. This behavior has also been witnessed in *Paratrechina longicornis* (Latreille) (Say-Piau and Chow Yang 2003). This leads to the speculation that it is not only genetics that aid in nestmate recognition, but also other factors such as diet. Polygyne colonies are a great example of this theory

because of the variety of genetic cues derived from having so many queens (Chen and Nonacs 2000).

Giraud et al. (2002) argue that it is the genetic origin of a colony rather than diet that allows ants to discriminate nestmates from non-nestmates. Aggression tests were performed on populations of Argentine ants along a 6000 km track around Spain, Portugal, France, and Italy. Two super colonies were distinguished, the main super colony and the Catalanian super colony. The tests showed, regardless of the distance between ants of the same super colony, there was no aggression. The genetic structure between the two super colonies was found to be largely different at eight microsatellite loci, and there was a great genetic diversity within each super colony. They concluded that unfound recognition loci remained constant in the each super colony.

There is no literature about how *N. fulva* discriminate nestmates from non-nestmates. Based on the information about *L. humile* and *P. longicornis*, it can be hypothesized that both recognition loci and diet play a role in recognition.

### **Feeding Habits**

*Nylanderia fulva* are opportunistic generalists that tend aphids and consume the honeydew secretions, prey on live animals, and scavenge from dead animals (Zenner-Polania 1990a, Ulloa et al. 2000, Campos-Farinha and Zorzenon 2005). In Brazil and Colombia, *N. fulva* workers have been observed interacting with aphids and scale insects, specifically tending them and collecting honeydew (Zenner-Polania 1990a, Campos-Farinha and Zorzenon 2005). In some cases, the ants transported the Hemipterans from infested plants to uninfested plants (Zenner-Polania 1990a). In Colombia alone, *N. fulva* is associated with over 25 species of Hemipterans (Zenner-

Polania 1990a). The association between the ants and Hemipterans is devastating to plant health. The plants have to combat the feeding Hemipterans as well as any diseases they may vector.

*Nylanderia fulva* has been recorded feeding on different forms of animal protein (Zenner-Polania 1990a, Zenner-Polania and Ruiz-Bolanos 1985). To capture small prey (other arthropods), workers may attack the prey and immobilize it (Zenner-Polania 1990a). Then the ants will either suck the hemolymph from the prey or transport pieces of the prey back to the nest (Zenner-Polania 1990a). *Nylanderia fulva* have also been documented to attack small animals such as rabbits, birds, lizards, and domestic animals (Zenner-Polania 1990a). The animal's death is usually attributed to asphyxia (Zenner-Polania 1990a). The ants then suck the liquids from the prey or take pieces of it back to the nest. In excavation of *N. fulva* permanent nests, Lepidoptera, Coleoptera, Isoptera, Hymenoptera, Arachnid, bird, and lizard remains were found (Zenner-Polania 1990a).

Cook et al. (2012) examined the macronutrient requirements for *N. fulva*. They found that the ants, when given a choice, consumed more food with a 1:2 protein to carbohydrate ratio. In no-choice tests, the ants regulated the amount of each nutrient that they took in to meet the requirements of the colony. In both experiments, there was a high rate of mortality. This may have been due to the use of solid food rather than liquid food in the experiments and a low larva to worker ratio that can lead to starvation due to the lack of enough larvae to break down the solid food to feed all of the workers.

### **Relationship with Other Species**

The introduction of the *N. fulva* to a new region causes the reduction and elimination of native ants and other insects (Zenner-Polania 1994, Aldana et al. 1995,

LeBrun et al. 2012). There have been direct observations, in Colombia, of competition between native ants and *N. fulva* (Zenner-Polania 1994, Aldana et al. 1995). Zenner-Polania (1994) compared the species richness of native ants in areas free of *N. fulva*, areas that border infestations of *N. fulva*, and in *N. fulva* infested areas. In areas free of *N. fulva*, the species diversity index was 0.77. The species index at the border of infestations dropped 88% to 0.09 compared to areas free of *N. fulva*. In areas that were completely infested, the species diversity index was 0.0088. This is a 98.85% reduction from areas that were not infested. The only ant species found in the *N. fulva* infested areas were *Monomorium floricola* Forel and *Dolichoderus diversus* Emery, making up 5% of the individual ants sampled. The success of these two species was related to their size and nesting habits (Zenner-Polania 1994).

Aldana et al. (1995) sampled the species richness of ants in the Laguna de Sonso Natural Reserve, Colombia, and in adjacent areas from the reserve. *Nylanderia fulva* were first found in the reserve in 1990. The areas sampled adjacent to the reserve were not infested with *N. fulva*. There were 28 ant species found in the adjacent areas. In the infested reserve area, only 7 species of ants were found. This is a 75% reduction in the number of ant species.

LeBrun et al. (2012) examined the effect of *N. fulva* on native ant and arthropod diversity in Texas grasslands. In *N. fulva* infested areas, the native ant densities were two orders of magnitude lower than the densities of *N. fulva*. Curiously, in areas with high densities of *N. fulva*, the red imported fire ant, *Solenopsis invicta* Buren, were absent and abundance of non-ant arthropods was greatly reduced.

Similarly, Zenner-Polania (1990a) documented the reduction of other arthropods in areas infested with *N. fulva*. There was a noticeable reduction in white grubs, leaf-feeding Lepidoptera, and termite colonies when compared to areas not infested with *N. fulva*. In Brazil, *N. fulva* was documented preying on the pupae of Lepidopteron pest, *Brassolis sophorae* (Linnaeus) (Campos-Farinha and Zorzenon 2005).

Besides affecting invertebrates, *N. fulva* has been documented attacking vertebrate fauna (Zenner-Polania 1990a, Aldana et al. 1995). On farms in Colombia, *N. fulva* has been blamed for the deaths of small animals, chickens, and other birds (Zenner-Polania 1990a). The death of these animals is believed to be asphyxia (Zenner-Polania 1990a). Larger animals were attacked around their eyes, nose, and hoofs. In the Laguna de Sonso Natural Reserve, *N. fulva* blinded an iguana and caused irreversible damage to a large bird, *Anhima cornutus* (Linnaeus) (Aldana et al. 1995). More quantitative research is needed to assess the effects of *N. fulva* on vertebrate fauna.

### **Pest Status and Control**

*Nylanderia fulva* is considered a serious pest in its native and introduced regions. Native to Brazil, *N. fulva* is considered a tramp species (Gusmão et al. 2008). It has been found infesting buildings, greenhouses, and tending honey-dew producing insects (Campos-Farinha and Zorzenon 2005, Gusmão et al. 2008). In Colombia, *N. fulva* was introduced into the coffee growing areas as a biological control agent for leaf-cutter ants (*Atta* spp.) and snakes (Zenner-Polania 1990a). It soon turned into a serious pest problem by tending sap-feeding insects, attacking domestic animals, entering structures, and displacing native ant species (Zenner-Polania 1990a, Zenner-Polania 1994). *Nylanderia fulva* has developed into a serious pest problem in the Caribbean and the

United States; especially in Florida and states along the Gulf of Mexico (Warner and Scheffrahn 2010, Pereira and Koehler 2007, MacGown and Layton 2010, Calibeo and Oi 2011). In the United States, *N. fulva* is considered a nuisance pest because the high densities of workers can affect day-to-day activities, and in extreme cases, makes outside activities impossible (MacGown and Layton 2010). In Texas, beekeepers have reported *N. fulva* killing honeybee larvae and taking over the hives to use as a nest once the bees had been decimated (Harmon 2009).

Many ants are attracted to electricity, and *N. fulva* is no exception. At the Jacksonville Zoo in Jacksonville, Florida, *N. fulva* infested electrical switch boxes causing shortages (Calibeo and Oi 2010). Meyers (2008), Drees et al. (2009), Nester and Rasberry (2011) also reported evidence of the ants causing electrical boxes and switches to fail.

*Nylanderia fulva* is usually found in high numbers when a pest management professional is called to a location. To manage the high numbers of ants, broad-spectrum bait is applied to the entire location (Culbert 2007, Pereira and Koehler 2007). If needed, a residual insecticide is applied to the outside of structures to deter the ants (Culbert 2007, Pereira and Koehler 2007). Control is usually seen for a short period of time before the ant population resurges (Gusmão et al. 2008, Drees et al. 2009, Nester and Rasberry 2011). The control of *N. fulva* is difficult due to their high numbers, weak foraging on most traditional ant baits, and the efficacy of pesticides.

There have been a variety of pesticides tested to see the effects on *N. fulva* populations. In Brazil, methoprene (insect growth regulator) granular bait reduced the number of foraging *N. fulva* around a hospital by more than half in an 18-week study

(Gusmão et al. 2008). Another insect growth regulator, pyriproxyfen, was tested in Texas for its efficacy on *N. fulva* (Nester and Rasberry 2011). Using a broadcast spray at the highest label rate, control was seen for 14 days then the population rebounded (Nester and Rasberry 2011). In both of these instances, a reapplication of pesticide would have to be done for continued control.

In Colombia, a bait mixture of corn bran, fish meal, 1% sugar solution, propionic acid, pork lard, and carbaryl 85 PM was applied after the rainy season, and the ants were controlled for approximately two months (Zenner-Polania 1990b). The density of ants, in Columbia, decline at the end of the rainy season and into the dry season. Given the natural decline in ant densities during the application of the bait, full control measures may not have been observed.

Meyers (2008) laboratory and field tested a variety of pesticides against *N. fulva*, but no long-term suppression of the ant populations was seen. In the laboratory, experiments were conducted on queenless colonies, making the data collected in those experiments questionable. Dinotefuran on corn bait matrix was field tested with an initial population reduction, but the population resurged within a week. The same population resurgence was seen after testing the effectiveness at the label rate of fipronil, chlorfenapyr, abamectin, acetamiprid, and bifenthrin. The overall conclusion of Meyers (2008) work was that more work needed to be done to find an effective chemistry that will control *N. fulva* for extended periods of time.

Scott (2012) examined bait size and attractants for *N. fulva*. This author found that the ants preferred foods that were between 0.85-1.00 mm in size and contained a high protein bait matrix. In the laboratory, fipronil was added to a protein bait matrix and

was highly efficacious in eliminating *N. fulva*. To fully test the effects of the formulated bait, field trials would need to be conducted.

When dealing with an *N. fulva* infestation, a single control method cannot be used because of the super colony structure (Zenner-Polania 1990b, Drees et al. 2009, Calibeo and Oi 2010). An integrated pest management plan is needed to control *N. fulva* infestations. Landscape debris, lawn debris, and trash need to be removed as they provide a place for temporary nest. Trash cans need to be emptied regularly and stored away from structures. To prevent entry into structures, landscape around the structure needs to be cut back so that no branches are touching the structure, and all cracks and crevices around the structure need to be sealed. Pest management professionals need to apply pesticides at the right times of the year for the toxicants to be most efficient (Zenner-Polania 1990b). More information about *N. fulva*'s biology and ecology is needed to design a better integrated pest management plan.

CHAPTER 3  
DISTRIBUTION AND DENSITY OF *NYLANDERIA FULVA* ON URBAN PLOTS AND  
THE EFFECT OF ABIOTIC FACTORS

**Introduction**

*Nylanderia fulva* (Mayr) is an invasive tramp ant that is polygynous (multiple queens per colony), polydomous (multiple nests per colony), and unicolonial (shows no aggression between neighboring colonies) (Zenner-Polania 1990a, Meyers 2008, Valles et al. 2012). Since ants from different nests and colonies are constantly interchanging members and resources, it may be more accurate to consider them a super colony ant (Hölldobler and Wilson 1990, Giraud et al. 2002). Super colonies are known to facilitate rapid population increases due to the high number of queens resulting in high brood production (Hölldobler and Wilson 1990). Little research has been done with the ecology of *N. fulva* in the United States, and most of the research has focused on the problems that the *N. fulva* causes and possible control measures (Meyers 2008, Valles et al. 2012).

*Nylanderia fulva* is a serious pest species in both its native and introduced areas. It is native to Brazil where it is often considered a tramp species; found infesting buildings, greenhouses, and tending honey-dew producing insects (Campos-Farinha and Zorzenon 2005, Gusmão et al. 2008). *Nylanderia fulva* was introduced into Columbia, the Caribbean, and along the Gulf states in the United States (Zenner-Polania 1990a, Pereira and Koehler 2007, MacGown and Layton 2010). It has developed into a serious pest problem by tending sap-feeding insects, attacking domestic animals, displacing native ant species, entering structures, and causing shortages in electrical boxes (Zenner-Polania 1990a, Zenner-Polania 1994, Pereira and Koehler 2007, Calibeo and Oi 2010, MacGown and Layton 2010).

Control of *N. fulva* is difficult and control is only temporary (Zenner-Polania 1994, Gusmão et al. 2008, Drees et al. 2009, Nester and Rasberry 2011). When a pest management professional is called to a location with a *N. fulva* infestation, the ants are usually in high numbers. In order to manage the infestation, broad spectrum bait is normally applied to the entire location (Culbert 2007, Pereira and Koehler 2007). If the ants are entering a structure, a residual insecticide is normally applied to the outside of the structure (Pereira and Koehler 2007, Drees et al. 2009). Both of these treatments are only adequate to manage the ant population for a short amount of time and the ants usually resurge in high numbers resulting in new insecticide applications (Drees et al. 2009, Nester and Rasberry 2011). The cost of the pesticide for these treatments has the potential to exceed the monthly cost of a pest control contract, making this treatment strategy uneconomical for the pest control company as well as the consumer. The amount of toxicant applied into the environment with this control strategy is higher than normal, and the environmental implications need to be considered. The super colony nature and lack of successful management strategies have caused *N. fulva* to become a pest of major concern for the consumer as well as the pest control industry.

The Argentine ant, *Linepithema humile* (Mayr) is also a super colony ant. Extensive work has been done on the biology, ecology, and control of this ant, and it is one of the best-studied examples of invasive super colony ants (Tsutsui and Suarez 2003). Similar to *N. fulva*, the colonies contain high numbers of workers which support and care for many queens which can reproduce at high rates (Horton 1918). Also, Argentine ants have high rates of reproduction that allow them to expand over large

geographical areas (Tsutsui et al. 2000, Tsutsui and Case 2001, Giraud et al. 2002, Heller 2005, Corin et al. 2007).

Environmental factors also encourage or suppress the spread of Argentine ants (Hölldobler and Wilson 1990, Krushelnycky et al. 2005, Schilman et al. 2005, Heller et al. 2008). Foraging workers are severely vulnerable to desiccation and temperature stress because they are traveling away from the safety of the nest (Hölldobler and Wilson 1990, Krushelnycky et al. 2010). Rainfall and soil temperature have been shown to affect the spread of Argentine ants (Krushelnycky et al. 2005, Schilman et al. 2005, Heller et al. 2008). Spatial pattern research suggests that Argentine super colonies expand in the spring and summer with increased soil temperature and rainfall, while in the fall and winter months the colonies contract (Krushelnycky et al. 2005, 2010, Heller et al. 2006, 2008).

Based on known similarities between *N. fulva* and the Argentine ant infestations, a study was designed to examine the spatial distribution and density of foraging *N. fulva* on urban lots over two years. The objectives were to compare the population distribution over the seasons and ground cover, compare ant density among locations and seasons, and correlate ant population with temperature and precipitation.

### **Materials and Methods**

Six urban lots, three in Gainesville, FL and three in Citra, FL, were chosen for the study because of the known presence of *N. fulva*. The locations in Gainesville were a recreational park and two urban house lots. The locations in Citra, a rural community, were three rural lots with houses. For all locations, sampling boundaries were set by using the portion of the lot with managed landscape; using the house as the center point and not extending over the property lines. The sample areas were gridded in 4 x 4 m

squares (Brenner 1988, Abbott 2005). The markers, placed at each vertex, were made by bending the metal stakes of surveyor flags into a U-shape and moving the flag to the bend in the stake. The markers were pushed into the ground so the U in the middle of the stake was flush with the ground and only the flag was showing. At each vertex, the ground cover type was recorded as: sand, loose litter (mulch, leaf debris, and loose organic matter), grass, or solid surfaces (asphalt, concrete, dirt roads).

Ant foraging in the lots was sampled during 2010 and 2011 between April and November. Sampling was done only when the air temperature reached a minimum of 21°C and was not over 32°C based on observations by Warner and Scheffrahn (2010) with *Nylanderia pubens* (Forel) in south Florida, which was believed to be the ant at the start of the study. The sampling period was divided into spring (April and May), summer (June, July, August), and fall (September, October, November) for analysis purposes.

Sampling was done by placing a 7.5 by 5.5 cm index card with approximately 5 ml of honey as flat as possible on the ground at each grid vertex. After 30 minutes, pictures were taken of the cards using a digital camera (Sony Cyber-shot, 8.1 megapixels). The sampling cards were collected and removed from the property after sampling was finished. The pictures were downloaded to a computer, and the ants were identified and counted on the pictures projected on the computer screen. This procedure allowed the pictures to be magnified as needed for better discrimination of the ant species and separate individuals.

The average daily temperature and precipitation for both Gainesville and Citra were compiled. For Gainesville, data used was from the Gainesville Regional Airport, which is approximately 10 km from the sampling sites and was compiled by the Florida

Climate Center in Tallahassee, Florida. Citra weather data, collected at a station approximately 6.5 km from the sampling sites, was compiled from the Florida Automated Weather Network maintained by the University of Florida.

**Data Analysis:** Only *N. fulva* data was used in the analysis. Analysis was performed on square-root transformed data (number of ants) for both distribution and density. The foraging distribution of *N. fulva* was analyzed using a mixed analysis of variance (GLIMMIX procedure in SAS 10 software) (SAS Institute 2012a) with the mean number of *N. fulva* at each sampling point as a response variable and the ground cover, season, monthly temperature averages, and monthly precipitation averages as fixed effects. For mean comparisons, a *P*-value of <0.05 was used to establish statistical differences. Contour maps of the ground cover at each sampling lot and the population densities were prepared using Proc Contour (SAS Institute 2012a). In the population density contour maps 0-1 represent low densities, 4-8 represent moderate densities, and 16-64 represent heavy densities.

A regression analysis (SAS Institute 2012b) was used to examine the effects of location, year, and month on foraging density with a *P*-value of <0.05 to establish statistical differences. To determine which weather parameters (average temperature, minimum temperature, maximum temperature, and average precipitation) affected the foraging density, the parameters were examined using a screening platform designed to determine factors with the largest effect on the response variable by effect sparsity which assumes that only a small number of the effects are significant and the others are not. The parameters with a significant *P*-value >0.05 were further analyzed using a regression analysis (SAS Institute 2012b).

## Results

**Foraging Distribution:** Results from the mixed model ANOVA showed that the ants were foraging more on sand than other ground cover types ( $F = 77.71$ ;  $df = 3$ ;  $P < 0.0001$ ) (Table 3-1). More *N. fulva* foraged in the summer months than in the spring and fall months ( $F = 34.46$ ;  $df = 2$ ;  $P < 0.0001$ ) (Table 3-2), but more ants foraged in the fall than in the spring. In the spring, the ants foraged mostly on sand, followed by loose litter, then grass and solid surfaces. In the summer and fall, the ants had no preference between foraging on sand or loose litter, but preferred these two ground covers over grass and solid surfaces (Figure 3-1). Results from the mixed model ANOVA showed that temperature had an effect ( $F = 10.75$ ;  $df = 1$ ;  $P = 0.002$ ) on where the ants foraged, but precipitation did not ( $F = 1.43$ ;  $df = 1$ ;  $P = 0.24$ ).

The Gainesville plots were an urban park and two urban residences. The relative location of the plots to each other is seen in Figure 3-2 and the ground covers are seen in Figure 3-3. In 2010, there were no other houses adjacent to Gainesville plot 1, an urban residence (Figure 3-4), but in 2011, construction began on residences on both sides of the location. In April and May in both years, the ants foraged in discrete areas on the plot, and ants were first seen foraging in the eastern side of the plot. In June and July, the ants foraged more broadly across the plot, but still in localized areas. In August, the ants reached the widest distribution. In September 2010, the ants foraged in similar discrete areas as in July. In September 2011, the ants foraged in a localized area in the southeast corner of the plot. Ants foraged in discrete areas in the spring and fall that had loose litter as a ground cover. This pattern was seen in 2010 and 2011, but there were fewer high density foraging areas in 2011 than in 2010.

Gainesville plot 2 (Figure 3-5) was an urban park, and the most natural sampling plot of all the plots included in this study. The north boundary of the sampling area had a creek running beside it and the south boundary was open grass. The wooded area of the plot had loose leaf litter around the base of the trees. The ground cover away from the trees was variable including loose leaf litter, sand, and grass. Ants foraged more broadly in 2011 than in 2010. In April of both years, the ants foraged in the area with trees close to the creek to the north. In July and August for both years, the ants foraged broadly and heavily across the plot. The areas where the ants did not forage as readily were covered with grass. In November 2010 and 2011, the ants foraged in locations where they were first seen in the spring.

Gainesville plot 3 (Figure 3-6) was also an urban residence, but it was unoccupied during the 2010 sampling period. In January of 2011, occupants moved into the residence and started irrigating the grass areas as well as landscaping around the house. During both sampling years, the house had mulch around its perimeter, but in 2011 more mulch was added to the existing mulched areas. In April of 2010, the ants were first foraging in discrete areas in the northeast corner of the plot. In May 2011, the ants foraged heavily across the north boundary of the plot and moderately on the south side of the plot. In July and August 2010, the ants foraged broadly and heavily across the plot area, and foraged in similar areas in June and September. In October and November 2010, the ants foraged again in discrete areas on the plot with the northeast corner being the location with the highest foraging. In April 2011, the ants foraged in discrete areas similar to May 2010. Both areas of heavy foraging were covered in mulch and leaf litter. From June until August 2011, the ants increasingly foraged more broadly

and heavily across the plot. Starting in September 2011 and going through November 2011, the ants gradually reduced their foraging area and foraged in fewer numbers.

The Citra plots were all rural residences. Each of the sample plots bordered the same orange grove, where *N. fulva* might have been introduced through transport of commercial orange containers from South Florida. The location of the plots in relation to each other is seen in Figure 3-7 and the ground cover at each location is seen in Figure 3-8.

The property of Citra plot 1 (Figure 3-9) shared its eastern property line with the orange grove. Also, the orange grove continued across the road on the northern side of the property. In May 2010, the ants foraged on the western side of the plot. In June, July, and August 2010, the ants began foraging closer to the house and were distributed in a wider area, with August being the month with the ants most widely distributed and having the heaviest foraging. In the fall months, the ants foraged in more localized areas and with a greater range of number of foragers. In April of 2011, the ants foraged on the east side of the plot and continued to forage across the southern boundary of the plot in the following month. In the spring of 2011, all of the landscaping around the house was removed and the trees and bushes in and around the plot area were severely cut back. In June and July of 2011, the ants foraged in more localized areas to the eastern side of the plot and not around the house as compared to the same months in 2010. In the fall of 2011, the ants foraged minimally in discrete areas on the eastern side of the plot.

Citra plot 2 (Figure 3-10) property shared its western boundary with the orange grove. In April, May, and June of 2010, the ants foraged in localized areas on the

northwestern corner and southern boundary of the plot. Both of these areas were covered in leaf litter. In July 2010, the foraging area became larger, and in August, the ants foraged heavily on most of the property. Throughout September, October, and November, the ants foraged in localized areas that became smaller over time. In 2011, the ants foraged in smaller more localized areas than in 2010. Also, in the spring of 2011, this plot was infested with fleas, with the worst infestation between the house and the southern plot boundary line. The homeowners treated the pets as well as all of the plot area several times throughout the 2011 sampling period with unknown pesticides.

The property of Citra plot 3 (Figure 3-11) shared half of its eastern-most boundary with the orange grove. There was a fence lined with trees separating the southern boundary from the adjacent property that backed up to the orange grove. In May and June 2010, the ants foraged on the southern side of the sampling plot. In July 2010, the foraging area increased and the ants foraged along the southern plot boundary, on the west side of the property, and in a small wooded area directly behind the house. In August 2010, the foraging area expanded to cover most of the plot, with several areas of heavy foraging concentration. In September 2010, the areas of foraging reduced in comparison to the previous month, and this continued in October and November, with the ants foraging in more restricted areas. Throughout the 2011 sampling period, the ants foraged in smaller localized areas than 2010. In 2011, the solid surface west of the northern most structure in this plot had heavy ant foraging where the homeowners fed their cats from June until August. The residents also had a fire pit where trash was accumulated and burned, around which the ants foraged from July to November in 2010 and June to October in 2011.

**Foraging Density:** Results from the regression analysis showed that location ( $F = 4.2$ ;  $df = 1$ ;  $P = 0.04$ ), year ( $F = 4.7$ ;  $df = 1$ ;  $P = 0.03$ ) and month ( $F = 3.9$ ;  $df = 1$ ;  $P = 0.001$ ) had an effect on the density of ants. Gainesville plots had significantly more ants than Citra plots. In 2010, there were more ants foraging than in 2011. There were significantly fewer ants foraging at Citra in 2011 than any other location/year combination. In both April and November, for both 2010 and 2011, there were significantly fewer ants foraging than the rest of the months sampled. August had significantly more ants foraging than the rest of the months sampled (Figure 3-12).

The average temperature was the only weather parameter that had a significant effect on the density of ants ( $t = 4.08$ ;  $P = 0.002$ ) with higher temperature associated with higher ant densities (Figure 3-12). The minimum temperature, maximum temperature, and average precipitation did not significantly affect the density of ants.

### **Discussion**

In super colonies of ants, a group of connected nests that share resources and reproduce together are considered a colony. The foraging strategy of *N. fulva* is most likely based on their super colony structure. The distribution and density of foragers in this study suggest that *N. fulva* colonies expand and contract seasonally.

The phenomena of expanding and contracting of ant colonies has been well documented in Argentine ants (Heller 2005, Heller and Gordon 2006, Heller et al. 2006, 2008). The ants aggregated in connected nests in the winter and dispersed in the summer (Heller 2005). The climate affected the rate in which the ants disperse in the summer. Heavy rainfall and high temperatures increased soil moisture and temperature which led to higher numbers of suitable nest locations (Heller 2005, Krushelnycky et al. 2005). This allowed the ants to disperse across the landscape (Heller and Gordon

2006). In the winter months that were drier and cooler, the ants contracted back into the aggregated nests that had a more stable microclimate (Heller 2005).

Zenner-Polania (1990a) documented the two types of nests *N. fulva* inhabited, permanent and temporary. Permanent nests were found in protected areas with controlled microclimates and allowed the ants to become established in an area. The temporary nests helped advance the colony into new areas where they could forage for nutritional resources. Permanent nests were inhabited year-round, but were essential in the late fall, winter, and early spring, as they were constructed in protected areas. Temporary nests were inhabited in late spring, summer, and early fall when brood production was at its highest and there was a high demand for nutritional resources. Temporary nests are abandoned for the permanent nest if the abiotic conditions are not right or if disturbed. In the spring and summer months, the colony network expanded to facilitate efficient collection of nutritional resources. Clustering in permanent nests in the fall and winter allowed for resources to be efficiently collected and utilized.

*Nylanderia fulva* foraging was distinctly seasonal in the sampled plots in north central Florida. Overall, in April and May, the foraging took place in localized areas of the plots with a small number of foragers. The workers gathered nutritional resources in those localized areas to aid in brood production. As the densities of ants increased, the ants began to inhabit temporary nests and the foraging distribution increased. The foraging distribution and density peaked in August. In September, the foraging distribution and density decreased, and with this decrease, the foraging areas became more localized. This was most likely due to the temporary nests being abandoned for the permanent nests. Brood production was reduced in the fall, and the need for

nutritional resources was not as great (Zenner-Polania 1990a). The permanent nests protected the colony from acute abiotic conditions. A seasonal pattern of high colony dispersal during the times of high reproduction followed by a contraction into few nests before winter is called seasonal polydomy and has been described for other super colony ant species (Cerdeña et al. 2002, Dillier and Wehner 2004, Elias et al. 2005).

In Colombia, on a 390<sup>2</sup>m coffee plantation infested with *N. fulva*, 80 permanent and 556 temporary nests were found (Zenner-Polania 1990a). This helps explain why there were several localized foraging areas in the spring months and even into the early summer months. The localized foraging areas most likely represent areas close to permanent nests. In the summer and early fall, these were areas of heavy foraging. The areas around permanent and temporary nests were most likely where the heaviest foraging was taking place. In the fall, foraging areas, again, become localized. The ants were most likely foraging close to permanent nests, as observed in the spring. In the winter, *N. fulva* foraged in discrete localized areas near permanent nests to gather enough nutritional resources to survive the colder months.

Knowing the ground cover that *N. fulva* foraged on given time of the year can aid in identifying where nests are located. In the spring (April and May), the ants preferred to forage on sand, then loose litter. At this time, the ants are most likely inhabiting their permanent nests located in areas with sandy or loose litter ground cover. At the coffee plantation sampled in Colombia, 39 of the permanent nest were found at root edges and 26 at the base of trees (Zenner-Polania 1990a). At the sampling plots in my study, the ground cover at the base of trees and at root edges, in the spring, was either sand or loose litter (mulch or leaf litter). In order to locate the permanent nest, it would be

advantageous to look around the base of trees and root edges in the spring. In the summer, there was no significant difference between sand, loose litter, and grass as ground cover. At the coffee plantation, Zenner-Polania (1990a) found temporary nests in soil crevices, in dead leaves, at the base of coffee trees, in banana stems, rotten roots, grass roots, and in empty snail shells. These nests could have been on or in a variety of ground covers. In the fall, sand and loose litter was again the most preferred ground covers, and, probably where permanent nests are found.

Temperature was the weather factor that was shown to affect the distributions and density of the ants. *Nylanderia fulva* have been documented surviving and reproducing at temperatures averaging between 13°C and 29°C (Zenner-Polania 1990a). In this study, as temperatures increased, the foraging distribution and density increased. August is one of the hottest months and was when the foraging distribution was most widespread, and the density was at its highest at all plots in both years.

Although not significantly correlated to ant density, precipitation, which was lower in 2011 than 2010, and water availability may affect *N. fulva* densities. The only sampling plot that did not see a decrease in ant density in 2011 was Gainesville plot 2. This is perhaps due to the presence of a creek along the north boundary of the sampling area. Precipitation was a good predictor of population size in Argentine ants (Heller et al. 2006, 2008).

By knowing seasonal characteristics of nesting and foraging patterns, better management programs could be designed for *N. fulva*. Control measures need to be applied in the winter and early spring when the densities of *N. fulva* are low and the ants are foraging in small discrete areas where permanent nests with reproductives are

present. This would allow for the least amount of control effort and cost to be used, reducing the economic and environmental impact of controlling this ant. This strategy contrasts the control techniques used currently in which broad-spectrum pesticides were used during the months when there was high ant densities (Culbert 2007, Pereira and Koehler 2007). Application of pesticide is usually dictated by customer complaints. Education of customers about *N. fulva* would be crucial to implementing a successful management program.

*Nylanderia fulva* was initially identified as *Paratrechina pubens* (Forel) before this study began. It wasn't until after the study was completed that the species was identified as *N. fulva*. *Nylanderia pubens* can forage at temperature no lower than 22°C (Warner and Scheffrahn 2010). *Nylanderia fulva* can forage at temperatures as low as 13°C (Zenner-Polania 1990a), but because the ant was incorrectly identified, sample procedures may not have covered the foraging season for *N. fulva*. In March 2011, one foraging sample was taken. It was not used in statistical analysis because of the lack of comparable data for 2010. Ant activity was seen during that sampling time. Year-round sampling may allow better identification of areas where permanent nests are present, which would allow better targeting for control measures.

Table 3-1. Mean number of *Nylanderia fulva* foragers per sampling station ( $\sqrt{x+0.5}$ ) on each type of ground cover in six sampling plots in North Central Florida.

Ground Cover	Mean Number of Ant Foragers ( $\sqrt{x+0.5}$ )	SEM
Sand	1.3 <i>a</i>	0.34
Loose Litter	1.0 <i>b</i>	0.27
Grass	0.7 <i>c</i>	0.19
Solid Surfaces	0.3 <i>d</i>	0.09

Means followed by the same letter are not significantly different (Tukey-Kramer  $P < 0.05$ ).

Table 3-2. Mean number of *Nylanderia fulva* foragers per sampling station ( $\sqrt{x+0.5}$ ) in each season at the six sampling plots in North Central Florida.

Season	Mean Number of Ant Foragers ( $\sqrt{x+0.5}$ )	SEM
Spring	0.3 <i>a</i>	0.08
Summer	1.7 <i>b</i>	0.50
Fall	0.9 <i>c</i>	0.27

Means followed by the same letter are not significantly different (Tukey-Kramer  $P < 0.05$ )

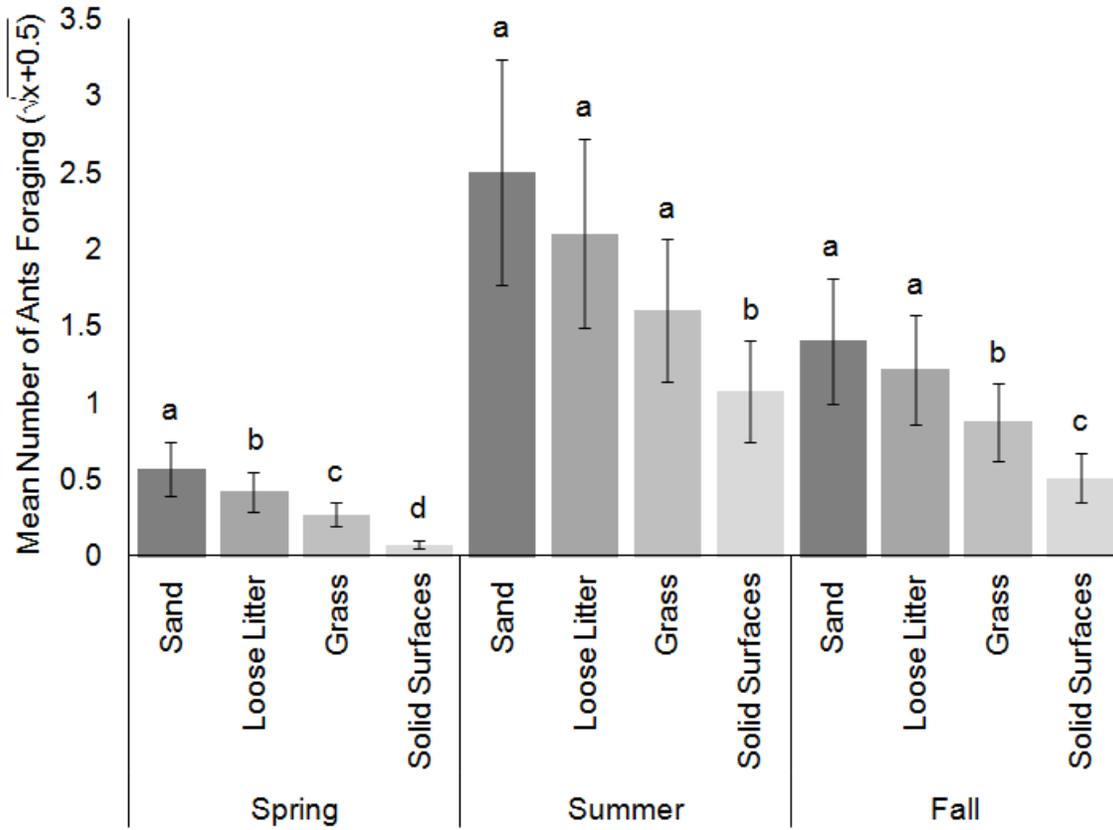


Figure 3-1. Mean number of foraging *N. fulva* ( $\sqrt{x+0.5}$ ) on different ground covers through the seasons. Within each season, means connected with the same letter are not significantly different by Tukey-Kramer ( $P < 0.05$ ).

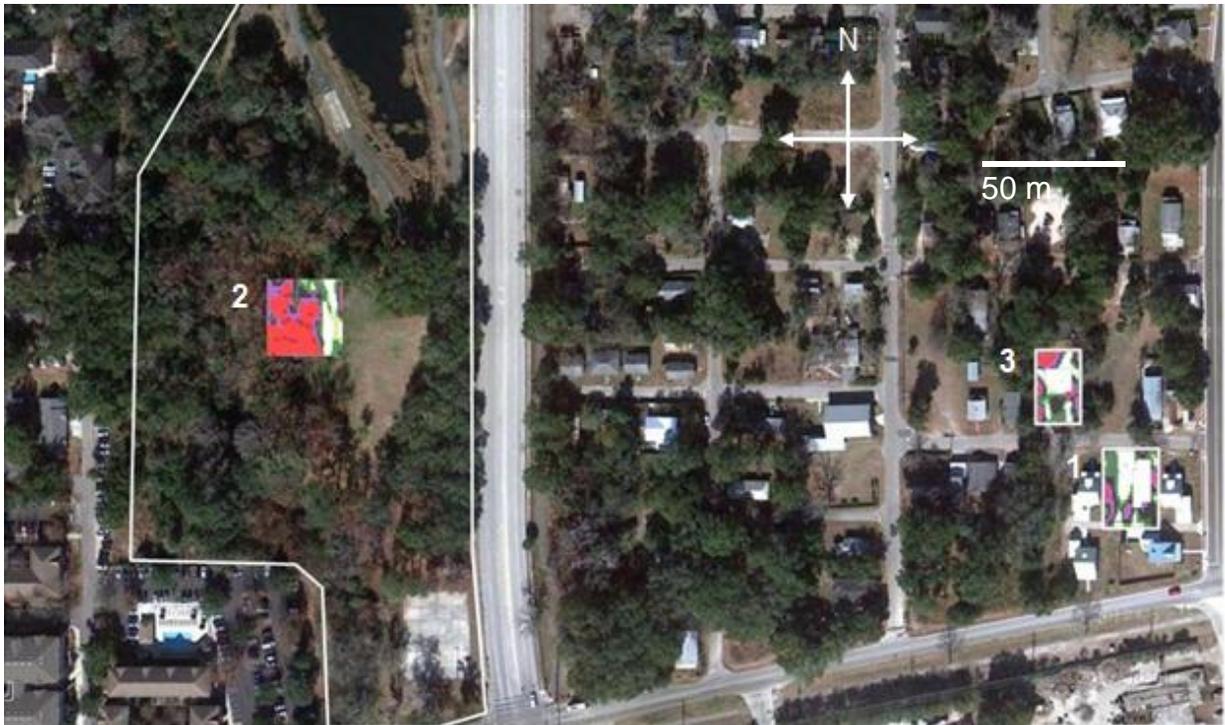


Figure 3-2. Aerial view of the Gainesville sampling locations in relation to one another. The white lines indicate property lines. Numbers correspond to the lots. The *N. fulva* population distribution is for August 2010 as shown in Figure 3-4, 3-5, and 3-6.

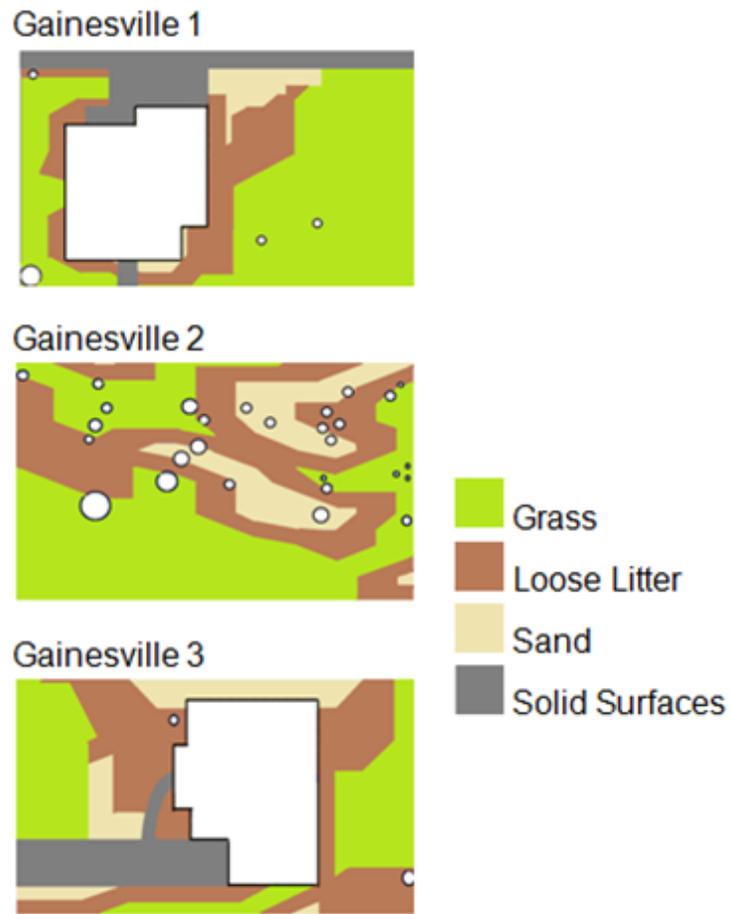


Figure 3-3. Ground cover at each of the sampling plots in Gainesville, FL. Solid lines represent solid structures and circles represent trees and bushes. The size of the circle indicates the relative size of the tree/bush.

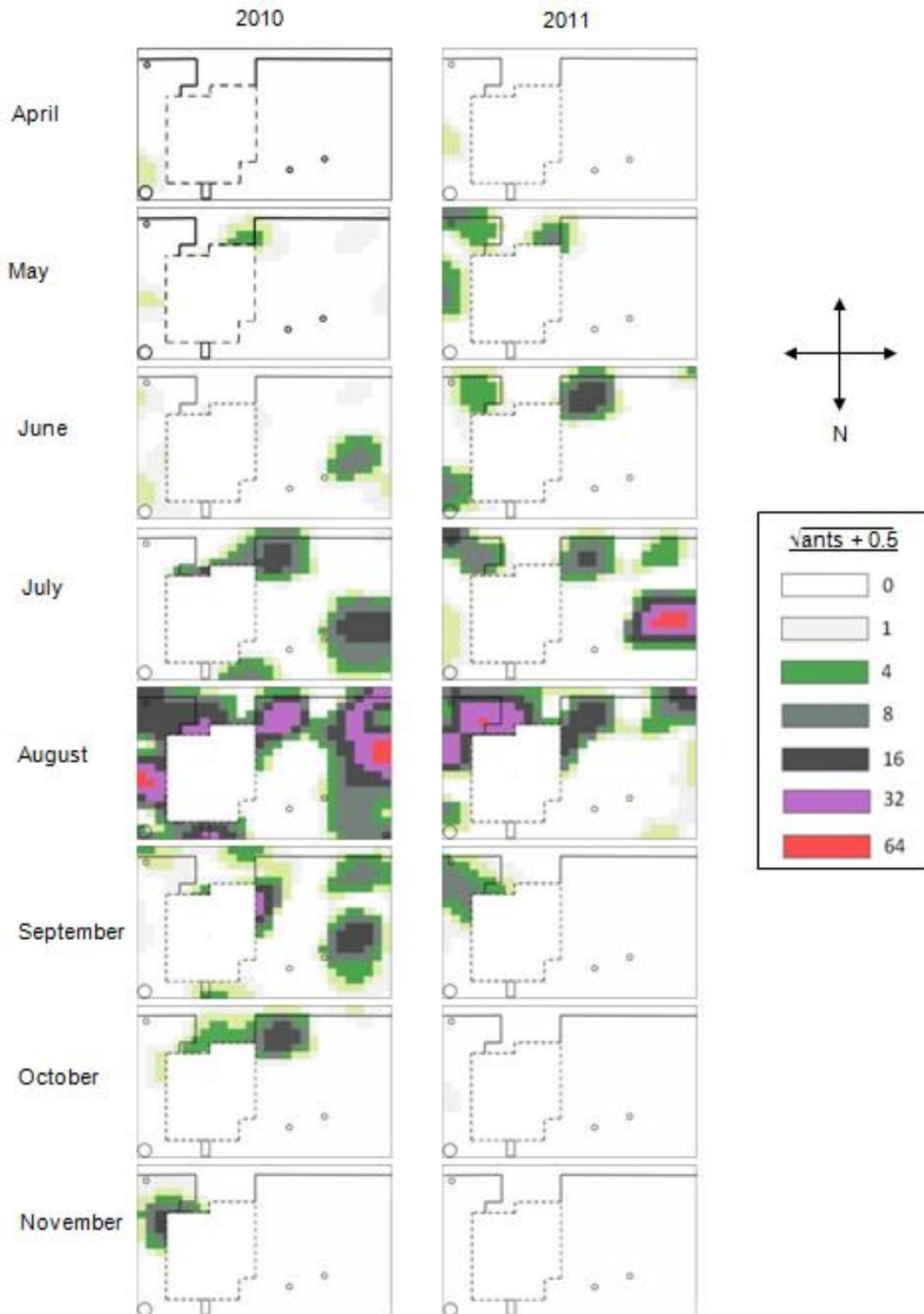


Figure 3-4. Spatial distribution of average monthly foraging counts ( $\sqrt{\text{ants} + 0.5}$ ) of *N. fulva* at Gainesville plot 1. Dashed lines represent buildings, solid lines represent driveways and roads, and circles represent trees and bushes. The size of the circle indicates the relative size of the tree/bush.

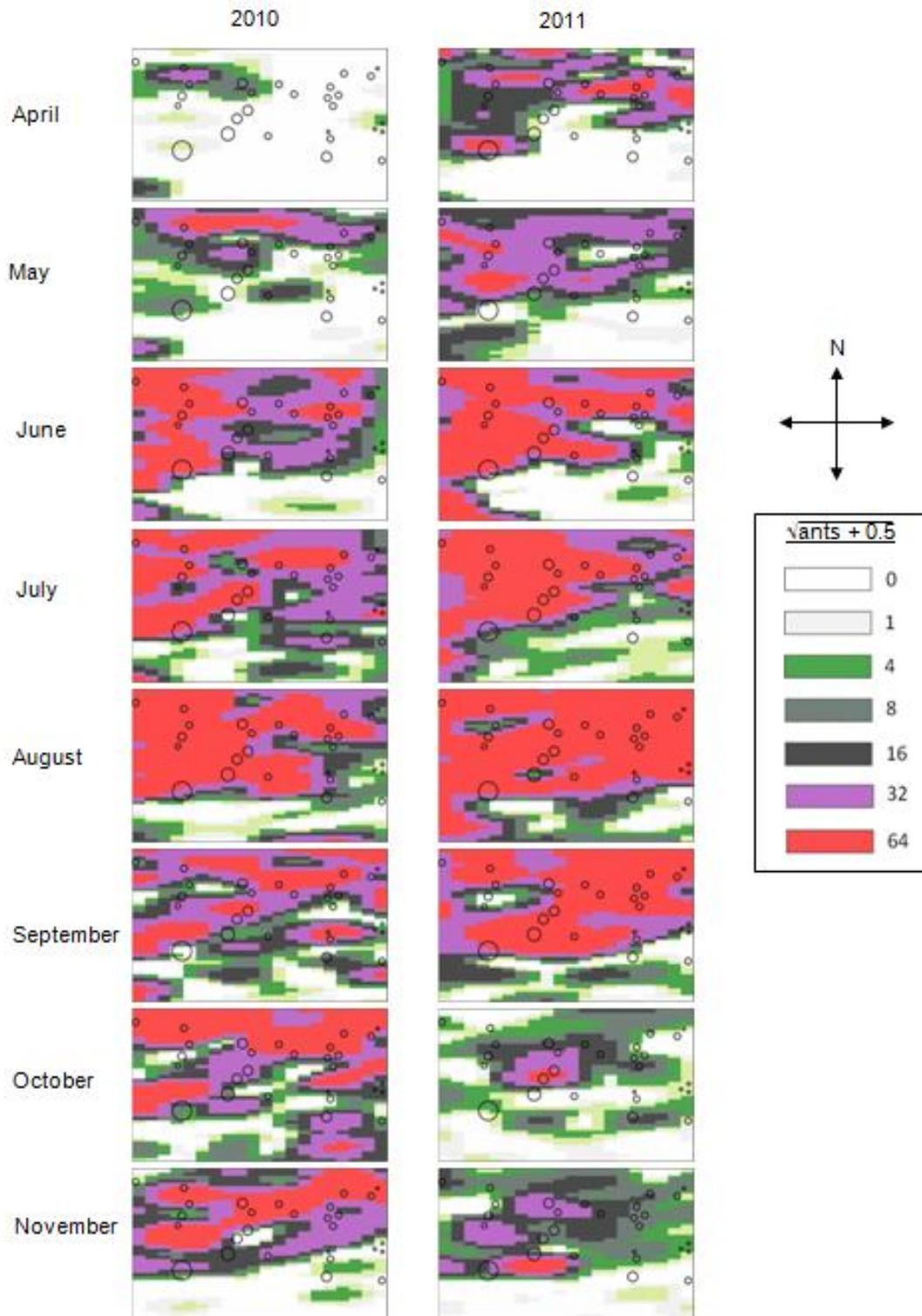


Figure 3-5. Spatial distribution of average monthly foraging counts ( $\sqrt{\text{ants} + 0.5}$ ) of *N. fulva* at Gainesville plot 2. Dashed lines represent buildings, solid lines represent driveways and roads, and circles represent trees and bushes. The size of the circle indicates the relative size of the tree/bush.

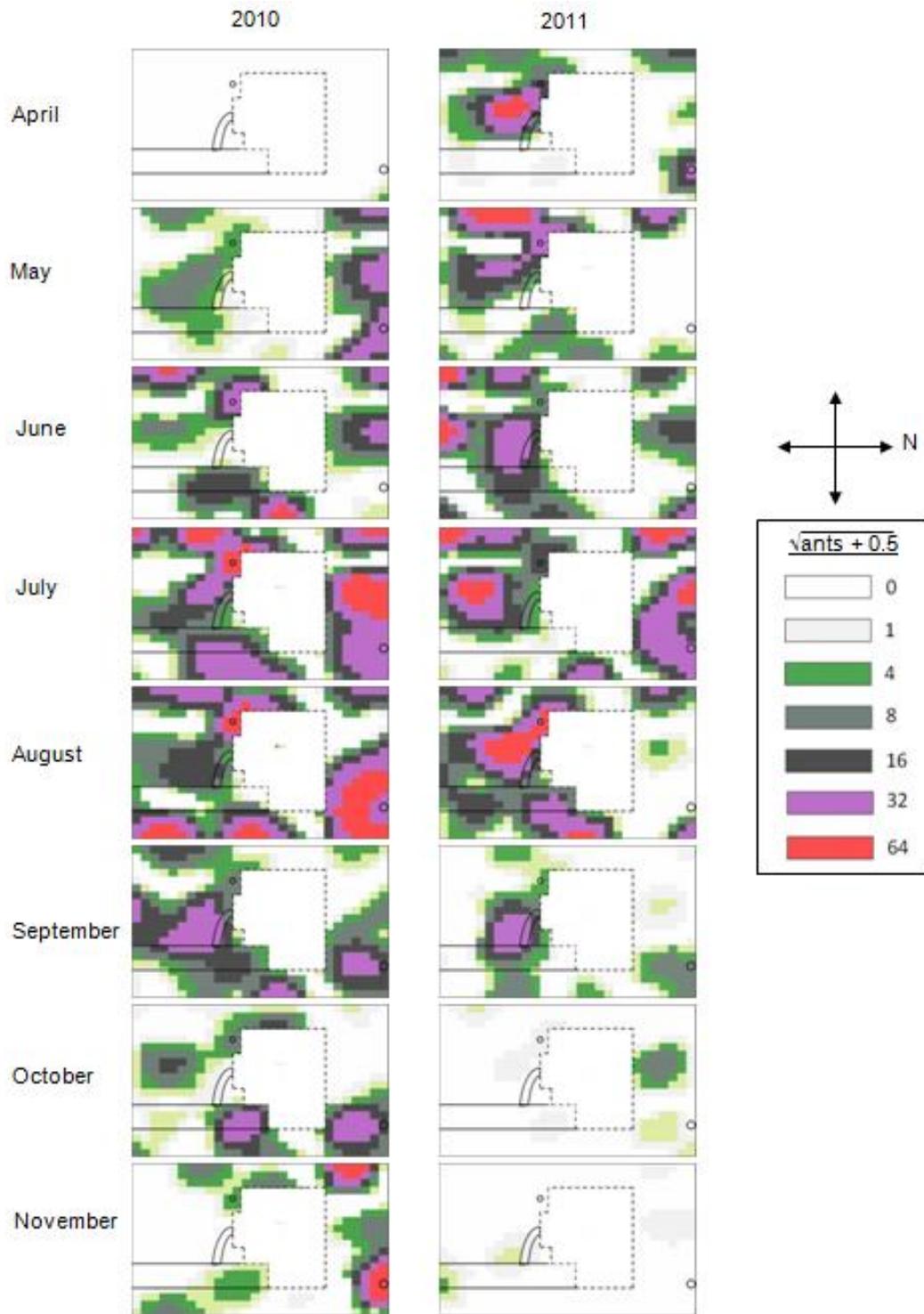


Figure 3-6. Spatial distribution of average monthly foraging counts ( $\sqrt{\text{ants} + 0.5}$ ) of *N. fulva* at Gainesville plot 3. Dashed lines represent buildings, solid lines represent driveways and roads, and circles represent trees and bushes. The size of the circle indicates the relative size of the tree/bush.

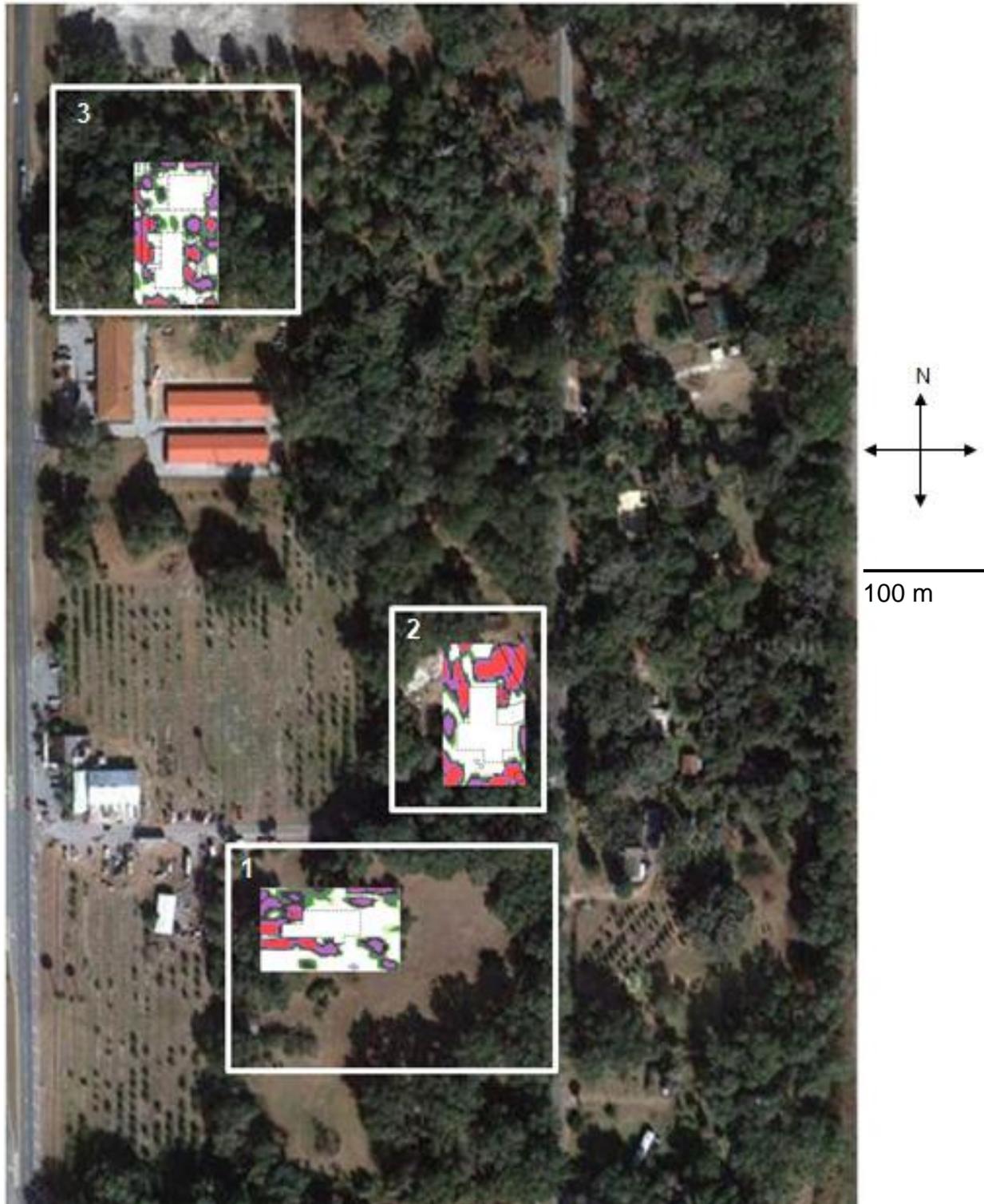


Figure 3-7. Aerial view of the Citra sampling locations in relation to one another. The white lines indicate property lines. Numbers correspond to the lots. The distribution is August 2010 as shown in Figure 3-9, 3-10, and 3-11.

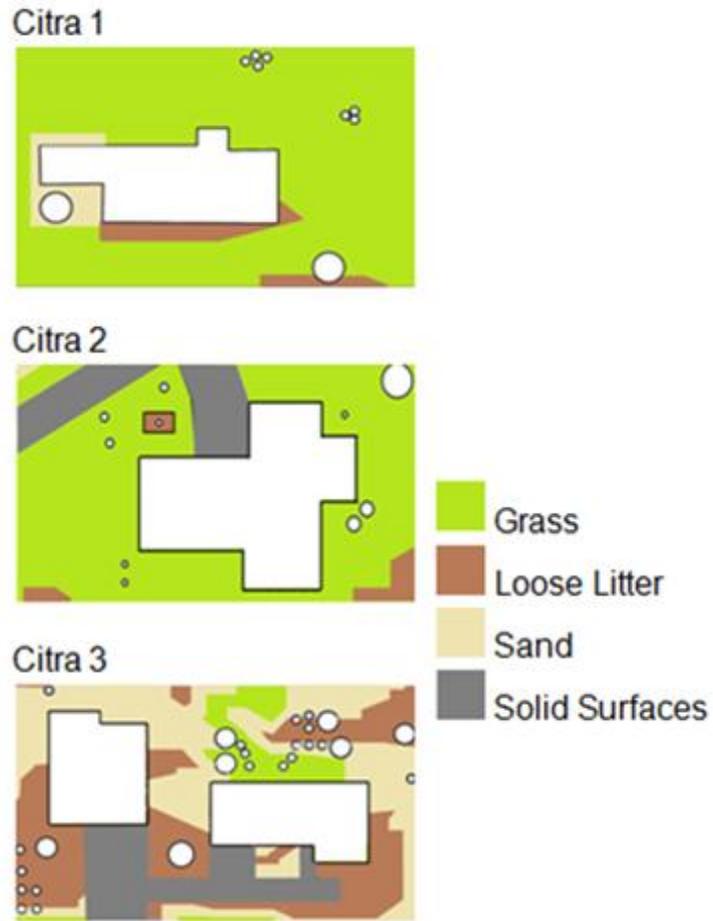


Figure 3-8. Ground cover at each of the sampling plots in Citra, FL. Solid lines represent solid structures and circles represent trees and bushes. The size of the circle indicates the relative size of the tree/bush.

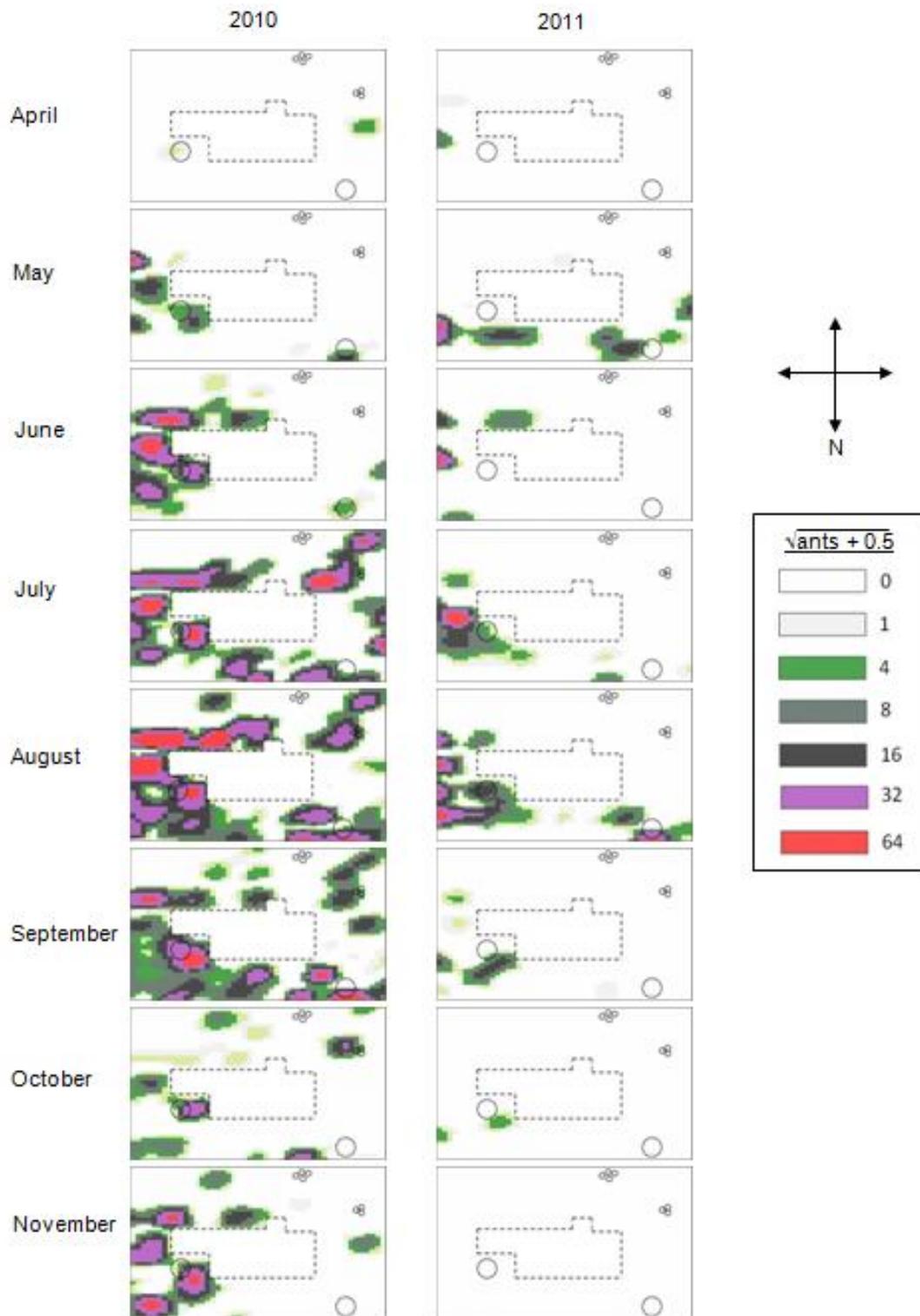


Figure 3-9. Spatial distribution of average monthly foraging counts ( $\sqrt{\text{ants} + 0.5}$ ) of *N. fulva* at Citra plot 1. Dashed lines represent buildings, solid lines represent driveways and roads, and circles represent trees and bushes. The size of the circle indicates the relative size of the tree/bush.

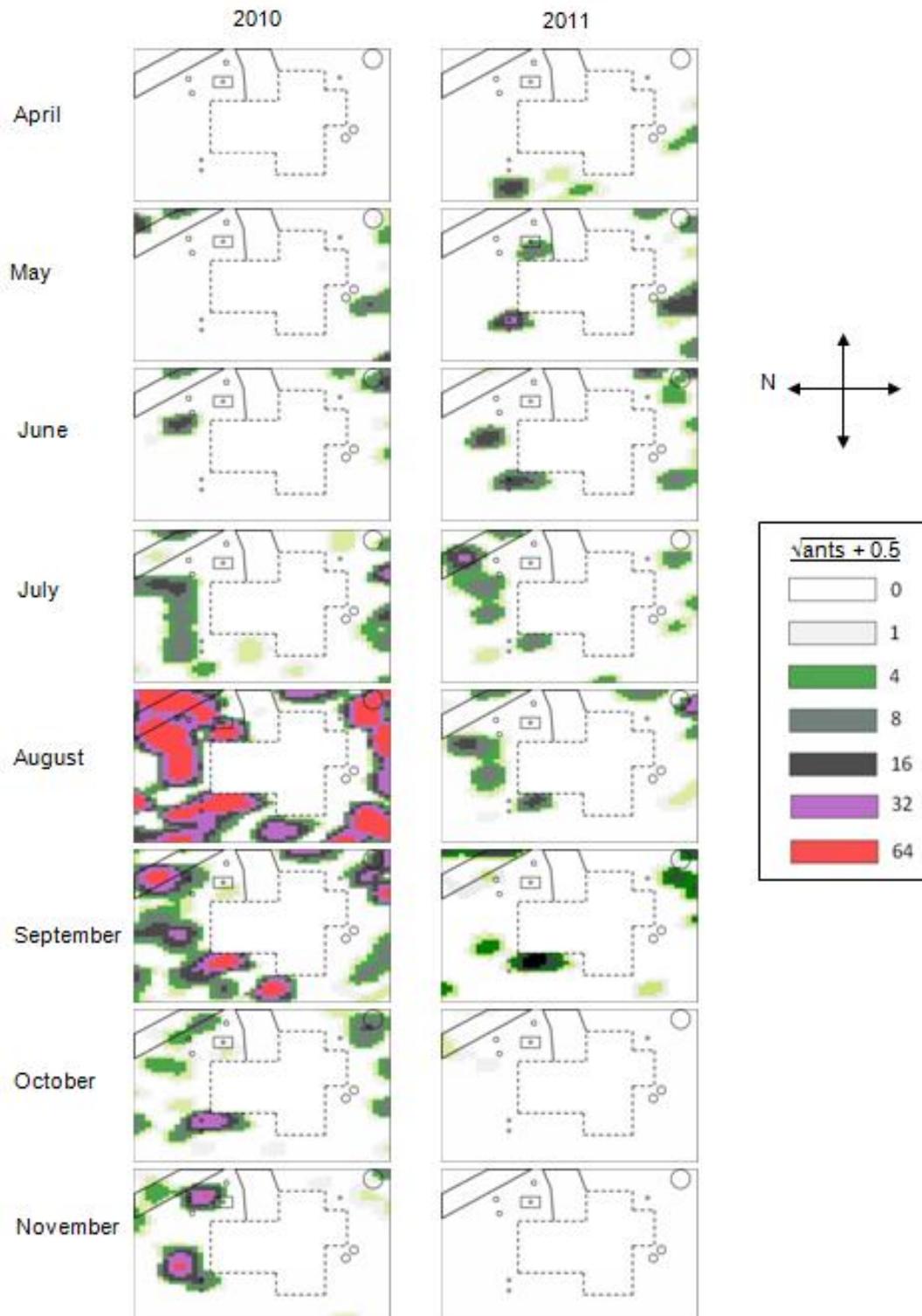


Figure 3-10. Spatial distribution of average monthly foraging counts ( $\sqrt{\text{ants} + 0.5}$ ) of *N. fulva* at Citra plot 2. Dashed lines represent buildings, solid lines represent driveways and roads, and circles represent trees and bushes. The size of the circle indicates the relative size of the tree/bush.

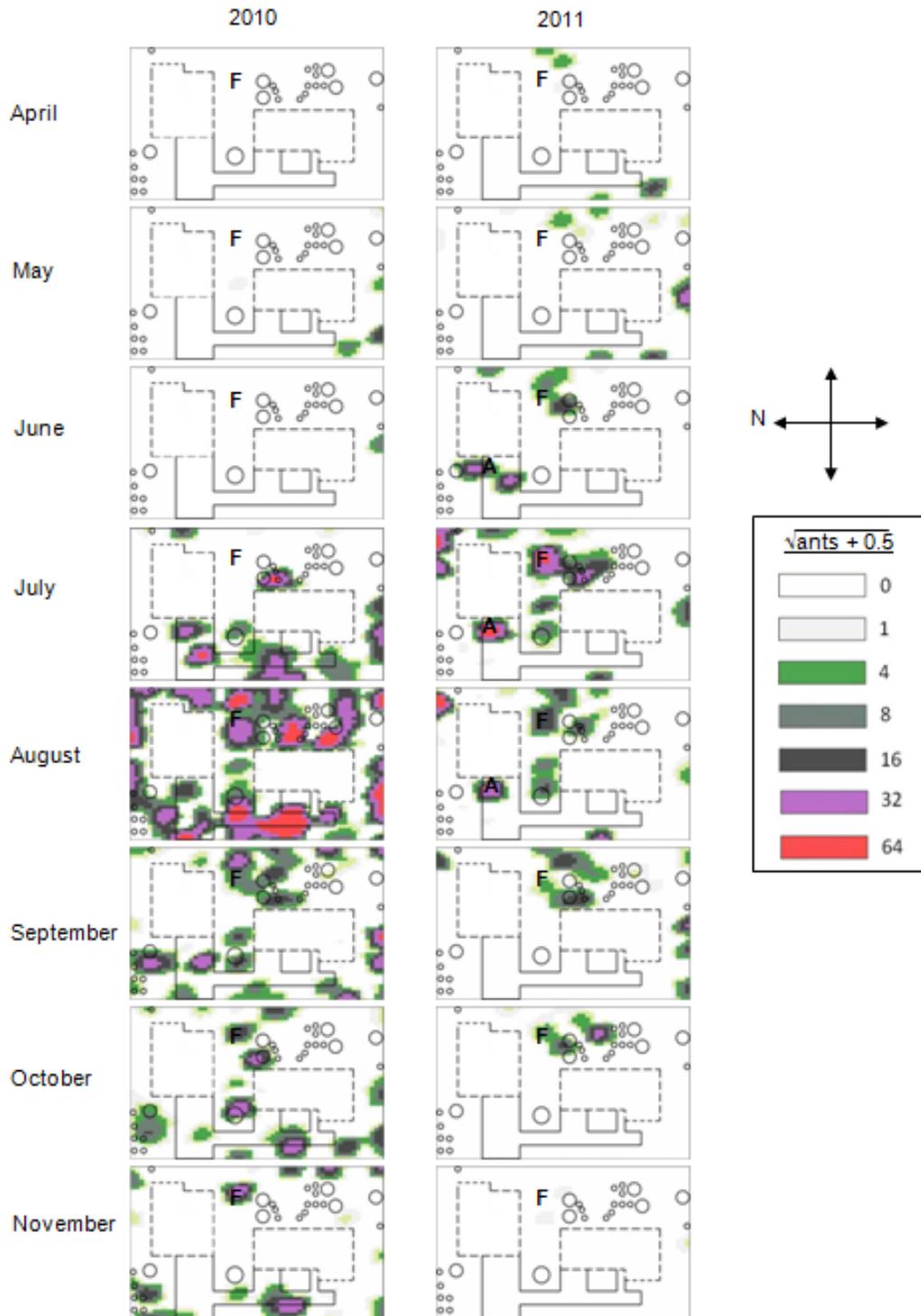


Figure 3-11. Spatial distribution of average monthly foraging counts ( $\sqrt{\text{ants} + 0.5}$ ) of *N. fulva* at Citra plot 3. Dashed lines represent buildings, solid lines represent driveways and roads, and circles represent trees and bushes. The size of the circle indicates the relative size of the tree/bush. The “F” represents the fire pit. In June, July, and August 2011, a cat feeding station was located at the site marked “A”.

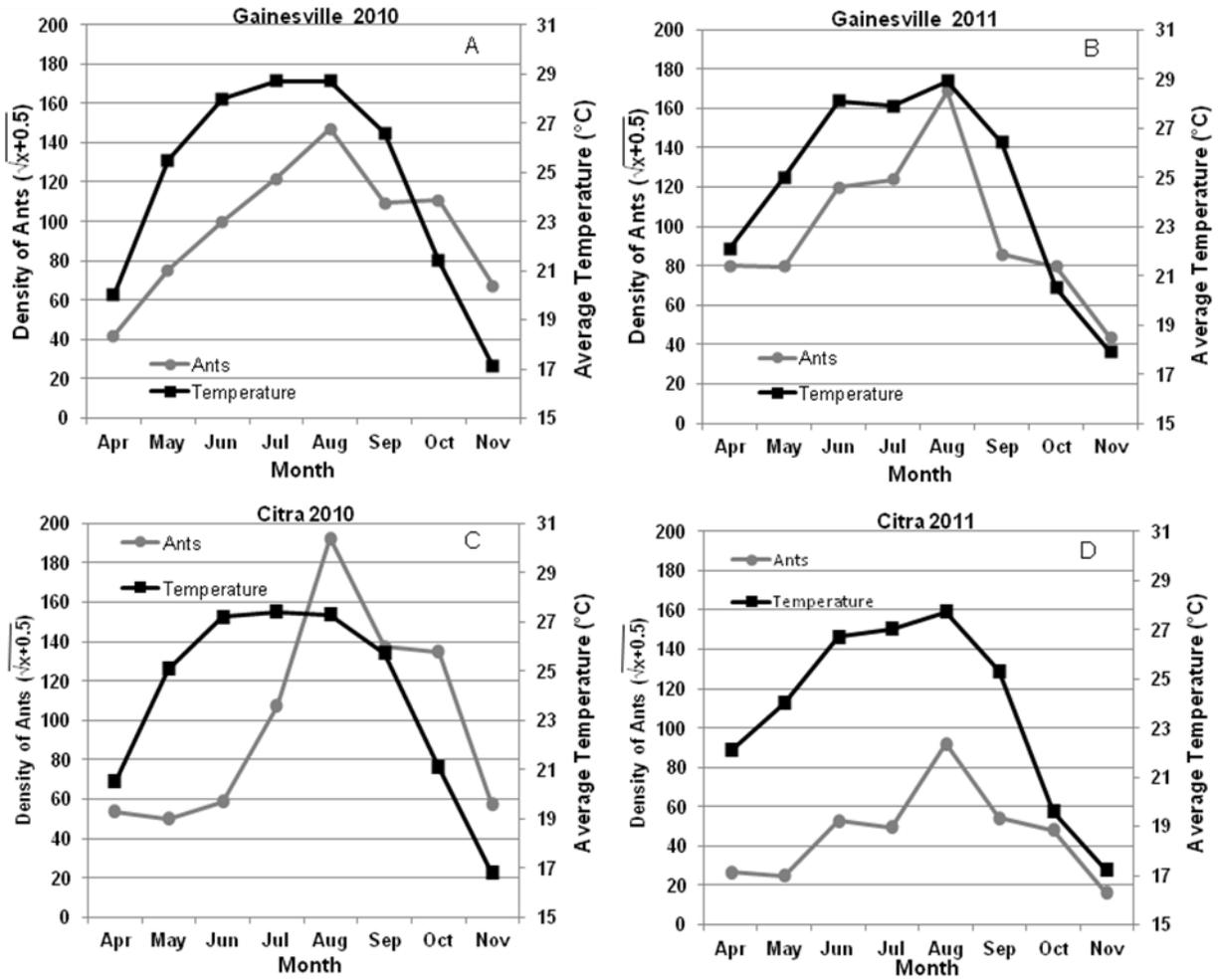


Figure 3-12. Mean monthly density of foraging *N. fulva* ( $\sqrt{\text{ants} + 0.5}$ ) and average temperature in Gainesville (A and B) and Citra (C and D) plots during 2010 (A and C) and 2011 (B and D).

CHAPTER 4  
AGGRESSIVE BEHAVIOR BETWEEN *NYLANDERIA FULVA* AND *SOLENOPSIS INVICTA* UNDER FIELD AND LABORATORY CONDITIONS

**Introduction**

The factors that direct the spread and success of invasive species are also critical in attempts to reduce their impact (Williamson 1996). There are numerous factors that allow invasive species to become dominant such as competitive abilities, resource allocation, lack of natural enemies, or human interaction. However, their competitive abilities are thought to be the reason invasive species are able to dominate native species to acquire territory and resources (Sax et al. 2007).

The *Solenopsis invicta* Buren and *Nylanderia fulva* (Mayr) are invasive species that were introduced from their native range of South America to the southern United States (Vinson 1997, Meyers 2008, Ascunce et al. 2011, MacGown 2012). *Solenopsis invicta* was introduced in the 1930s and has become established in the United States. It is not known when exactly *N. fulva* was introduced into the United States, but became a serious pest in the 2000's (Vinson 1997, MacGown 2010). Both species are known to displace native ants and other arthropod species as well as negatively affect vertebrate populations (Zenner-Polania 1990a, Zenner-Polania 1994, Aldana et al. 1995, Tschinkel 2006, Holway et al. 2002, LeBrun et al. 2012). In *S. invicta* and *N. fulva*'s native range of Brazil, their competitive behavior is not known.

Competition, as defined by Miller (1967) is “the active demand by two or more individuals of the same species (intraspecies competition) or members of two or more species (interspecies competition) at the same trophic level for a common resource or requirement that is actually or potentially limiting”. Competition then only comes about when there is a shortage or potential shortage of one or more resources. The

aggressive behavior between ant species is based on competition and is adaptive depending on the situation (Wilson 1970). In this study, the individual, small group, large group, and colony aggressive interactions as well as numerical dominance between *N. fulva* and *S. invicta* were examined to better understand each species' competitive strategies.

## **Materials and Methods**

### **Collection and Maintenance of Laboratory Colonies**

*Nylanderia fulva* were collected from two different locations, approximately 18 km apart, within Gainesville, FL: SW Depot Avenue (GNV1) and NW 50<sup>th</sup> Avenue (GNV2). *Solenopsis invicta*, were collected on the campus of the University of Florida at the Urban Entomology building in Gainesville, FL (UEL) and at the Vikal Building Complex in Citra, FL (VBC).

For *N. fulva*, single stock colonies consisting of 3,000-5,000 workers, 5-10 queens, and numerous brood were established. Single stock colonies of *S. invicta* were established consisting of 3,000-10,000 workers, 1 queen, and numerous brood. Colonies of both species were maintained in Fluon® (BioQuip Products, Rancho Dominguez, CA) coated trays containing nest cells composed of plaster-filled (Dentsply, York, PA) plastic Petri dishes (11 x 15 mm). Red acetate paper covered the nest cells to filter light and make it more attractive for brood rearing. Both species were provided *ad libitum* with water, 10% sugar water, and a variety of food including freshly killed American cockroaches (*Periplaneta americana*), hardboiled egg, orange, and honey. All colonies were maintained at  $27 \pm 1.5^\circ\text{C}$ ,  $40 \pm 7\%$  RH, and a 12:12 L:D cycle.

## **Aggression Test**

The level of aggression between four colony pairs was assessed (GNV1-UEL, GNV1-VBC, GNV2-UEL, and GNV2-VBC) using four different aggression bioassays similar to those designed by Buczkowski and Bennett (2008): a) worker dyadic interactions in a neutral arena, b) small worker group interactions in a neutral arena, c) large worker group interactions in a neutral arena, and d) intruder into an established resident territory. Because *S. invicta* workers are polymorphic while *N. fulva* workers are monomorphic, *S. invicta* workers chosen were approximately the same size as the *N. fulva* workers. An individual ant was not tested in more than one trial.

## **Dyadic Interactions**

Two workers of similar size, one from each stock colony, were selected at random on a wooden dowel ([152.5 mm x 2 mm] Fisher Brand, Pittsburg, PA) and placed into a plastic soufflé cup (30 ml [1 oz, Dart Manufacturing Company, Mason, MI]). Soufflé cups were coated with Fluon® within 1 cm of the bottom. This restricted the ants to a small area, maximizing the chance of the ants finding each other. The first interactions were scored on a 1-4 scale (Suarez et al. 2002, Buczkowski and Bennett 2008) [1= ignore, 2= avoid, 3=aggression (lunging, biting, and/or pulling- physical), 4= fighting (prolonged aggression and/or use of defensive compounds- physical and/or chemical)]. Only the first interaction was recorded to avoid compounding effects of more than one interaction (Buczkowski and Silverman 2005). The initiator was defined as the ant that made contact first. The interaction score was based on the initiator. Ten replicates were performed (Suarez et al. 2002, Buczkowski and Bennett 2008) for each colony pairing. For each replicate, the first interaction initiator, reaction or avoidance of the initiator, and the interaction score of the initiator were recorded.

A Chi Square test was performed in the JMP statistical package (SAS Institute 2012b) to determine if *N. fulva* and *S. invicta* were distributed differently as first interaction initiator and if the initiator showed a reaction or avoidance. The interaction score of the initiator for *N. fulva* and *S. invicta* were compared using a one-way ANOVA using JMP (SAS Institute 2012b).

### **Small Worker Group Interactions in a Neutral Arena**

Twenty workers of each species of similar size were randomly selected and transferred to plastic, Fluon®-coated vials (3 cm diameter, 8.5 cm high [Thornton Plastic Co. Salt Lake City, UT]). The ants were allowed to calm for 5 min, after which the two groups were combined by gently emptying the ants from the vials into a plastic Fluon®-coated arena (9 x 9 x 1.5 cm<sup>3</sup> high [Fisher Scientific, Pittsburgh, PA]). Three replicates for each of the four colony pairings were performed. The number of ants fighting (aggression level 3 or higher) was recorded at 1, 5, 10, 20, 40, 60 min and every hour until 8 h after the two species were combined. For each time point, the number of ants fighting, number of ants dead, and the ratio of workers of each species involved in fights were recorded.

To test for differences in survival between colony pairing and species, the univariate survival analysis from the JMP statistical package (SAS Institute 2012b) was used to fit the survival distribution to a linear model. A probit analysis was used to determine time to mortality for each species. The proportion of *N. fulva* and *S. invicta* dead at each sample time was compared using a one-way ANOVA and means separated by Tukey test. A regression analysis (SAS Institute 2012b) was used to examine the effects time and species on the number of ants fighting with a *P*-value of <0.05 to establish statistical differences. The proportion of *N. fulva* and *S. invicta*

fighting at each sample time was compared using a one-way ANOVA and means separated by Tukey test.

### **Large Worker Group Interactions in a Neutral Arena**

One hundred and fifty workers of each species of similar size were randomly selected and transferred to plastic, Fluon®-coated vials (4.5 cm diameter, 7.6 cm high [Fisher Scientific, Pittsburgh, PA]). The ants were allowed to calm for 5 min, after which the two groups were combined by gently emptying the ants from the vials into a plastic Fluon®-coated arena (23 x 23 x 2 cm<sup>3</sup> high [Fisher Scientific, Pittsburgh, PA]). Three replicates for each of the four colony pairings were performed. Data were collected and analyzed as described for small group interactions.

### **Intruder into an Established Resident Territory**

Individual intruder workers were collected on a wooden dowel ([152.5 mm x 2 mm] Fisher Brand, Pittsburg, PA) and introduced into the rearing trays (52 x 38 cm<sup>2</sup>) containing the stock resident colony of the opposite species. The initiator was defined as the ant that made contact first. The interactions score was based on the initiator. The first interactions were scored on a 1-4 scale (as previously described). Only the first interaction was recorded to avoid compounding effects of more than one interaction (Buczowski and Silverman 2005). The intruder was removed and discarded after each trial. Subsequent trials were performed when the resident colony was no longer visibly agitated (after approximately 10 min). Ten replicates with each of the four colony pairs were tested; five replicates with *N. fulva* workers acting as the intruder and five replicates with *S. invicta* workers acting as the intruder. For each replicate, the first interaction initiator, reaction or avoidance of the initiator, the interaction score for the

initiator, and the maximum number of workers collaborating to kill the intruder were recorded.

A Chi Square test was performed in the JMP statistical package (SAS Institute 2012b) to determine if *N. fulva* and *S. invicta* were distributed differently as first interaction initiator when residents and if the initiator showed a reaction or avoidance. The interaction score of the initiator and the number of resident colony collaborators for *N. fulva* and *S. invicta* were compared using one-way analysis of variance (ANOVA) using JMP (SAS Institute 2012b).

### **Field Study**

Data used was collected from field populations of ants as described in chapter 3. The ants were identified as *N. fulva*, *S. invicta*, *Pheidole* spp., *Dorymyrmex* spp., or others, counted, and square root transformed for each sampling time in 2010 and 2011 for each lot sampled. In order to determine if *N. fulva* was the dominant species at each of the lots, all groups identified except *N. fulva* were summed for each year and the sums compared using one-way ANOVA. For the locations and years that had significant statistical difference ( $P < 0.05$ ) between *N. fulva* and the summed other ants, the percentage of each group was calculated to determine the dominant ant group.

## **Results**

### **Dyadic Interactions**

Of the 40 interactions, *N. fulva* initiated 72.5% (29/40) interactions compared to 27.5% (11/40) initiated by *S. invicta* ( $\chi^2 = 8.1$ ;  $df = 1$ ;  $P = 0.004$ ). Of the interactions *N. fulva* initiated, a reaction was observed (scored 3 or 4) 62% of the time ( $\chi^2 = 2.8$ ;  $df = 1$ ;  $P = 0.09$ ), while *S. invicta* was avoided 38% of the time. When *S. invicta* was the initiator, they reacted 64% of the time ( $\chi^2 = 0.8$ ;  $df = 1$ ;  $P = 0.37$ ). There was no

statistical difference between the frequency of interaction types for the two species ( $F = 1.6$ ;  $df = 1$ ;  $P = 0.21$ ) (Figure 4-1), with *N. fulva*'s mean interaction score at  $3.2 \pm 0.17$  and *S. invicta* at  $2.8 \pm 0.28$ .

### **Small Worker Group Interactions in a Neutral Arena**

There was no statistical difference in the number of *N. fulva* and *S. invicta* that died ( $\chi^2 = 4.6$ ;  $df = 1$ ;  $P = 0.20$ ) after small group interactions (Figure 4-2). At 10 min there was a significant difference ( $F = 6.7$ ;  $df = 1$ ;  $P = 0.012$ ) between the numbers of dead ants for *N. fulva* and *S. invicta*, but there was no significant difference between the numbers of dead ants for each species at any of the other sample points. There was no statistical difference ( $\chi^2 = 0.6$ ;  $df = 1$ ;  $P = 0.43$ ) in the time to mortality for *N. fulva* and *S. invicta* (Table 4-1).

Only time ( $F = 131.2$ ;  $df = 12$ ;  $P < 0.0001$ ) had an effect on the number of ants fighting. There were significantly more *N. fulva* fighting than *S. invicta* at 5 min ( $F = 4.4$ ;  $df = 1$ ;  $P = 0.047$ ) (Figure 4-3), but all of the other sample points, there was no difference between the numbers of each species fighting. After 120 min, fighting ceased. There was no significant difference ( $F = 0.3$ ;  $df = 1$ ;  $P = 0.55$ ) in the number of ants cooperating in fighting. On average,  $2.3 \pm 0.28$  *N. fulva* workers cooperated in attacking *S. invicta* workers while  $2.0 \pm 0.28$  *S. invicta* workers cooperated in attacking *N. fulva* workers.

### **Large Worker Group Interactions in a Neutral Arena**

More *S. invicta* died than *N. fulva* ( $\chi^2 = 540.0$ ;  $df = 1$ ;  $P < 0.0001$ ) in large group interactions (Figure 4-4). At 1 ( $F = 1.0$ ;  $df = 1$ ;  $P = 0.33$ ), 5 ( $F = 0.1$ ;  $df = 1$ ;  $P = 0.75$ ), and 10 ( $F = 2.1$ ;  $df = 1$ ;  $P = 0.16$ ) min there were no significant differences between the numbers of ants dead for each species, but at all other times sampled, there were

significant differences (20 min [ $F = 5.7$ ;  $df = 1$ ;  $P = 0.03$ ], 40 min [ $F = 18.4$ ;  $df = 1$ ;  $P = 0.0003$ ], 60 min [ $F = 25.7$ ;  $df = 1$ ;  $P < 0.0001$ ], 120 min [ $F = 51.4$ ;  $df = 1$ ;  $P < 0.0001$ ], 180 min [ $F = 63.6$ ;  $df = 1$ ;  $P < 0.0001$ ], 240 min [ $F = 73.6$ ;  $df = 1$ ;  $P < 0.0001$ ], 300 min [ $F = 87.8$ ;  $df = 1$ ;  $P < 0.0001$ ], 360 min [ $F = 70.7$ ;  $df = 1$ ;  $P < 0.0001$ ], 420 min [ $F = 67.7$ ;  $df = 1$ ;  $P < 0.0001$ ], 480 min [ $F = 68.7$ ;  $df = 1$ ;  $P < 0.0001$ ]). (Figure 4-5). *Nylanderia fulva* had a faster time to 1% mortality than *S. invicta* (Table 4-2). At all other mortality levels, *S. invicta* had a faster time to mortality than *N. fulva*.

Time ( $F = 118.86$ ;  $df = 12$ ;  $P < 0.0001$ ), ant species ( $F = 28.84$ ;  $df = 1$ ;  $P < 0.0001$ ), and the interaction (time x species) ( $F = 4.43$ ;  $df = 12$ ;  $P < 0.0001$ ) had significant effects on the number of ants fighting. There were significantly more *N. fulva* fighting than *S. invicta* at 1 ( $F = 7.3$ ;  $df = 1$ ,  $P = 0.012$ ), 5 ( $F = 8.9$ ;  $df = 1$ ;  $P = 0.007$ ), and 10 ( $F = 9.8$ ;  $df = 1$ ;  $P = 0.005$ ) min (Figure 4-5), but not at the other sample times. After 240 min, there were no ants fighting. There was a significant difference ( $F = 5.1$ ;  $df = 1$ ;  $P = 0.03$ ) in the number of ants cooperating in fighting. On average,  $9.6 \pm 0.88$  *N. fulva* workers cooperated in attacking *S. invicta* workers while  $6.8 \pm 0.88$  *S. invicta* workers cooperated in attacking *N. fulva* workers.

### **Intruder into an Established Resident Territory**

When *N. fulva* was the resident, they initiated the interaction and reacted 100% (20/20) of the time. When *S. invicta* was the resident, there was no observed statistical difference between the two species as the initiator ( $\chi^2 = 1.8$ ;  $df = 1$ ;  $P = 0.18$ ), and *N. fulva* initiated 65% (13/20) of the interactions whereas *S. invicta* initiated 35% (7/20) of the interactions. Of the 65% of the interactions *N. fulva* initiated when *S. invicta* was the resident, *N. fulva* reacted (scored 3 or 4) 92.3% (12/13) of the time and avoided (scored 1 or 2) 7.7% (1/13) of the time. When *S. invicta* was the resident and initiator, they

always reacted (scored 3 or 4). There was no statistical difference between the form of interaction when *N. fulva* and *S. invicta* were compared ( $F = 1.6$ ;  $df = 1$ ;  $P = 0.2$ ) (Figure 4-6), with only a slight difference in mean interaction score between the species ( $3.7 \pm 0.34$  for *S. invicta* and  $3.2 \pm 0.16$  for *N. fulva*).

*Nylanderia fulva* and *S. invicta* workers cooperated in fighting the opposing species ( $F = 33.3$ ,  $df = 1$ ,  $P < 0.0001$ ). *Nylanderia fulva* workers fought in groups in 100% (20/20) of the encounters, whereas *S. invicta* workers received little help from their nestmates and fought in groups only in 30% (6/20) of the encounters. On average,  $6.7 \pm 0.59$  *N. fulva* workers attacked an intruding *S. invicta* worker (range 2-14 workers). In contrast, only  $1.9 \pm 0.59$  *S. invicta* workers attacked an intruding *N. fulva* worker (range 1-6 workers).

### Field Study

*Nylanderia fulva* was clearly the numerically dominant ant species at 4 of the 6 lots sampled for both years ([Citra 1, 2010:  $F = 22.7$ ;  $df = 1$ ;  $P < 0.0001$ ], [Citra 1, 2011:  $F = 22.9$ ;  $df = 1$ ;  $P < 0.0001$ ], [Gainesville 1, 2010:  $F = 36.3$ ;  $df = 1$ ;  $P < 0.0001$ ], [Gainesville 1, 2011:  $F = 18.0$ ;  $df = 1$ ;  $P = 0.0002$ ], [Gainesville 2, 2010:  $F = 89.4$ ;  $df = 1$ ;  $P < 0.0001$ ], [Gainesville 2, 2011:  $F = 148.9$ ;  $df = 1$ ;  $P < 0.0001$ ], [Gainesville 3, 2010:  $F = 98.8$ ;  $df = 1$ ;  $P < 0.0001$ ], [Gainesville 3, 2011:  $F = 55.7$ ;  $df = 1$ ;  $P < 0.0001$ ] (Figure 4-7). At Citra lot 2 in 2010, *N. fulva* was the dominant species compared to the summed other ants, but not significantly ( $F = 3.6$ ;  $df = 1$ ;  $P = 0.07$ ) (Figures 4-7 and 4-8). Through the all of the seasons in 2010, *N. fulva* was the numerically dominant species (Figure 4-8). In 2011 at Citra lot 2, the summed other ants were dominant ( $F = 28.8$ ;  $df = 1$ ;  $P < 0.001$ ) (Figures 4-7 and 4-8). Overall, the dominant ant was *S. invicta* (33.6%) followed by, *Pheidole* spp. (31.0%), *Dorymyrmex* spp. (17.6%), *N. fulva* (15.5%) and

others (2.3%) (Figure 4-8). The numerically dominant species changed every season (4-8). In the spring, *Pheidole* spp. were dominant and then *S. invicta* in the summer. In the fall, *Dorymyrmex* spp. was dominant. In 2010 at Citra lot 3, the summed other ants were dominant, but not significantly ( $F = 2.7$ ;  $df = 1$ ;  $P = 0.11$ ) (Figures 4-7 and 4-9). Overall, *S. invicta* (43.8%) and *N. fulva* (41.8%) were the numerically dominant species (Figure 4-9). In the spring, *S. invicta* was numerically dominant but the numerical dominance was reduced in the summer (Figure 4-9). In the fall, *N. fulva* was the numerically dominant species, followed by *S. invicta*, *Pheidole* spp., *Dorymyrmex* spp. and others (Figure 4-9). At Citra lot 3 in 2011, the summed other ants were significantly dominant ( $F = 62.9$ ;  $df = 1$ ;  $P < 0.0001$ ) (Figures 4-7 and 4-9). Overall, the dominant ant was *S. invicta* (82.8%) followed by *Pheidole* spp. (8.3%), *N. fulva* (7.9%), others (0.6%) and *Dorymyrmex* spp. (0.4%) (Figure 4-9). *Solenopsis invicta* was numerically dominant in every season. In the spring, *Pheidole* spp. was numerically dominant over *N. fulva*, but the dominance switched in the summer and continued in the fall (Figure 4-9).

## Discussion

In order to understand *N. fulva* and *S. invicta*'s competitive ability, aggressive interactions were examined. The use of aggression is dictated by competition for resources (Wilson 1970). In controlled arena bioassays, the tendency of individuals to fight becomes more evident. Results from dyadic interactions suggest that *N. fulva* workers have a higher tendency to fight even without the numerical advantage when compared to *S. invicta*. However, workers from both species were observed trying to escape from the arena, suggesting that self preservation may represent a stronger impulse than aggressive behavior in unfamiliar territory.

As group size increased, *N. fulva*'s tendency to fight increased, as predicted by the cost minimizer hypothesis (Buczkowski and Bennett 2008). The presence of nestmates increases the aggressiveness of individuals because of shared risk (Buczkowski and Bennett 2008). In small group interactions, more *N. fulva* engaged in fighting than *S. invicta*. It was not until 20 minutes into the interaction period that more *S. invicta* were fighting than *N. fulva*, but this occurred when there were more *N. fulva* dead than *S. invicta*. Cooperative fighting was not significant in small group interactions, and after five hours, all of the ants in both species were dead. These results suggest that individuals of both species were more motivated by self preservation than protection of small groups, as observed in dyadic interactions.

In large group interactions, cooperative fighting was a significant factor in determining the dominant species. There were more *N. fulva* workers fighting throughout the large group interaction study. *Nylanderia fulva* fought by projecting formic acid from the acidopore (MacGown 2012) and by pulling on *S. invicta*'s appendages. The release of formic acid may also act as an alarm pheromone, eliciting defensive responses from other *N. fulva* workers. *Nylanderia fulva* was able to create the numerical advantage by attacking in groups and systematically killing *S. invicta* creating a numerical advantage. This attack strategy is also consistent with the cost minimizer hypothesis and increases the likelihood of winning the fight. Fighting in groups may be one of the tactics *N. fulva* uses to outcompete other ant species and invade new areas. How individual *N. fulva* assessed group size in order to decide to fight or not is unknown. Most ants use semiochemicals, and those semiochemicals increase as group size increases (Buczkowski and Silverman 2005, Buczkowski and

Bennett 2008). Cooperative fighting was not seen as readily in *S. invicta*, even though they can release an alarm pheromone from their mandibular glands that induces cooperative fighting (Tschinkel, 2006). *Solenopsis invicta* workers fought more individually, pulling on appendages and acting defensively with their stinger.

Whole colony defensive ability was tested with the introduction of a worker to resident colonies of different species. *Nylanderia fulva* workers were highly aggressive when *S. invicta* workers were introduced into their colony and always initiated the first encounter. This suggests that *N. fulva* has strong nestmate cognition cues that allow them to quickly detect intruders. Upon detecting the intruder, *N. fulva* workers would project formic acid and pull appendages. The cost minimizer hypothesis was supported in *N. fulva*'s reaction to intruders by cooperative fighting and high aggression displayed toward intruders. When a *N. fulva* worker was introduced to a *S. invicta* colony, *N. fulva* initiated most of the interactions and reacted. Even with the numerical disadvantage, *N. fulva* engaged in fighting suggesting a high willingness to enter into aggression. When *S. invicta* was the initiator to the introduced *N. fulva*, *S. invicta* reacted to protect their colony from the intruder. The cost minimizer hypothesis was not supported by *S. invicta*'s behavior because cooperative fighting was not observed. Overall, both species would aggressively protect their colony in the case of an intruder, most likely killing the intruder and preventing recruitment workers of the opposite species.

The aggressive behavior in animals is often described Lanchester's (1916) *Theory of Combat* (Franks and Partridge 1993, Whitehouse and Jaffè 1996, Buczkowski and Bennett 2008). The square law and linear law are two combat models presented in the *Theory of Combat* that are often used when describing ant aggression (Franks and

Partridge 1993, Whitehouse and Jaffè 1996, Buczkowski and Bennett 2008). The square law states that the side with the most individual fighters wins the battle no matter the fighting ability of individual ants (Lanchester 1916, Whitehouse and Jaffè 1996). By creating the numerical advantage in large group interactions, *N. fulva* followed the square law model. The linear law focuses more on the individual fighter; if a fight is a series of one-on-one battles, a few good fighting individuals is better than many poor fighting individuals (Lanchester 1916, Whitehouse and Jaffè 1996). *Solenopsis invicta* fought individually more than in groups implying that individual power is favored over group size. Plowes and Adams (2005) analyzed the mortality rates of *S. invicta* fighting in different numerical ratios, and found that *S. invicta* follow the linear law.

Both Meyers (2008) and LeBrun et al. (2012) present primary evidence that *N. fulva* displaces *S. invicta* from overlapping invasive areas. *Nylanderia fulva* is a polygynous ant and colonies can reach tremendous numbers (Zenner-Polania 1990a, Meyers 2008, Valles et al. 2012). Given the high number of *N. fulva* workers, it is hypothesized that they follow the square law. *Solenopsis invicta* has the ability to form monogyne or polygyne colonies (Vinson 1997). Depending on the type of colony, would dictate the combat strategy. Monogyne *S. invicta* colonies would most likely follow the linear law while polygyne colonies would most likely follow the square law.

In the field study, at four of the six locations sampled, *N. fulva* was the numerically dominant species implying that *N. fulva* were able to out compete the other ant species for resources, because competition is density-dependent (Wilson 1970). The numerical dominance then suggests the use of the square law of combat as seen in the large group interaction.

Studies on a similar super colony ant species, *Linepithema humile* (Mayr), provided evidence of decreased prey availability during the course of an invasion (three years), suggesting that food availability is a limiting factor in ant density, and *L. humile* reached the carrying capacity for the invaded area (Ingram 2002). *Nylanderia fulva* has the ability to maintain high worker densities enabling them to be strong competitors and effective invaders, but high worker density can lead to quickly reaching the carrying capacity of an invaded area. At the four locations that *N. fulva* was numerically dominant, it is likely that the carrying capacity had not been met.

At Citra lot 2, *N. fulva* was the numerically dominant species in 2010, suggesting that *N. fulva* would be the numerically dominant species in 2011. From fall of 2010 to spring of 2011, the dominance changed from *N. fulva* to *Pheidole* spp. The numerical dominance was dynamic over each season suggesting competition for resources. Most likely, *N. fulva* had reached the carrying capacity at Citra lot 2. Depleting food resource availability and less than ideal weather conditions in 2011 (chapter 3) allowed the other ant species to out compete *N. fulva* and become numerically dominant. The field dynamic between *N. fulva* and *S. invicta* was not evident at this lot.

The field dynamic between *N. fulva* and *S. invicta* at Citra lot 3 is more evident. In the spring 2010, *S. invicta* was numerically dominant. *Solenopsis invicta* nest in more sunlight areas allowing them to forage and begin reproducing earlier in the year. *Nylanderia fulva* overwinters in less sunlight areas, and do not begin to actively forage and begin reproducing until the temperatures increase in the late spring (chapter 3). In the summer, *N. fulva* can produce and maintain high worker densities, allowing them to outcompete *S. invicta* because of numerical dominance (square law of combat). In the

fall, this trend continued. At Citra lot 3, *N. fulva* reached its carrying capacity in fall 2010. The less than ideal weather conditions and with the low densities of *N. fulva* allowed *S. invicta* to out compete *N. fulva* for food resources and territory.

Numerical strength usually dictates the winner of fights between groups of animals (Adams 1990, Traniello and Bechers 1991), and is supported by individual group members' willingness and ability to fight (Lanchester 1916). The evidence that *N. fulva* displaces *S. invicta* from overlapping invasive areas is most likely in the beginning of an invasion by *N. fulva*. *Nylanderia fulva* increases in density, and uses the large densities to numerically dominant over other ant species. The high food resource availability in newly invaded areas allows the large densities of *N. fulva* to be sustained. As food resources begin to deplete, the large densities of *N. fulva* are not sustainable, thus other ant species are able to compete for resources and territory.

Both *N. fulva* and *S. invicta* are urban pests of serious concern. The natural competition between these two species can be used in helping control them. A specific treatment, such as that suggested by Kabashima et al. (2007) can be applied first against *S. invicta*, so *N. fulva* can help in eliminating the *S. invicta*, which is a more dangerous species in interactions with humans. A second treatment, applied a few days later can be more directly active against *N. fulva*, so the second pest ant species is eliminated. Such strategy would only be possible in locations where both species exist, and *N. fulva* has not yet become the dominant species. In locations where *N. fulva* is numerically dominant, a treatment specific for *N. fulva* would first be applied reducing the ants' density. A reduction in *N. fulva* density would allow other ant species to begin to competing for food resources and territory, maintain low *N. fulva* densities.

Table 4-1. Mean time and 95% confidence interval to 1, 10, 50, 90, and 99 percent mortality of *Nylanderia fulva* and *Solenopsis invicta* in small group (20 individuals) interactions.

Mortality (%)	Mean Time in Min (CI 95)	
	<i>N. fulva</i>	<i>S. invicta</i>
1	1.8 (1.08-3.09)	2.2 (1.68-2.94)
10	5.8 (4.08-8.28)	6.8 (5.62-8.18)
50	23.9 (18.47-31.03)	26.6 (23.18-30.54)
90	98.6 (67.12-144.81)	104.4 (84.79-128.58)
99	312.56 (178.22-548.19)	318.3 (234.64-431.84)

Table 4-2. Mean time and 95% confidence interval to 1, 10, 50, 90 of *Nylanderia fulva* and *Solenopsis invicta* in large group (150 individuals) interactions. The predictions to 90% mortality for *N. fulva* and 99% mortality for both species mortality were exponentially outside of the sampled times, thus a very weak representation of actual time to mortality.

Mortality (%)	Mean Time in Min (CI 95)	
	<i>N. fulva</i>	<i>S. invicta</i>
1	1.3 (1.06-1.71)	3.7 (3.32-4.07)
10	14.6 (12.59-16.97)	12.1 (11.28-13.00)
50	272.6 (242.0-307.16)	52.26 (49.58-55.09)
90	*	225.6 (210.07-242.18)

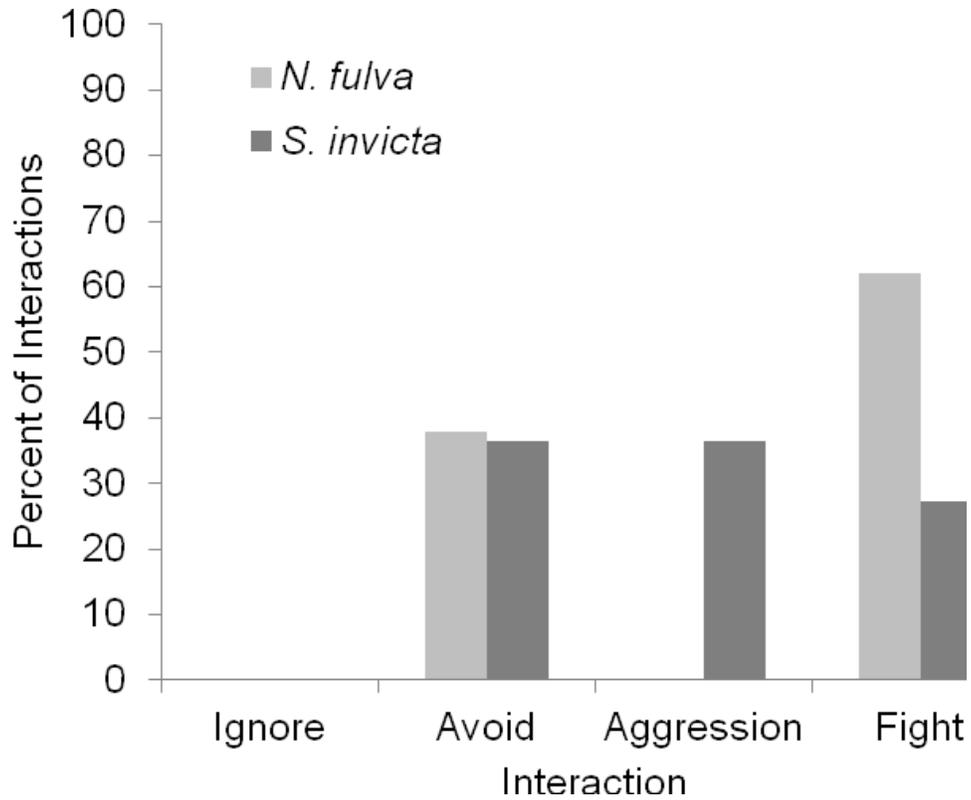


Figure 4-1. Frequency of interaction types when *N. fulva* and *S. invicta* were the initiators of the interactions when introduced to ant of the opposite species in dyadic interactions.

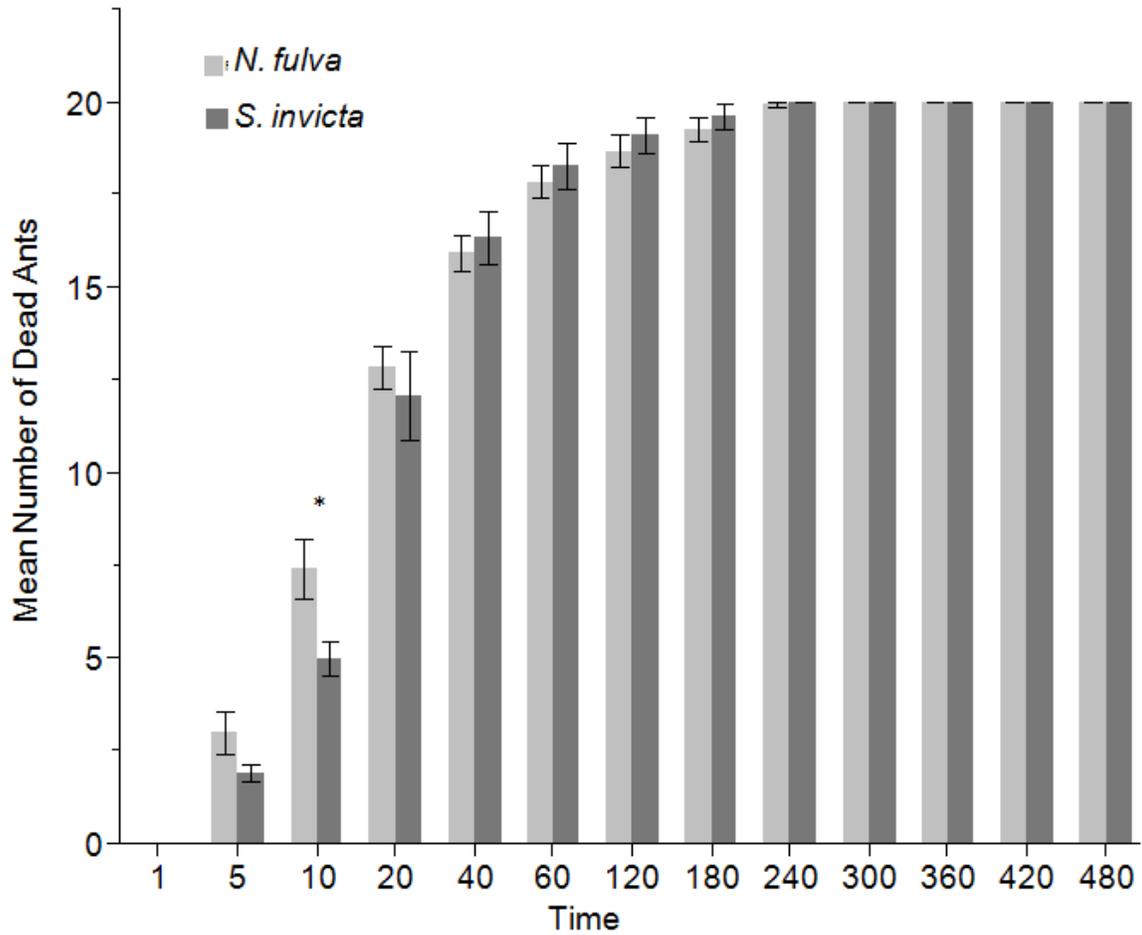


Figure 4-2. Mean number of *N. fulva* and *S. invicta* dead at sample times in small group (20 individuals of each species) interactions. Column pairs topped with an asterisk are significantly different ( $F = 6.7$ ;  $P = 0.012$ ) from each other. Each error bar is represents  $\pm 1$  standard error from the mean.

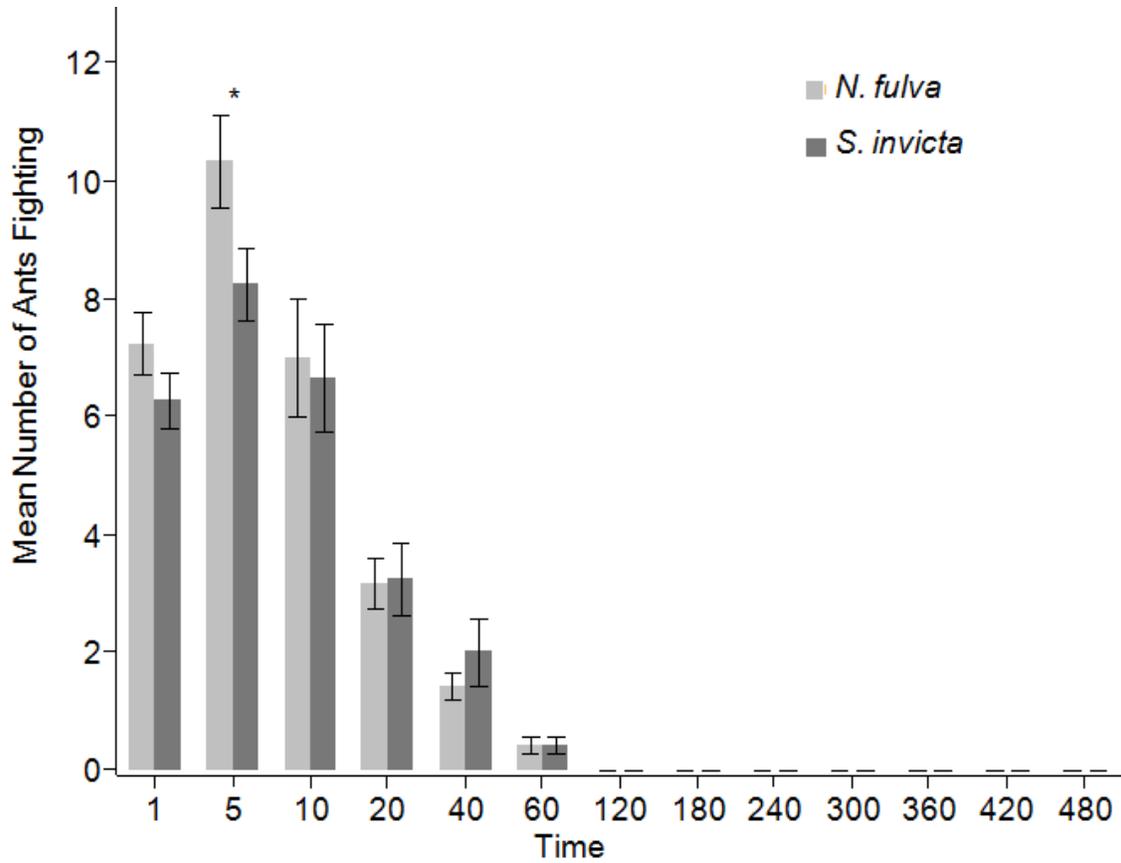


Figure 4-3. Number of ants fighting at each sample time for small group (20 individuals) interactions. Column pairs topped with an asterisk are significantly different ( $F = 4.4$ ;  $P = 0.047$ ) from each other. Each error bar is represents  $\pm 1$  standard error from the mean.

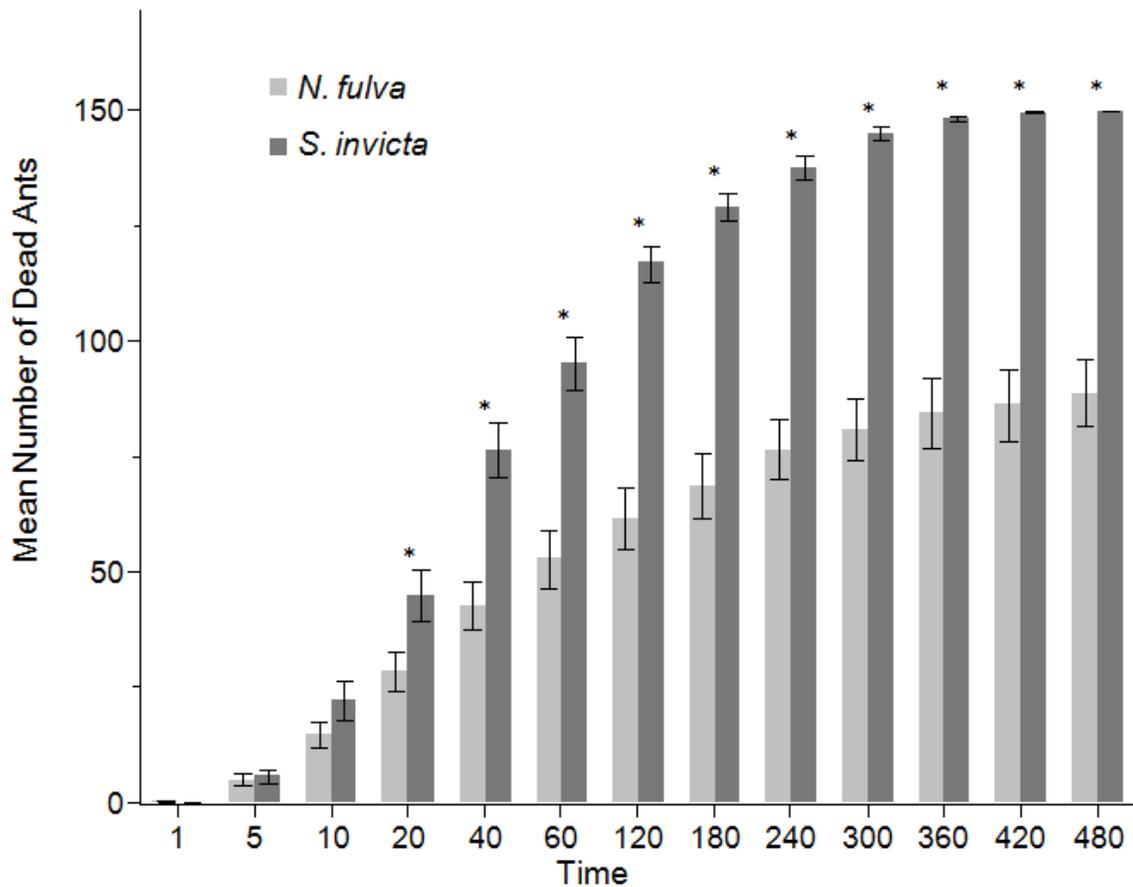


Figure 4-4. Mean number of *N. fulva* and *S. invicta* dead at each sample point for large group (150 individuals) interactions. Column pairs topped with an asterisk are significantly different from each other (20 min [ $F = 5.7$ ;  $P = 0.03$ ], 40 min [ $F = 18.4$ ;  $P = 0.0003$ ], 60 min [ $F = 25.7$ ;  $P < 0.0001$ ], 120 min [ $F = 51.4$ ;  $P < 0.0001$ ], 180 min [ $F = 63.6$ ;  $P < 0.0001$ ], 240 min [ $F = 73.6$ ;  $P < 0.0001$ ], 300 min [ $F = 87.8$ ;  $P < 0.0001$ ], 360 min [ $F = 70.7$ ;  $P < 0.0001$ ], 420 min [ $F = 67.7$ ;  $P < 0.0001$ ], 480 min [ $F = 68.7$ ;  $P < 0.0001$ ]). Each error bar is represents  $\pm 1$  standard error from the mean.

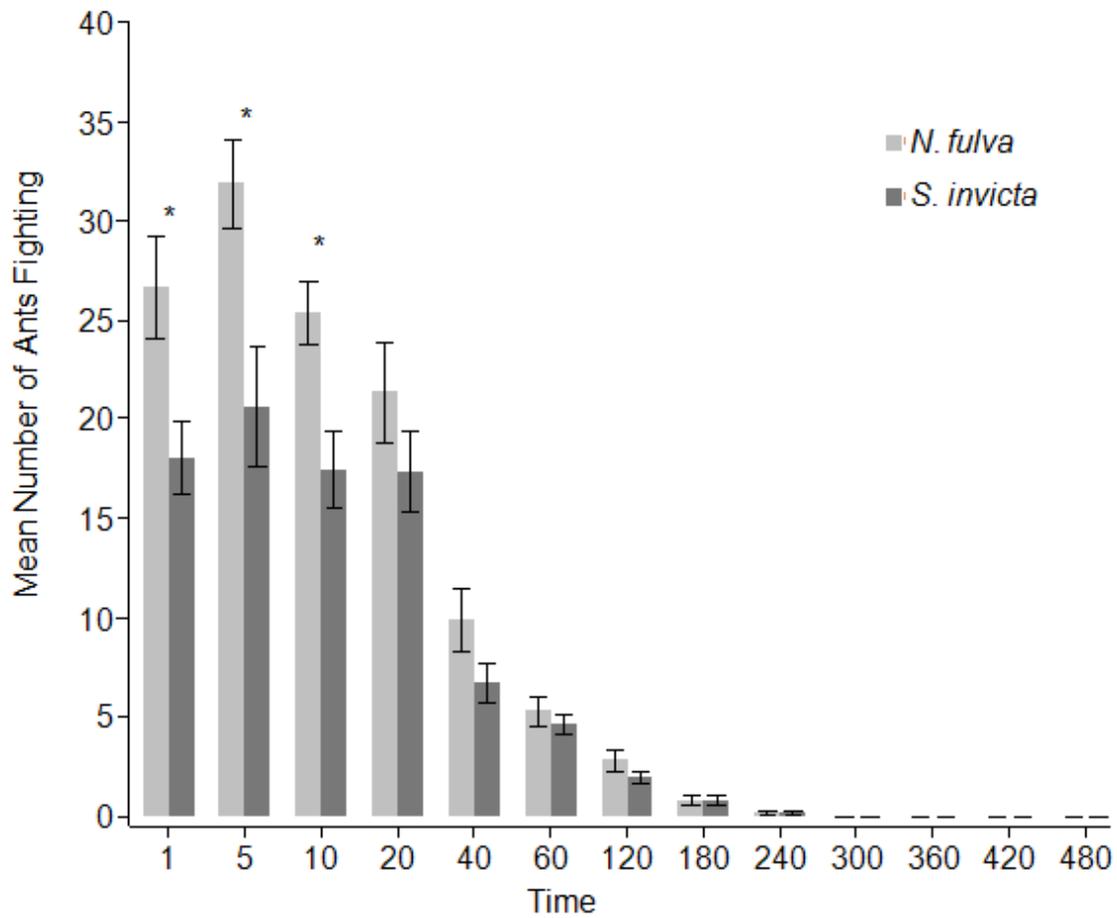


Figure 4-5. Number of ants fighting at each sample time in large group (150 individuals) interactions. Column pairs topped with an asterisk are significantly different from each other (1 min [ $F = 7.3$ ;  $P = 0.012$ ], 5 min [ $F = 8.9$ ;  $P = 0.007$ ], 10 min [ $F = 9.8$ ;  $P = 0.005$ ]). Each error bar is represent  $\pm 1$  standard error from the mean.

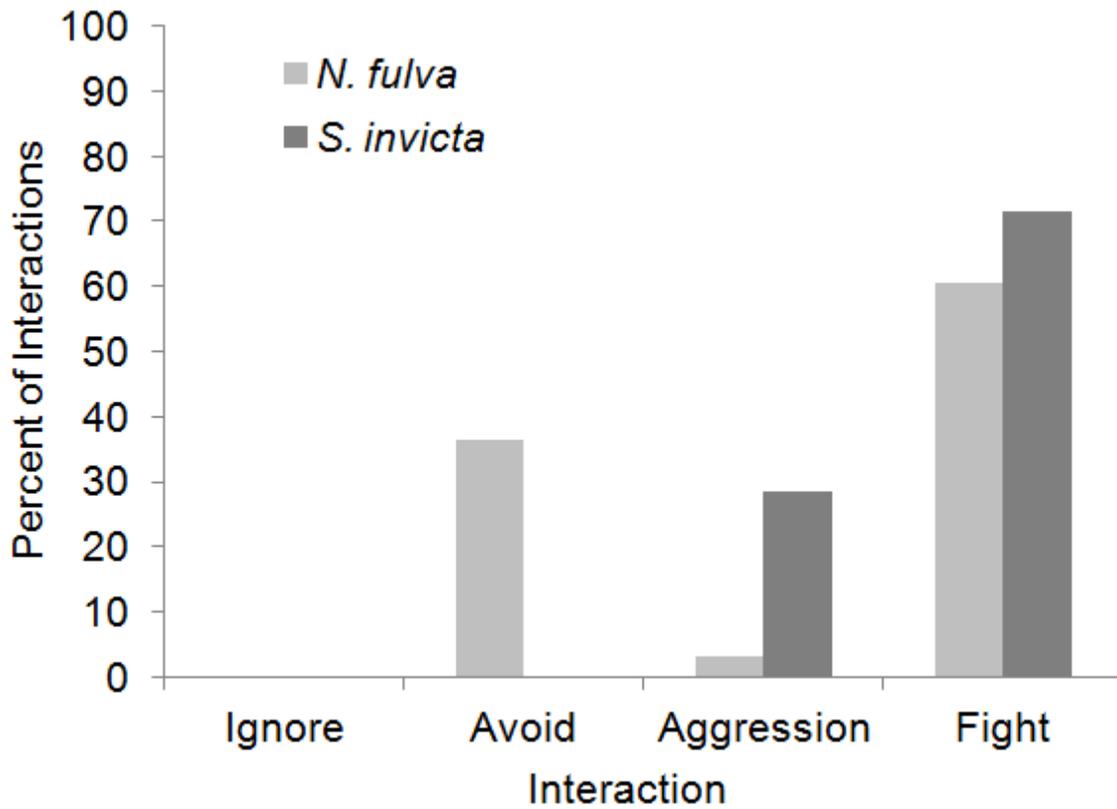


Figure 4-6. Frequency of interaction types when *N. fulva* and *S. invicta* were the initiators of the interactions after being introduced into resident colonies of opposite species.

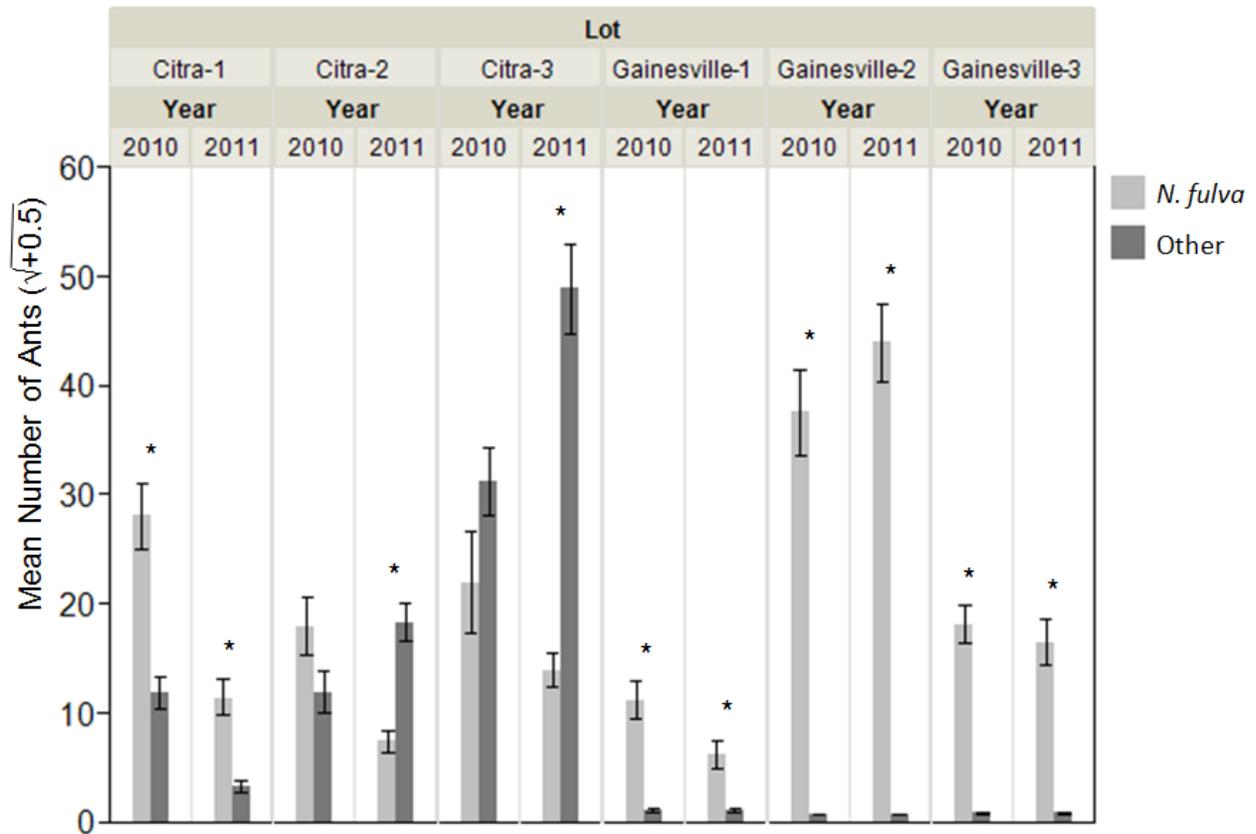


Figure 4-7. The mean number ( $\sqrt{x+0.5}$ ) of *N. fulva* and the summed other ants at each sampling plot for 2010 and 2011. The asterisks represent a significant difference between *N. fulva* and all the other ant species collected (*F*-test, *P* < 0.05). Each error bar is represents  $\pm 1$  standard error from the mean.

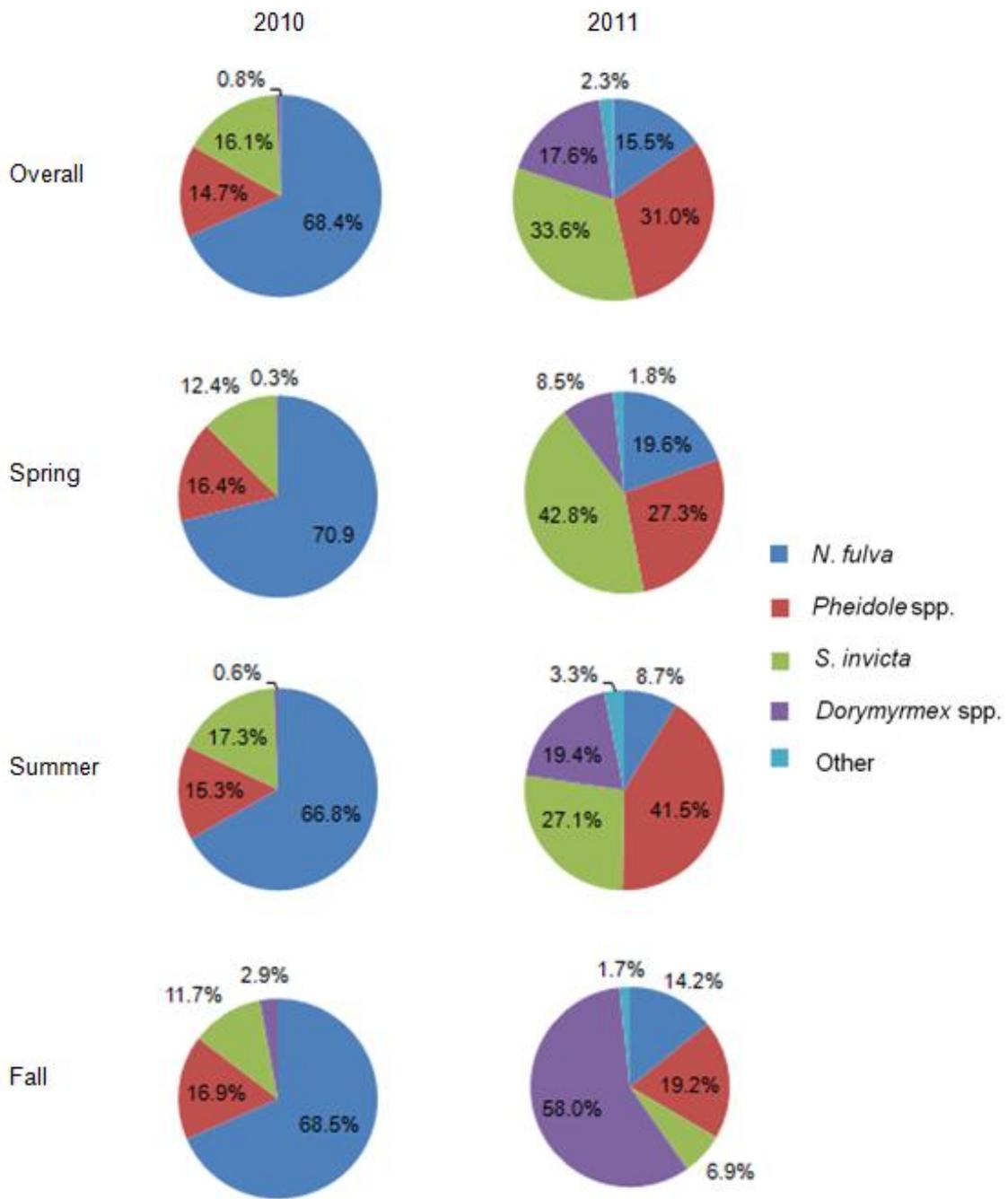


Figure 4-8. The ratio of ants found at Citra lot 2 in 2010 and 2011.

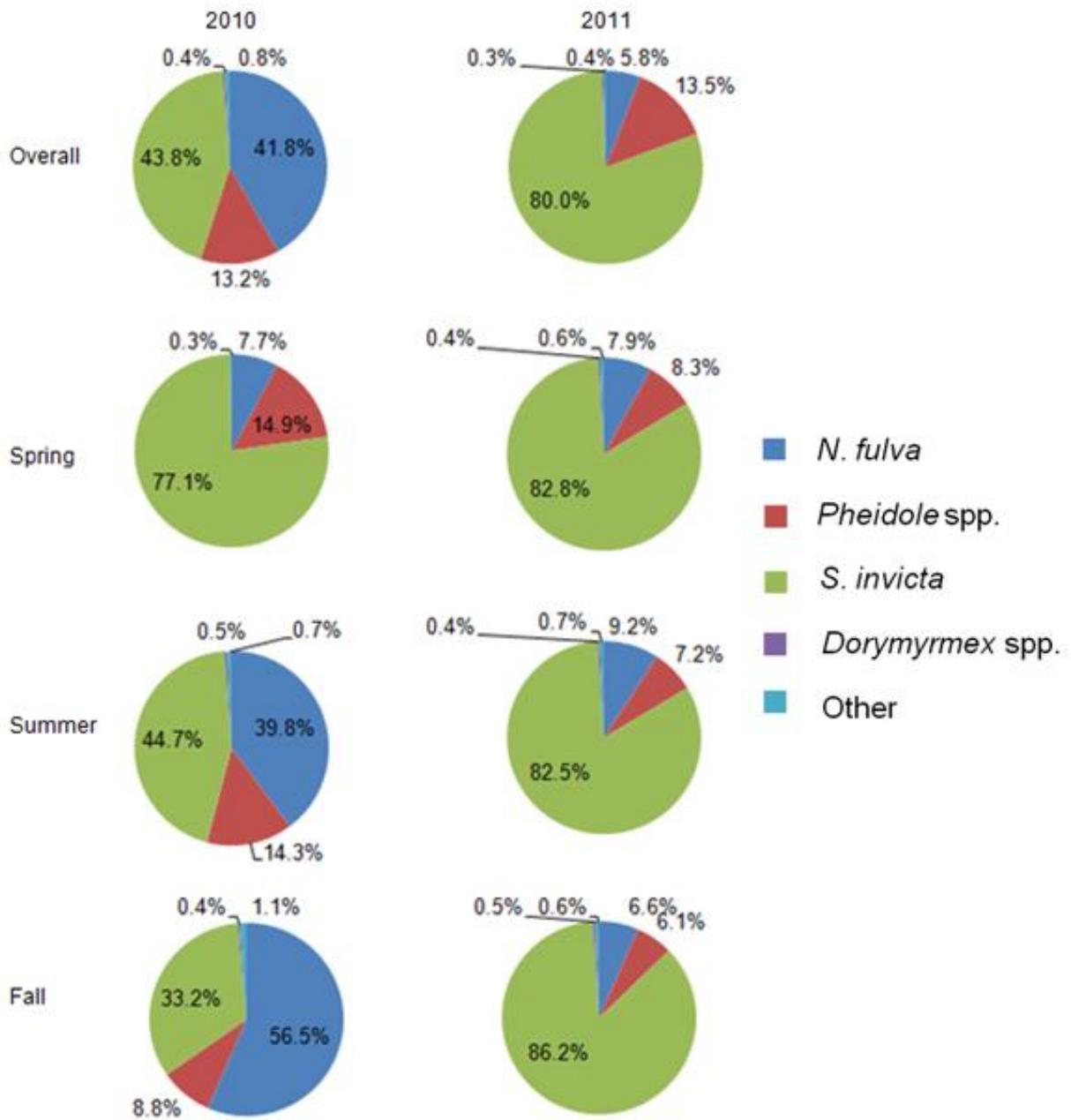


Figure 4-9. The ratio of ants found at Citra lot 3 in 2010 and 2011.

## CHAPTER 5 FOOD PREFERENCE OF *NYLANDERIA FULVA*

### Introduction

*Nylanderia fulva* (Mayr) is a polygynous, polydomous, supercolonial, tramp ant species. It is established throughout Florida and along the Gulf Coast (Pereira and Koehler 2007, Meyers 2008, Calibeo and Oi 2011, MacGown 2012). *Nylanderia fulva* adversely affects the fauna in ecosystems (Meyers 2008), agricultural production (Zenner-Polania 1990a, Campos-Farinha and Zorzenon 2005, Harmon 2009), and invades yards and occasionally structures (Zenner-Polania 1990a, Campos-Farinha and Zorzenon 2005, MacGown and Layton 2010, Calibeo and Oi 2011).

Extreme populations of *N. fulva* indicate copious food sources in the environment. *Nylanderia fulva* has been observed feeding on honeydew (Zenner-Polania 1990a), live and dead insects (Zenner-Polania 1990a, Meyers 2008, Warner and Scheffrahn 2004), as well as other dead animals (Meyers 2008). In research studies with *N. fulva*, the ants have been baited with hot dogs (Meyers 2008, Nester and Raspberry 2011) and 20% honey solution (Meyers 2008). These author's observations and baits used did not identify the food preference of these ants. In the field, the ants get carbohydrates from honeydew, but it is not known if the ants forage more on proteins or oils from animals. Cook et al. (2012) showed that laboratory colonies of *N. fulva* preferred a 1:2 protein: carbohydrate ratio of dry artificial food. High rates of mortality in this study failed to indicate much relevance (Cook et al. 2012). Opposingly, Scott (2012), showed that laboratory colonies of *N. fulva* preferred dry bait that was a 1.5:1 protein: carbohydrate ratio. The addition of a liquid protein (ground crickets)

caused the ants to forage more readily on the bait. When the dry bait with added protein was field tested, *N. fulva* did not show a preference for either (Scott 2012).

Seasonal shifts in food preference has been documented for *Camponotus pennsylvanicus* (DeGreer) (Tripp et al. 2000), *Linepithema humile* (Mayr) (Rust et al. 2000), and *Solenopsis invicta* Buren (Sorenson et al. 1985). Knowing of food preference shifts helps in designing and using the proper bait attractants given the season. However, little data exists regarding the season shifts in food preference for *N. fulva*. The objective of this study was to determine the food preference over time of *N. fulva* in a field setting, and to determine if there is a seasonal shift in food preference. Understanding of this species' food preferences in the field will be helpful with designing more effective baits.

### **Materials and Methods**

Food preference studies with multiple colonies of *N. fulva* were conducted on six urban lots; three in Gainesville, FL and three in Citra, FL. The locations in Gainesville were a recreational park and two urban house lots. The locations in Citra were three rural house lots. The sites were chosen because of the known existence of *N. fulva* and because these are representative of locations of where *N. fulva* can be found in Florida.

The lots were sampled biweekly, beginning in April 2011 and ending in November 2011. Sampling was done only when the air temperature reached a minimum of 21°C and was not over 32°C based on observations by Warner and Scheffrahn (2004). The sampling period was divided into spring (April and May), summer (June, July, August, September), and fall (October, November) for analysis purposes.

Three foods were used to determine food type preference; protein (tuna packed in water [StarKist, StarKist Co., Pittsburg, PA]), lipids (unrefined soybean oil [Organic Soybean Oil, Eden Foods, Inc., Clinton, MI]), and carbohydrates (honey [Great Value, Wal-Mart Stores, Inc., Bentonville, AR]). Two grams of each food was weighed out in sample tubes (100 mL hinged lid containers from Fisher Scientific, Pittsburg, PA) using a tabletop balance (Ohaus Cooperation, Parsippany, NJ). The tuna was well drained before weighing by pressing the water out of the can using the lid.

Three replications of the study were performed at each lot. The replications were at least 5 meters apart and placed near different ant trails. The sample tubes were 10 to 15 cm from the trail. The sample tubes were laid on their side with the hinge cap holding the tube in place near a trail of foraging *N. fulva*. In each replicate, each food (protein, lipid, and carbohydrate) was presented to the ants. The sample tubes were set up in a triangle, approximately 15 cm apart with the openings facing inward. The ants in the sample tubes were collected after 30 minutes by closing the hinged lid trapping the ants inside. The number of *N. fulva* in each sample tube was counted to determine food preference.

The data was normalized by square-root transformation. An analysis of variance (ANOVA) (SAS Institute 2012b) followed by a Student's t test was performed to determine which food was preferred by *N. fulva* (Hopper and Rust 1997). Percentages ants foraging on each food source and for each season were calculated. All tests of significance were evaluated at  $P = 0.05$ .

## Results

All of the food choices were fed on by the ants during the study period. Ants foraged significantly more ( $F = 41.4$ ;  $df = 2$ ;  $P < 0.0001$ ) on the protein ( $5.8 \pm 0.23$  ants)

than the carbohydrate ( $3.4 \pm 0.23$  ants) and the lipid ( $2.9 \pm 0.23$  ants) (Figure 5-1a). There was no significant difference between the number of ants foraging on carbohydrates and lipids ( $t = 1.96$ ;  $P > 0.05$ ). The same food preference was seen for the seasons as well (Figure 5-1b-d). In the spring, the ants foraged significantly more ( $F = 10.2$ ;  $df = 2$ ;  $P < 0.0001$ ) on the protein ( $3.9 \pm 0.36$  ants) than the carbohydrate ( $2.4 \pm 0.36$  ants) and the lipid ( $1.9 \pm 0.36$  ants) (Figure 5-1b). There was no significant difference between the number of ants foraging on carbohydrates and lipids ( $t = 1.97$ ;  $P > 0.05$ ). In the summer, *N. fulva* foraged significantly more ( $F = 29.1$ ;  $df = 2$ ;  $P < 0.0001$ ) on the protein ( $7.4 \pm 0.33$  ants) than the carbohydrate ( $4.4 \pm 0.33$  ants); and the lipid ( $4.1 \pm 0.33$  ants) (Figure 5-1c). There was no significant difference between the number of ants foraging on carbohydrates and lipids ( $t = 1.95$ ;  $P > 0.05$ ). In the fall, *N. fulva* foraged significantly more ( $F = 14.1$ ;  $df = 2$ ;  $P < 0.0001$ ) on the protein ( $3.2 \pm 0.29$  ants) than the carbohydrate ( $1.6 \pm 0.29$  ants) and the lipid ( $1.1 \pm 0.29$  ants) (Figure 5-1d). There was no significant difference between the number of ants foraging on carbohydrates and lipids ( $t = 1.97$ ;  $P > 0.05$ ).

At Citra 1 there was no significant difference between food source preferences until July (Figure 5-2). In July, the ants preferred the carbohydrate source more than the other foods sources ( $F = 7.9$ ;  $df = 2$ ;  $P = 0.004$ ). In the middle of September, the food preference changed from carbohydrates to protein, but there was no significant difference between the food preferences from the end of July until the end of the study.

The protein source only became significantly preferred at Citra 2 ( $F = 4.1$ ;  $df = 2$ ;  $P = 0.03$ ) in June. However, in July there was no significant difference between the food sources. In August, the protein started to be significantly preferred ( $F = 4.2$ ;  $df = 2$ ;

$P = 0.04$ ) and this preference lasted until November when there was no significant difference in the food preference ( $F = 0.6$ ;  $df = 2$ ;  $P = 0.56$ ).

June ( $F = 18.4$ ;  $df = 2$ ;  $P = 0.0001$ ) and July ( $F = 7.5$ ;  $df = 2$ ;  $P = 0.005$ ) are the only months when the protein source was significantly preferred at Citra 3. In August, the protein sources was significantly preferred over lipid source, but neither the protein and carbohydrate or carbohydrate and lipid were significantly different from each other ( $F = 4.2$ ;  $df = 2$ ;  $P = 0.04$ ).

At Gainesville 1, the ants significantly preferred protein in June ( $F = 10.6$ ;  $df = 2$ ;  $P = 0.0005$ ), August ( $F = 7.4$ ;  $df = 2$ ;  $P = 0.006$ ), September ( $F = 9.2$ ;  $df = 2$ ;  $P = 0.002$ ), and October ( $F = 5.8$ ;  $df = 2$ ;  $P = 0.01$ ). At Gainesville 3, the ants significantly preferred protein in May ( $F = 38.95$ ;  $df = 2$ ;  $P = 0.05$ ), June ( $F = 7.2$ ;  $df = 2$ ;  $P = 0.004$ ), and August ( $F = 12.9$ ;  $df = 2$ ;  $P = 0.0005$ ), September ( $F = 12.0$ ;  $df = 2$ ;  $P = 0.0008$ ). At both locations there was no significant preference in July ([Gainesville 1;  $F = 1.2$ ;  $df = 2$ ;  $P = 0.0$ ] [Gainesville 3:  $F = 3.3$ ;  $df = 2$ ;  $P = 0.07$ ]).

At Gainesville 2, the ants significantly preferred protein in May ( $F = 4.6$ ;  $df = 2$ ;  $P = 0.03$ ), July ( $F = 5.0$ ;  $df = 2$ ;  $P = 0.02$ ), and October ( $F = 5.5$ ;  $df = 2$ ;  $P = 0.01$ ). In August and November, the protein sources was significantly preferred ([August:  $F = 4.2$ ;  $df = 2$ ;  $P = 0.03$ ] [November:  $F = 3.9$ ;  $df = 2$ ;  $P = 0.04$ ]) over lipid source, but neither the protein and carbohydrate or carbohydrate and lipid were significantly different from each other.

## Discussion

Protein was highly preferred by *N. fulva* during the whole span of the study. The ants still foraged on the other two foods, but not as readily. Rust et al. (2000) found that Argentine ants, *L. humile*, also a polygynous, polydomous, supercolonial ant, preferred

proteins in the spring and the summer because of the need for protein for reproduction. They also found that carbohydrates were preferred in the fall and winter to sustain the workers activity as described also by Markin (1970). Argentine ant colonies need more protein when they are producing more brood in the summer months (Markin 1970), and foraging is a function of the colony's demand for nutrition.

Food preference of *N. fulva* may also reflect the nutritional needs of the colony throughout the year. *Nylanderia fulva* had a high demand for protein, especially during the colony growth phase in the spring. In the summer and fall, the ants still foraged more on protein suggesting that the colony was still growing. Because carbohydrates are used for energy, and given the potentially high number of workers in the summer and early fall, it was expected that *N. fulva* workers would switch from preferring protein to preferring carbohydrates, but this was not observed. This may indicate that *N. fulva* does not require as much carbohydrates to function or that there was a lack of the other proteins and lipids in the environment, thus *N. fulva* was feeding on those sources from the experiment and they were collecting carbohydrates elsewhere. Lipids are used to store energy and as expected, lipids were primarily collected in the fall in preparation for the winter months.

At Citra 1, the ants preferred carbohydrates over the other two food sources. The house was initially surrounded with thick vegetation, but in spring of 2011, the owners removed all the vegetation. The loss of vegetation may have caused a loss of sap-feeding insects, from which the ants collected honeydew, causing a reduction in carbohydrates in the ants' diet. As this nutrient is essential for survival, the ants foraged more on the carbohydrate source when presented in this experiment.

In a previous study, *N. fulva* demonstrated a change in food preferences (Meyers 2008). In a four-week field study, *N. fulva* switched from preferring 20% sucrose solution to preferring protein and lipids in the form of hot dogs. The study had to be stopped because the height of the grass made it impossible for the research to continue (Meyers 2008). The ants may have switched their food preference because of the availability of food in the environment as seen as Citra 1. Also, the short duration of the study does not provide information of a seasonal food preference. In this study, *N. fulva* did not have a season food preference, they preferred protein at all times. The odorous house ant, *Tapinoma sessile* Say also does not have seasonal food preference (Barbani 2003). *Tapinoma sessile* prefers carbohydrates year-round, but still feeds on proteins and lipids (Barbani 2003).

Little is known about the bait preference of *N. fulva*. The fact that *N. fulva* does not show a seasonal food preference and that protein was the preferred food choice here and by Scott (2012) suggests that a program incorporating protein baits would be effective year-round.

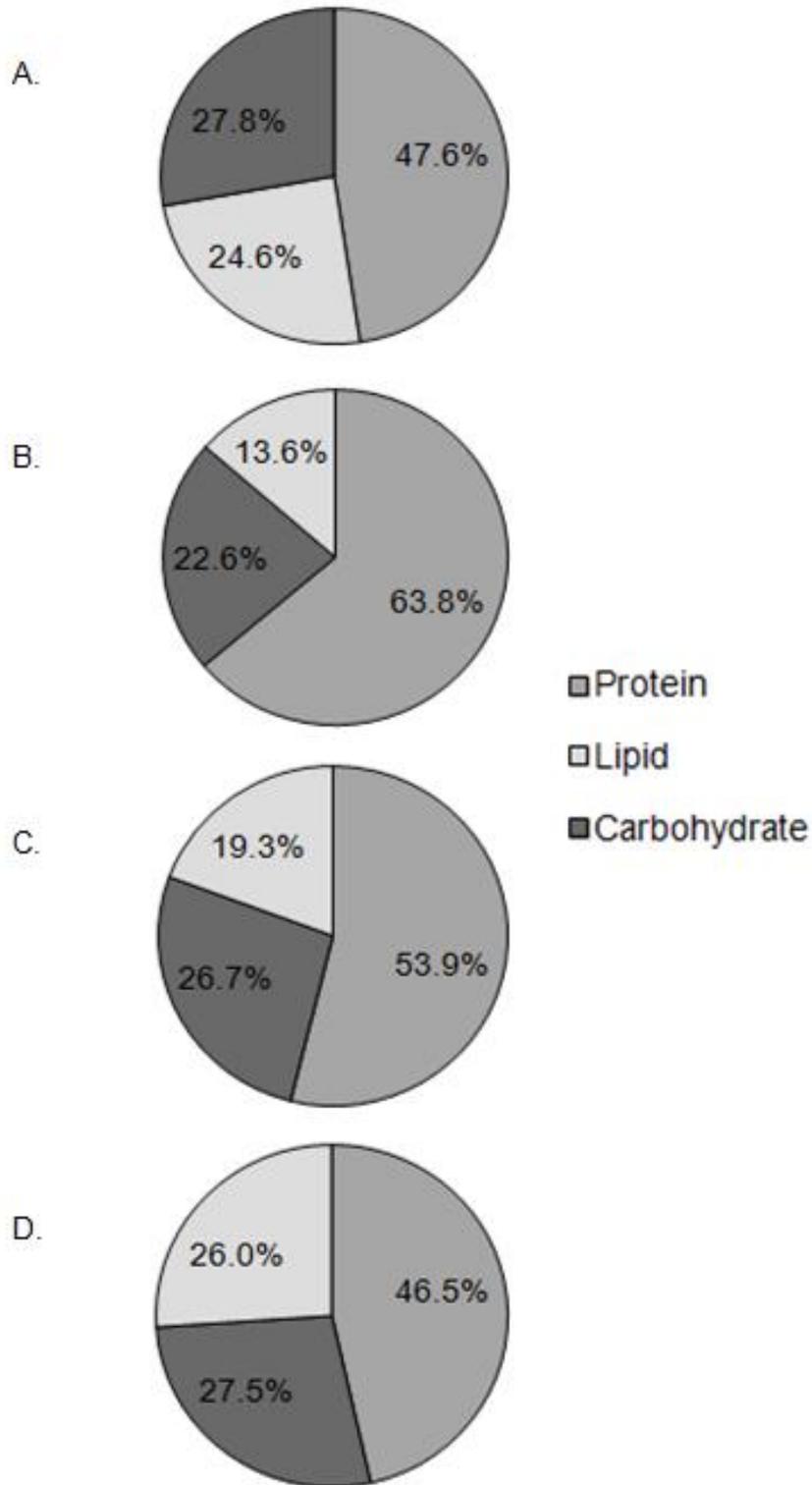


Figure 5-1. The percentage of *N. fulva* feeding on each of the food sources for the whole study (a), spring (b), summer (c), and fall (d).

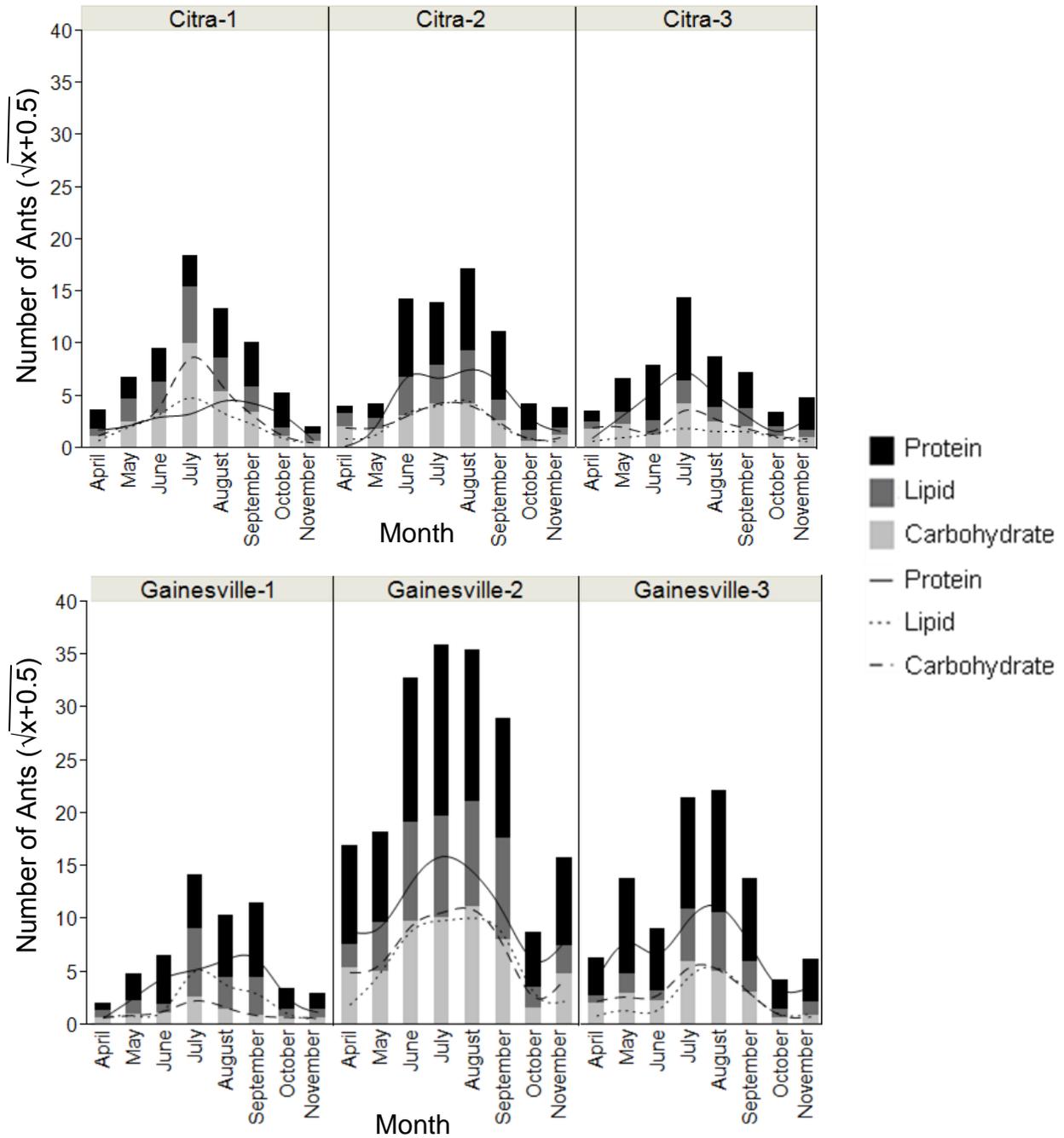


Figure 5-2. Mean number of *N. fulva* ( $\sqrt{\text{ants} + 0.5}$ ) collected in baited tubes containing a protein (tuna fish), lipid (soybean oil), and a carbohydrate (honey) in six sampling sites in north central Florida from April to November 2011.

## CHAPTER 6 CONCLUSIONS

The tawny crazy ant, *Nylanderia fulva* (Mayr) - formerly misidentified as the Caribbean crazy ant, *Nylanderia pubens* - is one of the more troublesome ants in Florida and throughout its introduced region in the southern United States. The tawny crazy ant is a difficult ant to control, and the discrepancy in proper identification over the last few years has contributed to failed control techniques, along with the super colony nature of the ant, and nesting strategies of the tawny crazy ant. The tawny crazy ant does not sting and only rarely bites when their nests are disturbed.

**Distribution and Naming:** The tawny crazy ant is originally from Brazil (Zenner-Polania 1990a). It was introduced into Colombia as a biological control agent against pests of the sugar cane, but later became a serious pest itself. Currently the tawny crazy ant can be found in several countries in South American and the Caribbean islands and is known as a pest in many locations.

It is not known when or exactly how the tawny crazy ant was introduced into the United States. The first reported identification of a similar ant was in the 1950s in Miami (Trager 1984). The ant was identified as *Paratrechina pubens* (Forel) which is now classified as *Nylanderia pubens*, the Caribbean crazy ant. At that time, the ant was not considered an important pest. The next report of *P. pubens* was around a Miami hospital in the early 1990s, but the ant was still not considered an important pest. Beginning in 2000, an ant with identical physical characteristics was observed in Fort Lauderdale, Port St. Lucie, Jacksonville, and Sarasota (Warner and Scheffrehn 2010) and quickly became a pest of concern. Given the proximity of the infestations to port cities, it is thought that the ants entered Florida through human trade, and that this

represented the same ant, *N. pubens*, previously identified in the Miami area. In 2002, an ant species we know to be the same one that became a pest in Florida was found in Texas (Meyers 2008). This ant was identified as a different species, *Paratrechina* species near *pubens* and known locally as the Raspberry crazy ant (Meyers 2008). In 2009, the ants were discovered in Mississippi, and then in 2010 in Louisiana (Hooper-Bui et al. 2010, MacGown and Layton 2010). These new infestations were first found, also, in and around port cities. In 2010, both *P. pubens* and *P. spp. nr. pubens* were moved to the genus *Nylanderia* based on new genetic evidence showing differences between the genera *Paratrechina* and *Nylanderia* (LaPolla et al. 2010). The Caribbean crazy ant was then referred to as *Nylanderia pubens*. Genetic comparison of ants found in Florida and Texas led to the conclusion, in the spring of 2012, that the ants in the two locations were the same species of ant (Zhao et al. 2010). Later in 2012, the ant was finally identified as *Nylanderia fulva* (Mayr). *Nylanderia pubens* and *Nylanderia fulva* are different species with differing biologies and ranges. The common name, tawny crazy ant, was approved by the Entomological Society of American in January of 2013 for *N. fulva*. The initial misidentification of *N. fulva* as *N. pubens* has contributed to failed control actions and caused problems in research. The ant misidentified from 2000 is *N. fulva*, and the ant in Miami from the 1950s supposedly was and is *N. pubens*.

### **Why Control of the Tawny Crazy Ant is Difficult**

- Tawny crazy ant colonies can have multiple queens and nests per colony. In the winter, the ants are usually found in only one or a few nests. These nests are referred to as permanent nests (Zenner-Polania 1990a). Permanent nests are always occupied and are the nests the ants leave from to form temporary nests in the late spring and summer. Permanent nests are found in protected areas and have been found extending 40 cm below the grounds surface (Zenner-Polania 1990a). In north central Florida, in the spring and summer, the queens produce large amounts of brood causing the ant population to increase rapidly leading to the formation of many temporary nests. These temporary nests allow

the ants to spread across the landscape to forage for resources. Temporary nests can be found in or under just about anything in the landscape. If temporary nests are disturbed, the ants will move to another nesting location. As the weather begins to cool in the late fall and winter, the ants may retreat back to the old permanent nest or may form new permanent nests (chapter 3).

- Having multiple queens per nest leads to a high reproductive potential. In permanent nests, up to 14 queens, 2300 workers, 1000 eggs, 1300, larvae, and 4500 pupae have been found (Zenner-Polania 1990a). Temporary nests are much smaller than permanent nests with varying numbers of castes and brood.
- Areas with a high amount to leaf debris and mulch are ideal places for tawny crazy ants to nest (chapter 3). These areas hold moisture that tawny crazy ants need to survive. Tawny crazy ants have also been found nesting in mulch piles, in rotting wood, in and under pieces of garbage, and under flowerpots. The potential abundance and diversity of nesting sites can make tawny crazy ant nests hard to characterize, especially in the summer months.
- The high reproductive potential, nesting strategy and the abundance of potential of temporary nesting locations allows tawny crazy ants to spread across a landscape. A single colony can cover acres of land, making them an area-wide problem, not just a single-lot problem.
- Unlike most ant species, tawny crazy ants from different colonies usually do not fight. Their non-territorial nature is a factor contributing to large populations. However, the tawny crazy ant is highly aggressive against other ant species, often displacing other ants and other arthropod species from areas (chapter 4).
- Tawny crazy ants prefer to feed on proteins year-round compared to other food sources (chapter 5). Protein sources in a typical north central Florida lot could include: termites, springtails, caterpillars, grubs, spiders, and other ant species (Zenner-Polania 1990a).

### **Integrated Pest Management Tactics for Tawny Crazy Ant Control**

An integrated pest management (IPM) method offers a safer way to control tawny crazy ants and leads to a greater chance of success. An IPM method incorporates prevention and suppression of problem-causing organisms through proper identification, inspection, and control techniques (sanitation, exclusion, and use of pesticides).

## Identification

The first step in an IPM method is proper identification of the pest. Without proper identification, control methods can be useless and potentially dangerous.

Tawny crazy ants are difficult to identify. At first glance, the tawny crazy ant looks like the red imported fire ant. Tawny crazy ants are reddish brown in color and 2.0-2.5 mm or 1/8<sup>th</sup> in length. Inspection under the microscope will reveal 12-segmented antennae, one node, and an acidopore, but these morphological characteristics alone will not lead to correct identification of *N. fulva*. For proper morphological identification, males are needed (Gotzek et al. 2012), and this is the reason why misidentifications occurred.

The foraging trails can aid in identifying tawny crazy ants. Tawny crazy ant foraging trails are wide (several ants) and loose. The ants move in a quick and erratic pattern, hence the classification as a “crazy ant”.

## Inspection

After proper identification as the tawny crazy ant, inspections will help reveal where the ants are foraging and nesting. Look for areas where the ants would have access to food, water, and shelter. Check the base of landscape plants to see if the ants are trailing up and down to collect honeydew from sap-feeding insects. Also, look around garbage containers, burn rings, and compost bins for foraging ants. It may be helpful to put down a tablespoon of honey or tuna fish in several different places around the lot to see where the ants are foraging (chapter 5). Tawny crazy ant nests are not distinct like red imported fire ant or pyramid ant mounds. Finding nests may require following foraging trails. Depending on the time of year, *N. fulva* nests and foraging can be in different locations (chapter 3). IN north central Florida, in the late fall, winter and

early spring, the ants are foraging in discrete locations, most likely around permanent nests. Permanent nests will be found in protected locations such as at the base of trees and at exposed root edges (Zenner-Polania 1990a). Start by looking for workers foraging in and around mulch and leaf debris around trees and structures. Shuffle mulch and leaf debris to get the ants to come out of the nest. In the late spring in early summer, the ants will spread out to temporary nests, increasing the area that must be inspected for nesting locations. Tawny crazy ant populations are highest in the summer months, when we can expect multiple foraging trails leading to different nests (chapter 3).

### **Control Techniques**

All animals need food, water, and shelter to survive. By reducing the amount of these three elements one can more easily reduce tawny crazy ant populations.

### **Sanitation**

Good sanitation practices should be used to restrict the ants' access to food, water, and shelter.

If possible, feed pets indoors. Besides feeding the ants, another possible side effect of feeding pets outside in heavily infested areas is inhalation of the ants by pets that could cause respiratory complications. Clean up animal waste and other items that would provide food for the ants. Avoid leaving garbage in burn cans or fire rings. Regularly washing out garbage and recycling containers will also reduce feeding sources. Trimming the landscaping around structures will reduce the number of honeydew-producing insects and help prevent the landscape plants being used as a bridge to enter structures.

Tawny crazy ants will form temporary nests in almost any lawn debris (fallen palm fronds, rotting wood, leaf debris, etc.). By diligently removing this debris, possible nesting sites are reduced, which will prevent extreme growth in tawny crazy ant populations.

Mulch and leaf debris provide food, water, and shelter to the tawny crazy ant. The mulch and leaf debris hold in moisture that the tawny crazy ant needs to survive. Proper drainage of mulched area will help in moisture reduction. Also, check irrigation systems in mulched areas; any leak will contribute to tawny crazy ant survival. Other arthropods and fungi in the mulch and leaf debris are a food source for the tawny crazy ant. In recent studies, mulch and leaf debris were the most likely place to find tawny crazy ants foraging year-round (chapter 3). Move mulch back away from structures, keep it dry, and if possible avoid using mulch and leaf debris in landscaping.

Tawny crazy ants usually enter structures to find food and water when populations are extremely large. Good indoor sanitation will help reduce the presence of this ant as well as other pest species indoors. Store food in tightly closed containers, dispose of food waste promptly, and clean up spills. Regularly vacuuming will remove any food particles that maybe on floors or carpets and serve as food for ants.

Good sanitation practices by home owners/inhabitants in infested areas will help reduce tawny crazy ant populations. Also, good sanitation will allow pest control specialist to more easily access the pest problem and effectively apply control measures.

## **Exclusion**

Exclusion techniques are designed to prevent pests from entering structures. Check that doorways and windows have tight fitting doors and screens with properly

installed weather stripping. Sealing cracks, crevices, and other entry points in the foundation and walls is essential in reducing tawny crazy ants and other pests inside.

## **Chemical Control**

Pesticides are the most common and recognized method of pest management. When handling any pesticide be sure to read and follow the label for maximum control and to reduce safety hazards.

Currently, there are no over-the-counter pesticides that effectively control tawny crazy ants. It is best to call a pest control specialist to help in managing tawny crazy ant populations. Before the pest control specialist arrives, it would be beneficial to follow the sanitation and exclusion methods provided here.

Pest control specialists have a limited amount of pesticides available and labeled for tawny crazy ant control, making sanitation and exclusion methods even more important. Once a pest control specialist arrives, they will assess the scope of the problem and design a management strategy. Some techniques they may use, but are not limited to, include:

- Applying a residual pesticide, which the ants must come in contact with to be controlled, around structures to prevent tawny crazy ant entry. If the pest control specialist applies a residual pesticide, be sure not to use water or a broom to remove the dead ants. Instead, use a leaf blower to ensure that the pesticide is not washed or scraped away and will continue to help protect the structure.
- Recent studies have shown that populations of the tawny crazy ant are the worst in the late spring, summer, and early fall (chapter 3). The sooner in the year a management plan is put into place, the better the chances are the control actions will produce desirable results.
- Pesticide baits on which the tawny crazy ant feeds on, or a contact pesticide may be broadcasted across the landscape. These pesticides are available in granular and liquid form. The pest control specialist may decide to use bait stations to dispense baits to the ants. The goal with any pesticidal bait is to use the ants' behavior of sharing food throughout the colony via trophallaxis (oral exchange of nutrients between colony members). The foraging ants take the bait back to the

nest and share the bait throughout the colony. Liquid baits are shared by adult workers, while solid bait granules are taken back to the nest for the larvae to digest and then workers feed to the colony the digested granules. This is because workers cannot digest solid food.

- If the pest control specialist notices tawny crazy ants trailing up landscape plants, they may apply a systemic pesticide to the plants to control honeydew-producing insects. This helps in eliminating the honeydew as a food source for the ants, but does not affect nectar production.

### **Things to Remember**

- Tawny crazy ants are an area-wide problem, not individual-lot problems. The reduction of a tawny crazy ant population on one lot does not guarantee that they the ants will not reinvade from adjacent areas. Tawny crazy ants affect entire communities.
- Follow the guidelines set forth by the pest control specialist. They can tell you how to maximize the potential of their management plan to control tawny crazy ants.

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## BIOGRAPHICAL SKETCH

Stephanie Hill, daughter of Paula Larrick, was born and raised in southeast Ohio. She graduated from Buckeye Trail High School in 2003 and went on to continue her studies at Hiram College where she earned a Bachelor of Arts in 2007. Stephanie entered the graduate program at the University of Florida in the entomology and nematology department in the fall 2007. She was a master's student under the supervision of Dr. Roxanne Connelly. In 2009, she received her master's in entomology and nematology. Stephanie stayed at the University of Florida, under the direction of Dr. Phil Koehler, to complete her doctorate in entomology and nematology.