RECRUITMENT TO AND DEFENSE OF APHIDS BY FIRE ANTS AND NATIVE

ANTS AND AN ESTIMATE OF THEIR TROPHIC POSITIONS USING

STABLE ISOTOPES

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RECRUITMENT TO AND DEFENSE OF APHIDS BY FIRE ANTS AND NATIVE

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THESIS ABSTRACT

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The red imported fire ant, *Solenopsis invicta*, is an invasive ant known to form

facultative mutualisms with aphids. Fire ants significantly reduce the abundance of aphid

predators and herbivores on plants infested with aphid colonies. However, to develop a

broader understanding of the ecological consequences of facultative mutualisms

involving invasive ants, differences in the recruitment to aphid colonies by fire ants and

native ants must be known. Furthermore, it is necessary to quantify and compare

differences in aphid defense between fire ants and native ants to determine if fire ants are

more effective mutualists than native ants. I used two field sites, one at Tuskegee

National Forest and the other at Auburn University's Mary Olive Thomas Tract to compare recruitment to aphids by fire ants and native ants. I used a choice field experiment using plants with and without cotton aphids to identify other ant species that respond to the presence of cotton aphid colonies and to estimate the number of workers per species that recruit to aphids. At Tuskegee National Forest, the native pyramid ant, *Dorymyrmex bureni*, was the only native ant species to recruit to cotton aphids. Fire ants were far more abundant at aphid colonies than native ants. Fire ants averaged almost 8 workers per aphid colony while pyramid ants averaged only 2 workers per aphid colony. At the Mary Olive Thomas Tract, the native ant, *Camponotus pennsylvanicus*, was the only native ant to recruit to aphids, but averaged less than one ant per aphid colony. Fire ants averaged more than 3 workers per aphid colony. At both sites fire ants were far likelier to recruit to aphids than either of these native ants. I also suppressed fire ants in half of my plots at each site to determine if fire ants were competitively excluding native ants from aphids. After fire ant suppression, the recruitment of *D. bureni* to aphid colonies increased 5-fold and *Camponotus pennsylvanicus* recruitment to aphids also significantly increased, although to a lesser extent. These data suggest that the presence of fire ants inhibits native ants from recruiting to aphid colonies, but the mechanism of this inhibition remains unknown. In my aphid colony defense experiment, aphid colonies tended by fire ants increased by more than 80 aphids per aphid when aphid predators were present, suggesting that fire ants were effective defenders of the aphids. Conversely, *D. bureni* tended aphid colonies declined by more than 100 aphids per colony when aphid predators were present, suggesting that this native ant was not an

effective defender of aphids. My results suggest that fire ants are better than some native ants in aphid colony defense and may be better mutualistic partners for aphids.

I also performed a stable isotope analysis to estimate the trophic level of fire ants in Alabama. Stable isotope analysis measures the ratio of heavy to light, biologically important isotopes and is a quick method to ascertain the trophic level of an organism in a food web. Three native ant species occupy a trophic level above fire ants, suggesting a more carnivorous diet than fire ants. Three other native ant species occupied a nearly identical trophic level to fire ants, suggesting they exploit similar resources.   Most importantly, fire ants occupied an intermediate trophic level between arthropod leaf-chewing herbivores and predacious, non-ant arthropods. This suggests that carbohydrates acquired from facultative mutualisms with honeydew-producing insects are an important component of the diet of fire ants and probably contribute substantially to colony growth and maintenance.

Style manual or journal used: Ecological Entomology (Chapter 1), Oecologia (Chapter 2)

Computer software used: Microsoft Excel, Microsoft Word, SigmaPlot, SAS 9.1

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INTRODUCTION

Two phylogenetically different organisms cooperatively interacting with each

other characterize a mutualism (Boucher et al 1982, Stachowicz 2001, Bronstein 2001).

Mutualisms can either be obligate or facultative. An obligate mutualism is when the host

requires a partner for survival. In a facultative mutualism, both organisms can survive

without the presence of the other, but both organisms gain from an interaction. Ant-aphid

mutualisms are an example of a facultative mutualism. The host aphid can survive

without ants, but may also have many different ant species as partners. Ant-aphid

mutualisms are generally characterized as food-for-protection mutualisms (Way 1963).

The aphid produces a secretion rich in carbohydrates and amino acids known as

honeydew. Ants collect the honeydew and in return defend the aphids from aphid

predators. However, ant-aphid mutualisms are highly conditional. For example, smaller

aphid colonies are thought to gain more benefit from ant attendance than larger aphid

colonies because ants tending smaller colonies will be more likely to encounter an aphid

predator than ants tending a larger colony (Addicott 1979). Another factor that is highly

conditional in ant-aphid mutualisms is the efficacy of various ants as mutualists. Kaneko

(2003) studied cotton aphids, *Aphis gossypii*, tended by two ant species, *Lasius niger* and

Pristomyrmex pungens, on Mandarin trees in Japan. He reported that *A. gossypii* colonies

tended by *L. niger* were parasitized less by the parasitoid *Lysiphlebus japonicus* than

colonies tended by *P. pungens*. In addition, not all ant species tend aphid colonies.

Many ant species are accidently introduced to locations outside of their native ranges. Broadly, these ants are referred to as introduced ants. Invasive ants are a subset of introduced ants that are capable of penetrating ecosystems beyond urban areas (Holway et al 2002). The six most widespread invasive ants are characterized with an omnivorous diet and high worker densities (Holway et al 2002). In some cases the presence of these invasive ants are correlated with outbreaks of honeydew producing insects. For example, the invasion of the yellow-legged crazy ant, *Anoplolepis gracilipes*, is linked to the outbreak of several scale species on Christmas Island (O'Dowd et al 2003). On Cameroon, the invasive big-headed ant, *Pheidole megacephala*, is correlated with outbreaks of the coccid *Peregrinus maidis* on maize plants (Dejean et al 1997).

My research focuses on the invasive ant *Solenopsis invicta*, the red imported fire ant. Fire ants are abundant in the southeastern United States. Fire ants are known for their ability to locate and recruit large numbers of workers to food resources quickly. Fire ants are also known to form facultative mutualisms with aphids. Aphid colonies tended by fire ants increase in abundance (Kaplan and Eubanks 2002). Furthermore, fire ants significantly reduce the presence of aphid predators at aphid colonies (Kaplan and Eubanks 2002) and decrease the abundance of non-aphid herbivores on aphid-infested plants (Kaplan and Eubanks 2005).

Native ants also tend aphids. For example, Nielson et al (1971) listed 18 ant species associated with 34 aphid species in Florida. The efficacy of native ants as aphid mutualists, however, is unknown in almost all cases. This leads to three questions: 1) Are there differences in the recruitment of fire ants and native ants to aphid colonies? 2) Do

fire ants competitively exclude native ants from aphid colonies? 3) Are there differences between fire ants and native ants in the defense of these colonies from aphid predators? I conducted a series of experiments to answer these questions. First, I conducted a field experiment to quantify and compare differences in the recruitment to aphid colonies by native ants and fire ants. Second, I conducted a field experiment to test if fire ants competitively exclude native ants from tending aphid colonies. Third, I performed a greenhouse and field experiment to quantify and compare differences in the defense of aphid colonies between native ants and fire ants.

I focused on the native pyramid ant, *Dorymyrmex bureni* in my experiments. Very little information is published on the genus *Dorymyrmex* and even less on *D. bureni*. However, Wojcik (1994) reported *D. bureni* was one of the few native ants whose abundance increased after fire ant invasion. At my field sites, *D. bureni* colonies were frequently observed in close proximity to fire ant colonies. Furthermore, fire ant workers were frequently found in the trash piles *D. bureni* workers create outside of their colonies. Most importantly, *D. bureni* workers were observed on cotton plants, possibly for the purpose of honeydew retrieval.

Few studies have compared the diets of fire ants and native ants. A few studies have attempted to quantify a fire ant colony's diet with visual observations. Vogt et al (2002) reported 30% of workers returning to the colony carried honeydew. Helms and Vinson (2002) estimated honeydew comprises 80% of a fire ant colony's diet. These studies suggest honeydew is a very important resource for fire ant colonies. However, the importance of honeydew to fire ant colonies relative to native ant colonies remains unexplored. Comparing diets between fire ants and native ants is difficult. Many native

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ant species are cryptic or not very abundant. This makes collecting foraging workers returning to the colony with food difficult. Furthermore, collecting foraging workers is very time intensive. Another obstacle in comparing diets is identifying food particles. Vogt et al (2002) at a field site reported 46% of the food particles they recovered were unidentifiable. An alternative method used to estimate trophic position, and thus diet, is stable isotope analysis.

Stable isotope analysis measures the ratio of heavy to light isotopes of biologically important elements, eg. $^{14}N:^{15}N$ (Ehleringer et al 1986). One element of particular interest is nitrogen (N). The amount of ^{15}N in an organism relative to other organisms in the community can be used to infer its trophic level, e.g. producer, herbivore, carnivore, in that community (Peterson and Fry 1987). In this study, I use stable isotope analysis to estimate the trophic position of fire ants and native ants. Because honeydew is estimated to comprise 80% of a fire ant colony's diet, I predict that fire ants will occupy a trophic position slightly above arthropod herbivores at my field sites.

CHAPTER 1: TENDING AND DEFENDING: COMPARING APHID-ANT MUTUALISMS INVOLVING NATIVE AND INVASIVE ANTS

Abstract. 1. Previous experiments quantify the abundance and defense of invasive ants tending aphid colonies but do not quantify differences between invasive ants and native ants tending aphid colonies. We conducted a series of experiments to quantify and compare differences in tending and defending between the invasive red imported fire ant, *Solenopsis invicta*, and native ants.

2. We conducted a choice field experiment with cotton plants with and without cotton aphids to quantify and compare the recruitment of fire ants and native ants to the presence of cotton aphid colonies. In Tuskegee National Forest, there were 4 fire ant workers on aphid-infested plants for every worker of the native pyramid ant, *Dorymyrmex bureni*. At the Mary Olive Thomas Tract fire ants averaged a little more than 3 workers per aphid-infested plant for every native ant worker of *Camponotus pennsylvanicus*.

3. Next, we suppressed fire ant abundance in half of our plots to compare differences in native ant recruitment after fire ant suppression. The recruitment of pyramid ants to cotton aphids after fire ant suppression significantly increased by 10x. The number of *C. pennsylvanicus* workers at aphid colonies also significantly increased.

4. We also performed an experiment to quantify the defense of aphid colonies by fire ants and pyramid ants. Aphid colonies tended by fire ants increased by nearly 80 aphids when aphid predators were present but declined by more than 100 aphids when tended by pyramid ants and aphid predators were present.

5. This is the first study to quantify differences in the recruitment to aphid colonies and the defense of these colonies between fire ants and native ants. This study shows the efficacy of invasive ants as food-for-protection mutualists is higher than it is for native ants.

Introduction

Mutualisms are characterized by phylogenetically different species cooperatively interacting with one another (Boucher et al 1982, Stachowicz 2001, Bronstein 2001). A widely recognized and well-documented mutualism is found between ants and aphids (Stadler and Dixon 2005). Myrmecophilic or 'ant loving' insects (e.g. scales and aphids) produce honeydew-like secretions rich in carbohydrates and amino acids (Way 1963). Ants collect the honeydew and aphids benefit with protection from predators, increased fecundity (Stadler and Dixon 1999), and decreased maturation time (Stadler and Dixon 1999, Flatt and Weisser 2000). Numerous studies document that a single aphid species can host multiple ant species (Addicott 1979, Bristow 1984, Novgorodova 2005) and the consequences of the mutualism for the aphid can vary with the attending ant species. For example, the aphid *Aphis gossypii* is tended by the ants *Lasius niger* and *Pristomyrmex pungens* on mandarin trees in Japan (Kaneko 2003). Aphid colonies tended by *L. niger*

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had lower levels of parasitization by the parasitoid *Lysiphlebus japonicus* than colonies tended by *P. pungens* (Kaneko 2003).

Ant-aphid mutualisms are hypothesized to be significant in facilitating the abundance and spread of invasive ants (Holway et al 2002, Helms and Vinson 2002, O'Dowd et al 2003, Ness and Bronstein 2004). Many ant species are accidently introduced to locations beyond their native ranges. A subset of introduced ants is invasive ants and they are capable of penetrating ecosystems outside of urban areas (Holway et al 2002). Invasive ants frequently obtain high densities (Holway et al 2002). One hypothesis for how invasive ants maintains high densities is by shifting diets to more plant-based resources in their introduced range compared to their native range (Holway et al 2002). One example of an invasive species shifting its diet is the Argentine ant, *Linepithema humile*. Tillberg et al (2007) reported a decline in trophic level to a more herbivorous diet of Argentine ants in their introduced range of California compared to their native range of Argentina. Aphid honeydew is a plant-based resource that could allow invasive ants to sustain high densities in their introduced range (Holway et al 2002). Several studies report facultative mutualisms between invasive ants and honeydew-producing insects such as scales and aphids (Kaplan and Eubanks 2002, Daane et al 2007, Grover et al 2008) and some honeydew-producing insect outbreaks can be correlated to the presence of invasive ants (Dejean et al 1997, O'Dowd et al 2003). However, comparisons between the attendance and defense of aphid colonies by native ants and invasive ants are poorly documented (Ness and Bronstein 2004). Information on differences in attendance and defense is important to developing a better understanding of the ecological consequences of invasive ant-aphid mutualisms.

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One of the most notorious invasive ants is the red imported fire ant, *Solenopsis invicta*. Fire ants are known to have widespread negative effects on vertebrate (Allen et al 2004) and invertebrate animals (Porter and Savignano 1990). Many studies have also found negative effects on the abundance of fire ants on native ants (Porter and Savignano 1990, Wojcik 1994, Morrison 2002, Gotelli and Arnett 2000, Helms and Vinson 2005). In their introduced ranges, fire ants form mutualisms with honeydew producing insects. For example, the red mealybug is an introduced scale found on grass roots in Texas. Honeydew from the mealybug colonies is estimated to comprise up to 80% of a fire ant colonies diet, leading to the speculation that the presence of one species facilitates the spread of the other (Helms and Vinson 2002). Fire ants also form a mutualistic relationship with the brown citrus aphid, *Toxoptera citricida*, reducing the emergence of the parasitoid *Lysiphlebus testaceipes* by 75% (Persad and Hoy 2004). Fire ants also tend cotton aphids in high numbers and reduce the presence of aphid predators by as much as 96% (Kaplan and Eubanks 2002).

Differences in the abundance and defense of aphid colonies by fire ants and native ants are unknown. Quantifying these differences is important to establishing the importance of the ecological consequences of invasive ant-aphid mutualisms. We performed a field experiment to assess native ant species' recruitment to cotton aphids compared to fire ant recruitment to cotton aphids. We then suppressed fire ant abundance to test if the presence of fire ants inhibits the recruitment of native ants to aphid colonies. We also performed a greenhouse and field experiment to compare the efficacy of predator defense of aphid colonies by fire ants and the native pyramid ant.

Materials and Methods

We used the cotton aphid, *A. gossypii*, because fire ants recruit to their colonies in large numbers (Kaplan and Eubanks 2002). We used cotton plants and cotton aphids to determine native ant recruitment because cotton aphids quickly establish large colonies on cotton plants and they could readily be transferred to the field. To test the effects of native ants on aphid predators, experiments focused on pyramid ants, *Dorymyrmex bureni,* because they were the most abundant native ants at the field sites (see results). Pyramid ants are commonly found in sandy soils from Maryland to Florida and west to Texas (Snelling 1995).

Numerical response of ants to aphids

We determined if the abundance of native ants increased in plant canopies with the presence of aphids using a choice field experiment. Cotton plants were grown from seed in a greenhouse and selected for the experiments when they were ~ 1 m tall. Plants were randomly assigned one of two treatments: aphids present or aphids absent. Plants with aphids present were infested with ~300 cotton aphids, a density commonly encountered in the field (Eubanks 2001). Aphids were selected from a greenhouse colony and allowed to acclimate to plants for 48 hours. Plants with and without aphids were then transported to the field for the experiment.

We used two field sites during two different months for this experiment. In June, the site was an old field at Tuskegee National Forest (TNF). In July, the second site was a planted longleaf pine stand at Auburn University's Mary Olive Thomas Tract (MOTT). A prescribed burn was applied at MOTT in early April. At each site, we established six

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plots measuring 6 x 6 m that were separated by a minimum of 100 m. In the corner of

each plot, we placed one cotton plant with and one cotton plant without aphids one meter

apart. Cotton plants remained in pots. Pots were placed in the ground and were covered

with excavated soil, leaving only the stems and leaves exposed. The upper and lower

surface of the leaves were visually searched for ants at 8:00 AM, 9:30 AM, and 11:00

AM during the following two days. The ant species and number of workers were

recorded. We performed a repeated measures analysis of variance to compare ant

abundance on plants with and without aphids (all statistical analyses used SAS, version

9.1; SAS Institute 1995).

Many ant species do not respond to the presence of aphids. I wanted to determine

the ant species at each site that did not recruit to aphids. To do this, I used a combination

of pitfall traps and visual surveys. Two pitfall traps (2 cm diameter) with 5 ml of

ethylene glycol were placed one meter apart between the two cotton plants, so the cotton

plants and pitfall traps formed a square. After three days, pitfall traps were returned to

the lab and ants were identified to species. We spent fifteen minutes visually searching

the perimeter of the plot for ants. Foraging ants were collected and returned to the lab for

identification.

Next, we determined the numerical response of native ants in plant canopies to the

presence of aphids after fire ant suppression. We randomly selected three plots at each

field site for fire ant suppression. We established a 30 x 30 m plot from the center of the

6 x 6 m plot for fire ant removal. The spatial territory of a fire ant colony can occupy up

to 197 m^2 and a 30 x 30 m removal zone ensured a fire ant free space (Adams 2003).

Fire ant colonies were removed with boiling water. Thirty liters of boiling water was

heated in a 37-liter turkey boiler and poured onto each colony. Each colony was treated two times and very large colonies three times. Previous studies document a significant reduction in fire ant abundance with this method (King and Tschinkel 2006, Lebrun et al 2007). To determine the effectiveness of fire ant suppression, I used bait stations with 1 g of tuna on an inverted lid of a plastic 4 x 10 cm container. After thirty minutes the container was placed on the lid, trapping the ants inside. The container was placed in the freezer at 0° C and the ants counted at a later date. I performed a t-test comparing the abundance of fire ants at bait stations pre- and post treatment to determine the effectiveness of fire ant suppression. I then placed cotton plants with and without aphids back in the corners of the 6 x 6 m plots and recorded ant species and workers. A split-plot analysis of variance was performed with fire ant suppression as the whole-plot treatment and the presence/absence of aphids as the sub-plot treatment. We analyzed the difference in the averages from the six recordings (three times a day for two days) from the pre- and post treatment. The analysis was performed using PROC mixed procedure in SAS. We also determined ant diversity and fire ant abundance as before. To determine if fire ant suppression was effective, we compared fire ant abundance from bait stations before and after treatment with a one-way analysis of variance.

Effect of ant-aphid mutualism on aphid predators

I performed greenhouse and field experiments to compare differences on aphid predators by a native ant and an invasive ant participating in an ant-aphid mutualism. For both experiments, cotton plants were grown from seed in the greenhouse to a height of ~0.5 m.

Cotton aphid colonies with ~200 individuals were established on plants from a greenhouse colony.

For the greenhouse experiment, we first counted the number of aphids per plant. Plants were then placed in a plastic bin measuring 53 cm x 43 cm x 13 cm. To prevent ant escape, the rims of the plastic bins were lined with liquid teflon. Plants were randomly assigned one of three ant treatments: fire ants, pyramid ants, or a no-ant control. Ant colonies were collected from nearby fields and placed in the plastic bins in the greenhouse for 24 hrs. After 24 hours, half the plants were assigned a predator or a no predator treatment. Predators were 3rd instar green lacewing larvae, *Chrysoperla carnea*, a common aphid predator in Alabama cotton fields, and were obtained from a commercial supplier (Beneficial Insectary, Redding, CA, USA). Two predators were placed on the upper two leaves of the plant. After 36 hours, surviving predators and aphids were counted. To estimate ant colony abundance, ant colonies were placed in a freezer at 0° C for eight hours. Colonies were removed and sifted for 20 minutes with a No. 18 Newark Standard Testing Sieve. Workers were removed and counted. This experiment was replicated thirteen times in three blocks of three replicates each and one block of four replicates. A one-way ANOVA was performed with each ant species to test for aphid predator survival. The effect of ant species on aphid colony growth was tested with an ANCOVA with ant and green lacewing larvae treatments as the main effects and ant density as a covariate.

In late June, we performed a field experiment to compare differences between aphid tending pyramid ants and fire ants on aphid predators at the E.V. Smith Research Station in Tallassee, AL. The field we selected measured 80 x 100 m and was divided

into two 40 x 100 m plots. Fire ants in one plot were suppressed with boiling water using the same methods as described above. We then established a 70 m transect 15 m from the 40 m side and 20 m from the 100 m side. A cotton plant with aphids was then placed in the ground every 7 m. We waited 24 hours to allow ants to locate the aphid-infested plants and then placed two 2nd instar green lacewing larvae on the top two leaves of each plant. The next morning surviving green lacewing larvae were counted and the ant species and number of workers present were recorded. Logistic regression analysis was performed to determine the effect of the abundance of pyramid ants on green lacewing larvae survival. Fire ant abundance in each plot was measured with pitfall traps. Two pitfall traps with circumference of 2 cm and 5 cm deep with 5 ml of ethylene glycol were placed one meter apart on a diagonal with the cotton plant in the middle. A t-test was performed to compare fire ant abundance between the two plots.

Results

Numerical response of ants to aphids

We identified twenty ant species at the Mary Olive Thomas Tract and nine species were recorded on cotton plants (Table 1). Three of these nine preferentially foraged on plants with aphids: (Fig 1a.) Fire ants $F_{1,47} = 21. 40$, $P < 0.0001$, (Fig. 1b.) *Camponotus pennsylvanicus* $F_{1,47} = 4.31$, $P = 0.04$, and (Fig. 1c.) *Brachymyrmex patagonicus* $F_{1,47} = 20.45$, $P < 0.0001$. After fire ant suppression, fire ant abundance at bait stations declined significantly ($F_{1,22} = 20.62$, $P = 0.0002$). The abundance of *C. pennsylvanicus* on aphid-infested plants in fire ant suppressed plots increased significantly (Fig. 2), but the abundance of other ant species did not.

We identified six ant species at Tuskegee National Forest (Table 2). Three ant species were recorded on plants and two preferentially foraged on plants with aphids: (Fig. 3a) fire ants $F_{1,47} = 21.02$, $P < 0.0001$ and (Fig. 3b.) pyramid ants $F_{1,47} = 10.14$, $P = 0.003$. After fire ant suppression, fire ant abundance at bait stations did not significantly decline ($F_{1,22} = 1.75$, $P = 0.20$). However, after fire ant suppression pyramid ant abundance on plants with aphids significantly increased (Fig 4. $F_{1,40} = 19.96$, $P < 0.0001$).

Effect of ant-aphid mutualism on aphid predators

In all ant treatments, aphid abundance in colonies without green lacewing larvae increased (Fig. 5). Aphid abundance in colonies tended by pyramid ants had a larger increase than aphid colonies tended by fire ants (Fig. 5). Aphid abundance in colonies not ant tended or tended by pyramid ants declined when predators were present (Fig. 5).

We also compared aphid tending pyramid ants and fire ants against aphid predators in the field. Pyramid ants did not have a significant effect on the survival of green lacewing larvae ($\chi^2 = 0.2661$, $P = 0.60$). However, in the control plot, fire ants were not abundant on aphid-infested plants (0.11 ± 0.08) and so we did not test the effects of fire ants on aphid predators.

We also compared the numerical response of pyramid ants to cotton aphids on cotton plants between the fire ant control and treatment plots. Fire ant abundance from pitfall traps in the treatment plot (12.88 ± 15.6) was significantly less than in the untreated plot (48.88 ± 57.7) ($t (238) = 9.03$, $P < 0.001$). Pyramid ant abundance in pitfall traps in fire ant treated plots (22.25 ± 31.9) did not significantly vary from untreated plots (26.91 ± 31.9) ($t (238) = 0.00$, $P = 1.00$). Interestingly, pyramid ant

abundance on aphid-infested plants in the fire ant treatment plot was significantly higher than in the fire ant control plot ($F_{1,235} = 4.34$, $P = 0.04$).

Discussion

Our results indicate native ants do not protect aphid colonies from aphid predators, suggesting invasive ants are more effective food-for-protection mutualists. Furthermore, invasive ants were more than twice as abundant on aphid-infested plants as native ants. This is significant because previous studies have correlated the presence of invasive ants with honeydew-producing insect outbreaks (Haines and Haines 1978, Dejean et al 1997, de Souza et al 1998, O'Dowd et al 2003). Because the availability of ant mutualists can be a limiting resource for mutualisms with honeydew-producing insects (Addicott 1978, Cushman and Addicott 1989, Cushman and Whitham 1991), the combination of increased attendance and a higher efficacy in the defense of aphid colonies by invasive ants could lead to honeydew-producing insect outbreaks.

We identified twenty-two ant species at two field sites in central AL, USA. In a choice field experiment four of these ant species, including two introduced species *S. invicta* and *Brachymyrmex patagonicus*, preferentially foraged on plants infested with aphids. Ants are known to collect nectar from cotton plants' extrafloral nectaries (Rudgers and Straus 2004), but because extrafloral nectaries were present on plants with and without aphids, aphids appear to be the most likely stimulus for attracting foraging ants into the plant's canopy. Empirical evidence from our greenhouse experiment indicates the presence of fire ants and pyramid ants in the plant's canopy does not

negatively impact aphid survival. This suggests that workers of both species were

engaged in honeydew retrieval rather than aphid predation.

In the field, aphid predator abundance did not significantly decline in aphid

colonies tended by pyramid ants. Furthermore, in the greenhouse, pyramid ant-tended

aphid colonies declined when aphid predators were present. This suggests pyramid ants

do not participate in the food for protection mutualism that typifies ant-aphid mutualisms

(Stadler and Dixon 2005), even though they appear to collect honeydew. This is

significant because pyramid ants had the largest numerical response to aphid-infested

plants of the native ants we recorded. Our results suggest a high numerical response

alone is not a requisite for the food-for-protection mutualism. This suggests the high

abundance of invasive ant species alone does not lead to honeydew-producing insect

outbreaks, that a high efficacy in the defense of these colonies is also very important.

Our results also suggest fire ants exclude some native ants from tending aphid

colonies. After fire ant suppression, the abundance of pyramid ants on aphid-infested

plants increased significantly. Whether the exclusion occurs from an interaction on the

ground causing a disruption of recruitment to aphids or on the plant is unclear.

Interestingly, during our aphid protection field experiment at E.V. Smith, pyramid ants

were also significantly less abundant on plants in the fire ant control plot than in the fire

ant treatment plot, but fire ants were not abundant on aphid-infested plants, suggesting

the interaction may occur on the ground. Native ant abundance at aphid colonies did not

increase as dramatically following fire ant suppression at the Mary Olive Thomas Tract

as at Tuskegee National Forest. One possibility for this is ants tend aphids with more

attractive honeydew (Fischer et al 2001, Blüthgen et al 2003). Other honeydew-

producing insects in the same proximity of an ant-tended aphid colony can reduce the numerical response of ants to that colony by producing more favorable honeydew (Cushman 1991, Fischer et al 2001). For example, *Lasius niger* prefers trisaccharides such as melizotose (Kiss 1981, Völkl et al 1999). However, *Anonychomyrma gilberti* prefers disaccharides such as sucrose (Blüthgen et al 2004). At the Mary Olive Thomas Tract, we collected *Camponotus americanus* tending psyllid nymphs, fire ants tending the mealy bug *Eurycoccus blanchardi* (King and Cockerell) on the roots of oak trees, and several ant species (*Paratrechina* sp., *C. americanus*, *B. patagonicus, Crematogaster ashmeadi*, and fire ants) tending unidentified aphid colonies on grass suggesting these ant species may prefer other honeydew sources. Furthermore, ants, such as *Crematogaster ashmeadi,* that are known to collect honeydew did not respond to the presence of cotton aphids at our field sites (Nielsson et al 1971). Future studies should focus on more than one aphid species.

We report a higher efficacy of invasive ants than native ants in the defense of aphid colonies from aphid predators. The combined effects of increased ant attendance and a higher efficacy of defense could contribute to honeydew-producing insect outbreaks. To further develop our understanding of the ecological consequences of invasive ant-aphid mutualisms, future work should focus on naturally occurring aphid populations over a season in non-agricultural settings in the presence and absence of invasive ant species.

Table 1: Ant species recorded at the Mary Olive Thomas Tract.

| Species Names	Workers Recruited to Aphids
Subfamily Dolichoderinae Tribe Dolichoerini	
Forelius mccooki (McCook)	
Tribe Plagiolepidini	
Brachymyrmex patagonicus	X
Paratrechina faisonensis (Forel)	X
Paratrechina parvula (Mayr)	X
Prenolepis imparis (Say)	
Tribe Camponotini	
Camponotus americanus Mayr	X
Camponotus nearcticus Emery	X
Camponotus pennsylvanicus (DeGeer)	X
Tribe Formicini	
Formica pallidefulva Latreille	
Subfamily Pseudomyrmecinae Tribe Pseudomyrmecini	
Pseudomyrmex seminole (F. Smith)	
Subfamily Ponerinae Tribe Ponerini	
Hypoponera opacior (Forel)	
Subfamily Myrmicinae Tribe Attini	
***Cyphomyrmex rimosus* (Spinola)**	
Tribe Soleopsidini	
Monomorium minimum (Buckley)	X
***Solenopsis invicta* Buren**	X
Tribe Pheidolini	
Aphaenogaster fulva Roger	
Pheidole sp.	
Tribe Crematogastrini	
Crematogaster ashmeadi Mayr	
Crematogaster minutissima Mayr	X
Tribe Formicoxenini	
Temnothorax pergandei	
Tribe Myrmecinini	
Myrmecina americana Emery	

Introduced species are in bold (McGlynn 1999)

Table 2: Ant species recorded at Tuskegee National Forest.

| Species Names	Workers Recruited to Aphids
Subfamily Dolichoderinae Tribe Dolichoerini	
Dorymyrmex bureni Buren	X
Tribe Plagiolepidini	
Brachymyrmex patagonicus	X
Subfamily Ponerinae Tribe Ponerini	
Hypoponera opacior (Forel)	
Subfamily Myrmicinae Tribe Attini	
Cyphomyrmex rimosus (Spinola)	
Tribe Soleopsidini	
Solenopsis invicta Buren	X
Tribe Pheidolini	
Pheidole bicarinata	

Introduced species are in bold (McGlynn 1999)

Fig. 1. Mean abundance of ant workers ±SE on plants with and without aphids present at the Mary Olive Thomas Tract. Fire ants and *B. patagonicus* are introduced species. Asterisks indicate a significant difference in abundance between plants with and without aphids (*P < 0.05, **P < 0.005).
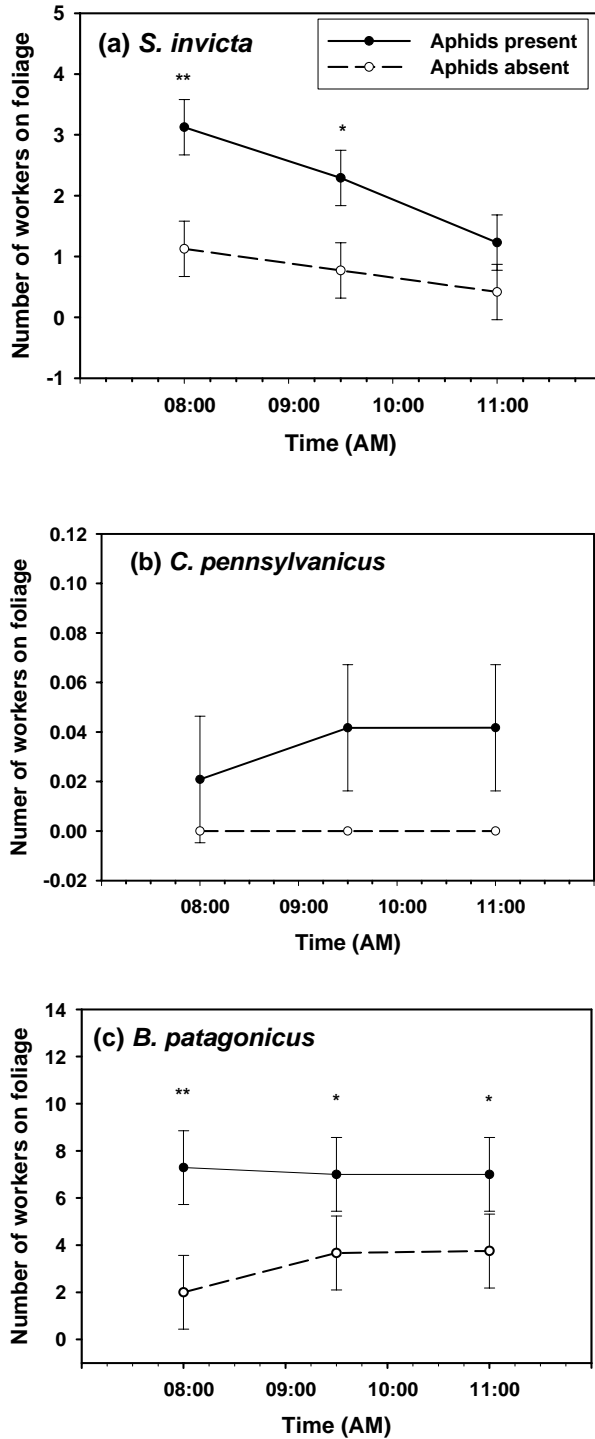
Fig. 2. The mean abundance of ant workers ± SE on cotton plants in fire ant suppressed plots at the Mary Olive Thomas Tract. The mean was calculated from the difference in the number of workers on cotton plants between pre- and post fire ant suppression. Asterisks indicate a significant difference (*$P < 0.05$).
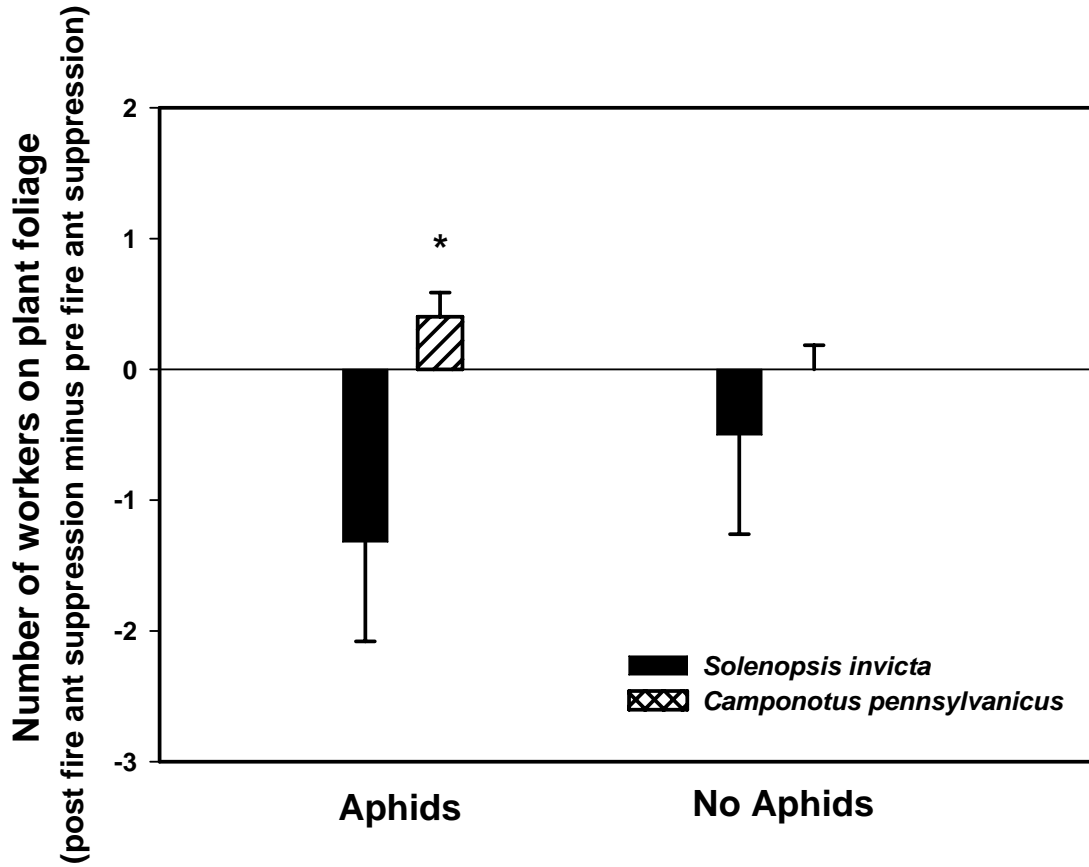
Fig. 3. Mean abundance of ant workers ±SE on plants with and without aphids present at Tuskegee National Forest. Asterisks indicate a significant difference in abundance between plants with and without aphids (*P < 0.05, **P < 0.005).
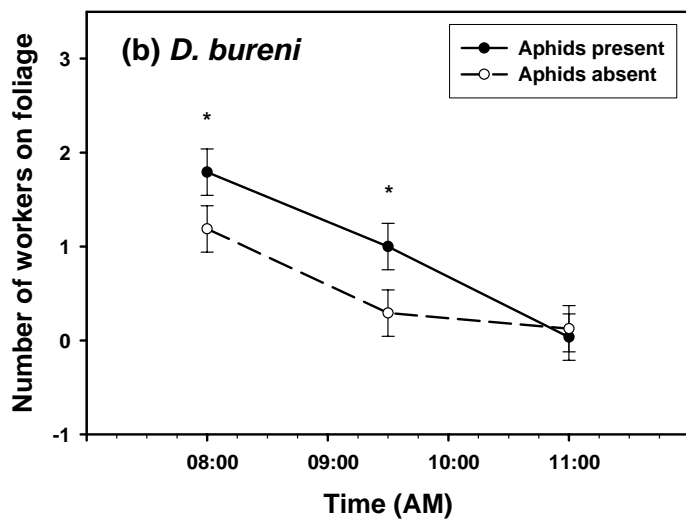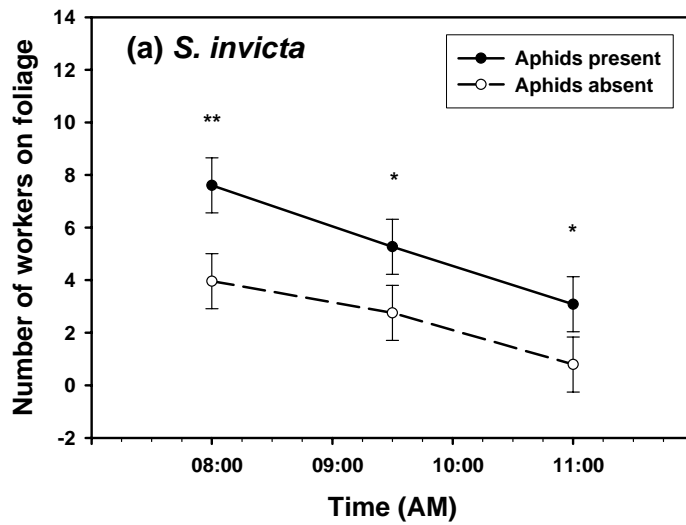
Fig. 4. The mean abundance of ant workers ± SE on cotton plants in fire ant suppressed plots at Tuskegee National Forest. The mean was calculated from the difference in the number of workers on cotton plants between pre- and post fire ant suppression. Asterisks indicate a significant difference in the abundance of workers between pre- and post fire ant suppression (**$P < 0.005$, ***$P < 0.0001$).

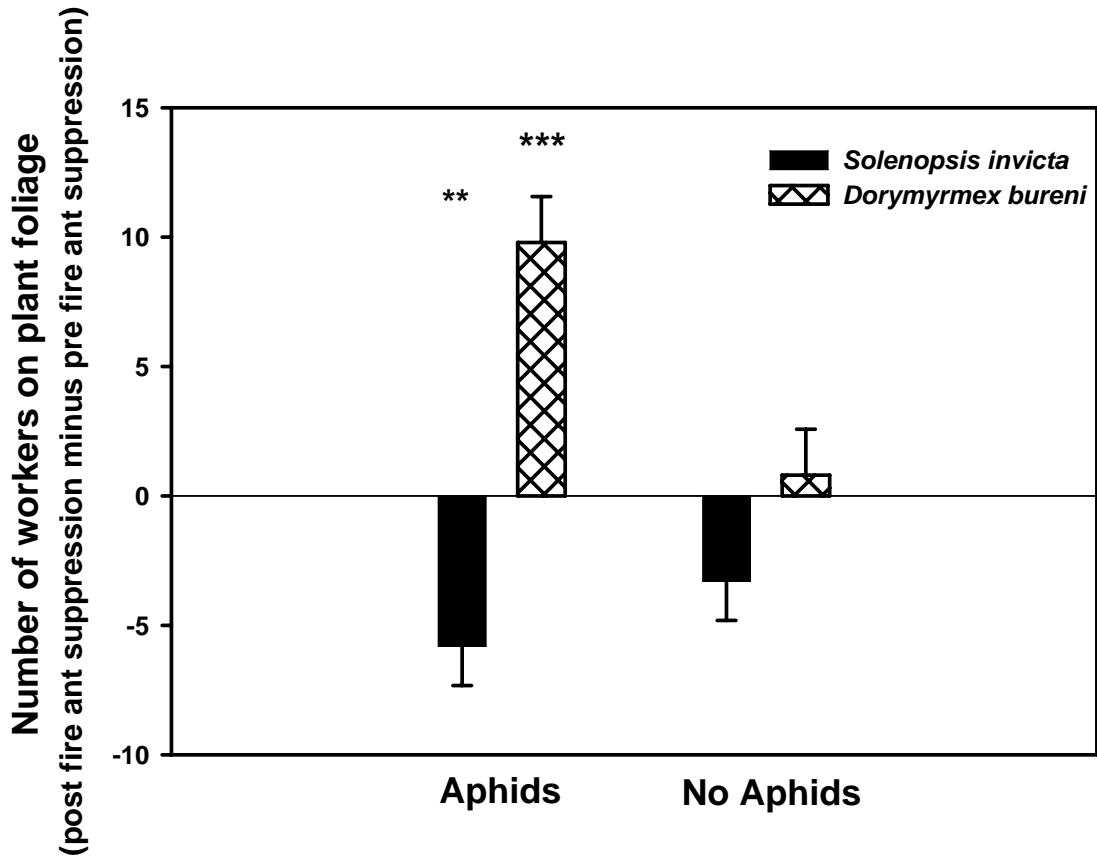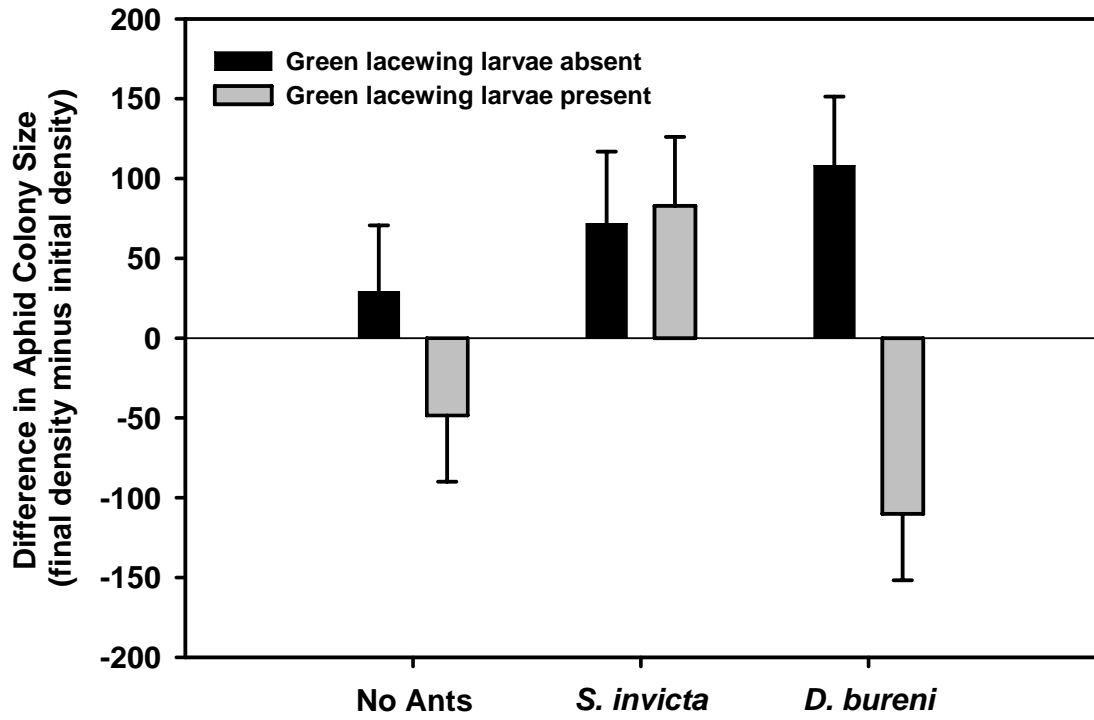**Fig. 5.** The mean ± SE difference in aphid colony size after 36 hours.

CHAPTER 2: A DIETARY COMPARISON BETWEEN FIRE ANTS AND NATIVE ANTS USING STABLE ISOTOPES

Abstract Fire ants reach high worker densities within their introduced range. A flexible diet could allow a trophic shift to a more herbivorous diet relative to their native range allowing the maintenance of high worker densities. To test this, I perform a stable isotope analysis at two sites in Alabama, USA to identify the trophic level of fire ants relative to the arthropod community, particularly native ants. Fire ants occupy a trophic level intermediate of predacious, non-ant arthropods and leaf-chewing herbivores, with a significant depletion in ^{15}N relative to predacious, non-ant arthropods. My results also suggest a decline in the trophic level of fire ants relative to their native range. I also report fire ants share a similar trophic level with three native ant species, suggesting fire ants and these native ant species exploit similar resources. Overall, my results support the hypothesis that dietary flexibility can facilitate the success of an invasive ant invasion.

Introduction

Many ants are accidentally introduced to new locations outside of their native range. Broadly, these ants are known as introduced ants, but are further divided into tramp ants

and invasive ants. Tramp ants are associated with human activity and are confined to urban areas (McGlynn 1999). Invasive ants are characterized by their ability to enter ecosystems, independently from human disturbances, in their introduced ranges (Holway et al 2002). Six of the most widespread invasive ants are omnivorous and frequently reach high worker densities (Holway et al 2002). One hypothesis for how invasive ants reach high worker densities is through dietary flexibility. Ecological theory predicts declining biomass at higher trophic levels. A shift to a lower trophic level could result in a population of an invasive species supporting increased biomass. One hypothesis for how invasive ants reach and sustain high worker densities is through the consumption of carbohydrate-rich resources such as plant and insect exudates, e.g. extrafloral nectar and honeydew, that native ants either fail to exploit or do so less efficiently relative to invasive ants (Holway et al 2002). This hypothesis is also used to explain how arboreal ants maintain high worker densities that prey resources alone could not sustain (Tobin 1994, Davidson 1997, Davidson et al 2003). Evidence supporting this hypothesis for invasive ants is sparse and usually correlative. On Christmas Island, the yellow-legged crazy ant, *Anoplolepis gracilipes,* is associated with scale insect outbreaks in the forest canopy (O'Dowd et al 2003). Yellow-legged crazy ant workers returning to the forest floor were also frequently observed with swollen, translucent gasters, suggesting these workers were participating in honeydew retrieval in the canopy (O'Dowd et al 2003). However, the composition of the liquid in their gasters could be carbohydrate-rich honeydew or protein-rich insect hemolymph, and therefore, remains unknown. One study did report a decline in the trophic level of the invasive Argentine ant, *Linepithema humile*, in its introduced California range relative to its native range (Tillberg et al 2007).

The trophic level of Argentine ants declined one year after invasion, a decline that was attributed to Argentine ants exhausting higher trophic level food resources such as native ant colonies, and shifting to a diet with more plant-based resources such as the honeydew produced by aphids and scales (Tillberg et al 2007). Other invasive ants may also sustain high worker densities by shifting their diets to more plant-based resources, but this remains unexplored.

In the southeastern United States, the red imported fire ant, *Solenopsis invicta*, is a notorious invasive ant species. Densities of fire ants in the United States are higher than in their native ranges in South America (Porter et al 1992) and their presence in the United States is associated with negative, widespread ecosystem effects. For example, fire ants are implicated in the reduction of bobwhite quail (Allen et al 1995) and other vertebrate populations (Allen et al 2004). Fire ants also attack and kill colonies of native ants such as *Pogonomyrmex badius* (Hook and Porter 1990). Fire ants are generally considered omnivorous, consuming insects (Vogt et al 2001, Vogt et al 2002) and frequently forming facultative mutualisms with honeydew-producing insects such as scales and aphids (Kaplan and Eubanks 2002, Helms and Vinson 2002). Facultative mutualisms between fire ants and honeydew-producing insects are considered a very important resource to the diet of a fire ant colony. For example, Tennant and Porter (1991) estimated 80% of a fire ant colony's diet is derived from honeydew resources. Vogt et al (2002) at a site in Oklahoma estimated 30% of workers returning to the colony returned with honeydew. Fire ants forage extensively for liquids (Tennant and Porter 1991), but the composition and identity of the liquid contents in the crops of the foraging

workers returning to the colony remains unclear (e.g. nectar, honeydew, hemolymph etc.).

One method that could more fully elucidate the importance of honeydew resources to a fire ant colony's diet is stable isotope analysis. Stable isotope analysis measures the ratio of heavy isotopes to lighter isotopes, with higher trophic organisms (e.g. predators) more nitrogen enriched than lower trophic organisms (e.g. producers). Stables isotope analysis was recently used to further understand the dietary flexibility and determine the trophic level of Argentine ants in California (Tillberg et al 2007). Stable isotope analysis was also previously used to identify the trophic level of ants relative to other arthropods (Blüthgen et al 2003, Davidson et al 2003). In all three studies, highly abundant ants occupied a trophic level intermediate between predacious, non-ant arthropods and leaf-chewing herbivores, suggesting the reliance of these ant species on plant-based resources.

The objective of my study is to investigate the trophic relationship between fire ant colonies and arthropods, particularly native ant colonies, at two locations in Alabama, USA. Fire ants in their native range exist at the same or higher trophic level as predatory arthropods (LeBrun et al 2007). Because fire ants reach higher worker densities in the United States than in their native ranges (Porter et al 1992), I predict the trophic level of fire ants will be lower than predatory arthropods. I also tested the trophic position of fire ants with the most abundant native ant, as measured by pitfall traps, at each field site to test for similar resource consumption. If two ant species exploit similar resources, the possibility of interspecific competition exists. My study will further develop our understanding of the importance of plant-based resources to invasive ant invasions.

Materials and Methods

Stable Isotope Analysis

Stable isotope analysis measures the ratio of heavy and light isotopes of biologically important elements such as carbon and nitrogen. Biologically important elements generally occur in two isotope forms, heavy and light, with one form more common than the other (Ehleringer et al 1986). For example, ^{12}C accounts for 98.89% of carbon and ^{13}C accounts for the other 1.11% (Ehleringer et al 1986). The heavy to light ratio is reported as 'per mil' (‰) and is calculated with the equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

Where δ is the difference between the sample and the standard, X is the element, R_{sample} is the ratio of heavy isotope to light isotope in the sample and $R_{standard}$ is the ratio of the heavy and light isotope in an international standard. The standard changes depending on the isotope of interest. For example, nitrogen's standard is N_2 atmospheric concentration and the standard for carbon is PeeDee belemnite.

Carbon is used to determine an organism's basal dietary carbon sources (DeNiro and Epstein 1978). Different photosynthetic pathways (e.g C3 and C4) have different ^{12}C:^{13}C values. For example, the δ^{13}C value for C3 plants is -20 to -32 ‰ and C4 values range from -9 to -17 ‰. Overlapping δ^{13}C suggests two organisms share the same basal diet. Nitrogen is used to determine an organism's trophic level (e.g. producer or consumer). The concentration of the heavy nitrogen isotope, ^{15}N, accumulates, called enrichment, in organisms at higher trophic levels, usually at a rate of 2-4‰ per trophic level, although this varies by age (Minagawa and Wada 1984), taxon (McCutchan et al

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2003) and variation in dietary C:N (Vanderklift and Ponsard 2003). An organism's trophic position is characterized by comparing its $\delta^{13}C$ and $\delta^{15}N$ values relative to other organisms in the community.

Sample collection and preparation

I selected two sites, an old field at Tuskegee National Forest (TNF) and a planted long leaf pine stand at Auburn University's Mary Olive Thomas Tract (MOTT). The old-field site is tilled and fertilized with 13-13-13 fertilizers once a year. Vegetation is dominated by grasses and a *Passiflora* sp. The long leaf pine stand is dominated by mature long leaf pines (*Pinus palustris*) and oaks, with grass in the under story. At MOTT, prescribed fire is used as a management tool to maintain open stand structure and a burn was applied in early April of the current study.

Six plots measuring 6 x 6 m were selected at each site with at least 50 m separating each plot. I visually searched the ground and vegetation, including tree trunks, in the 6 x 6 m square and collected ants with an aspirator. In June, I collected at Tuskegee National Forest and in July at the MOTT. Because ^{15}N is reported to vary among ant colonies (Tillberg et al 2006), I collected workers from at least three colonies. To ensure samples were from different colonies, only one sample of each species was taken from a plot. To establish an herbivore and predator baseline, I collected arthropod generalist herbivores and generalist predators. Species were kept separate, brought to the lab and placed in a freezer at 0° C until further processing. Samples were not stored in ethanol because ethanol is reported to change $\delta^{13}C$ (Tillberg et al 2006).

After 48 hours, each specimen was removed from the freezer. Ants were identified to species and other arthropods to family. The specimen was dried in an aluminum cup at 50° C for 48 hrs. After drying, the ants' abdomens were removed to ensure honeydew in the crop did not affect $\delta^{13}C$ (Blüthgen et al 2003; Tillberg et al 2006). Ant samples consisted of 3 to 45 workers, depending on worker size. Specimens were ground to a fine powder with a mortar and pestle, placed in a 3.5 x 5 mm tin capsule and weighed to the nearest thousandth of a microgram. Samples were analyzed in a Thermo-Finnigan Delta^{Plus} Advantage gas isotope-ratio mass spectrometer at the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University.

Statistical Analysis

To test for a significant difference in $\delta^{15}N$ between fire ants and predacious, non-ant arthropods, I performed a paired t-test with fire ants and green lynx spiders, *Peucitia viridans*. In an earlier experiment, the most abundant native ant species in pitfall traps at TNF was *Dorymyrmex bureni* and at the MOTT, *Paratrechina faisonensis* (Barnum unpublished data). Furthermore, these ants were the most abundant ants on cotton plants infested with aphids (Barnum unpublished data). Because these native ant species exploit plant-based resources, and *D. bureni* occupied bait stations during the same experiment, the possibility for these ant species to be exploiting similar resources exists. To test for this, I selected these two native ant species for a multivariate analysis (MANOVA)(SAS Proc GLM) to compare their $\delta^{13}C$ and $\delta^{15}N$ with fire ants. In the event of a significant effect, a univariate test was used to identify the significant element.

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Our understanding of an organism's trophic level improves if multiple systems are compared across time or space (Kling et al 1992, Post et al 2000). However, considerable variation in basal $\delta^{15}N$ exists between sites. To compare between systems, I must establish a standard equation to compute $\delta^{15}N$. I adopted Post's (2002) equation to calculate trophic level:

$$\delta^{15}N_{fcl} = \lambda + \frac{(\delta^{15}N_{secondary\ consumer} - \delta^{15}N_{base})}{\Delta_n}$$

Where $\delta^{15}N_{fcl}$ represents the trophic level of the secondary consumer, $\delta^{15}N_{secondary\ consumer}$ and $\delta^{15}N_{base}$ are directly measured, λ is the trophic level (e.g., $\lambda = 1$ for a producer, $\lambda = 2$ for an herbivore) of the $\delta^{15}N_{base}$ organism and Δ_n is the enrichment of $\delta^{15}N$ per trophic level, also called a trophic step.

Post (2002) reviewed the literature to estimate the $\delta^{15}N$ between trophic levels and estimated a trophic step of 3.4‰. McCutchan et al (2003) estimated the trophic step for consumers fed invertebrate diets as 1.3‰ and in a meta-analysis of the literature Vanderklift and Ponsard (2003) estimated the trophic step as 2.54‰. Because of this large variation between studies, I calculated the ΔN between trophic levels from my samples. For the predator, I selected the green lynx spider and for the herbivore a Chrysomelidae. I chose the Chrysomelidae because all larval instars and the adult are leaf-chewing herbivores. I did not calculate a mean of arthropod herbivores because the ^{15}N in phloem-feeding insects such as aphids is either equal or depleted relative to their plant hosts (Yoneyama et al 1997, Oelbermann and Scheu 2002, Sagers and Goggin

2007). At TNF, the δ^{15}N of the green lynx spider was similar to a Chrysomelid, an herbivore, from this site (see results). To account for this, I calculated the trophic step for the MOTT and applied it to TNF. For the δ^{15}N$_{base}$ I selected the Chrysomelidae because I was able to collect the same species, *Leptinotarsa juncta*, the false Colorado potato beetle, at each site. After calculating this new trophic position for fire ants, I used a paired t-test to compare the trophic position of fire ants at TNF and the July sampling at the MOTT.

Results

The broad range of δ^{15}N at both sites suggests ants occupy several trophic levels. At the MOTT, the δ^{15}N of the ant species sampled spanned 4.54‰ from maximum to minimum and the δ^{13}C spanned 7.24‰ (Fig 1). The δ^{15}N of the ant species sampled at TNF spanned 4.26‰ and δ^{13}C spanned 6.55‰ (Fig 2). The trophic level of a few ant species at both sites occupied a trophic level similar to herbivores while other ants occupied a trophic level closer to predacious, non-ant arthropods. The δ^{15}N of fire ants was significantly depleted relative to the predacious, non-ant arthropods (green lynx spiders) at the MOTT (t (2)= -4.19, P = .05).

I also found evidence for the exploitation of similar resources by *S. invicta* and *D. bureni* at TNF. The trophic position of *D. bureni* and *S. invicta* was not significantly different (Wilks' lambda 0.605, $F_{2,9}$ = 2.93, P = 0.10). Furthermore, the trophic level (δ^{15}N) of fire ants and *D. bureni* were nearly identical ($F_{1,10}$ = 0.00, P = 0.98), but with more variation in δ^{13}C ($F_{1,10}$ = 2.60, P = 0.13). However, *S. invicta* and *P. faesonensis* do

not appear to exploit similar resources. The trophic position of *P. faisonensis* and *S. invicta* was different (Wilks' lambda, 0.156, $F_{2,4}$ = 10.84, P = 0.02), and the $\delta^{15}N$ ($F_{1,5}$ = 5.00, P = 0.08) and $\delta^{13}C$ ($F_{1,5}$ = 0.73, P = 0.43) were significantly different between the two species.

I also compared native ants with a higher trophic level than fire ants to fire ants. The trophic levels of three native ant species at the MOTT were higher than fire ants (Fig 1). Because the sample size of these three species were small, I pooled them and compared the mean $\delta^{15}N$ of these three ant species to fire ants, but they were not significantly enriched ($F_{1,8}$ = 2.40, P = 0.16).

I used Post's (2002) equation to estimate a trophic level for fire ants at both sites. The trophic level of fire ants at TNF (2.85) was higher than fire ants from MOTT (2.72). However, a paired t-test revealed no significant difference between the trophic level of fire ants between the two sites (t (4) = 0.42, P = 0.69).

Discussion

My results support the hypothesis that the exploitation of plant-based resources can contribute to the success of an invasive ant species. The $\delta^{15}N$ of fire ants was significantly depleted compared to the $\delta^{15}N$ of predacious, non-ant arthropods. This depletion suggests plant-based resources are an important component of a fire ant colony's diet in their introduced range. These results also support prior dietary analysis that suggested a strong reliance by fire ant colonies on plant-based resources (Vogt et al 2002, Helms and Vinson 2002). However, it is a mistake to consider fire ants primarily as herbivorous. Fire ants occupy a trophic level intermediate of predacious, non-ant

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arthropods and leaf feeding herbivores. This suggests other arthropods and carrion are still important resources for fire ant colonies in their introduced ranges.

The $\delta^{15}N$ depletion of fire ants compared to predacious, non-ant arthropods is also significant because fire ants occupy a trophic level equal to or greater than predacious, non-ant arthropods in their native range (LeBrun et al 2007). The separate trophic levels fire ants occupy between their native and introduced range suggests fire ants are capable of subsisting on resources from multiple trophic levels. The ability to exploit resources from multiple trophic levels could facilitate their success in their introduced range. This dietary flexibility also appears to facilitate the spread of Argentine ants in California (Tillberg et al 2007) suggesting dietary flexibility is important for the successful establishment of invasive ants in their introduced ranges.

My comparisons of $\delta^{15}N$ revealed the trophic level of fire ants and pyramid ants, *Dorymyrmex bureni*, were statistically indistinguishable suggesting these two species exploit similar resources. However, the foraging behavior of these two ant species differs. Fire ants recruit large numbers of workers to stationary food sources such as dead vertebrates, and exclude other ant species from the source (Porter and Savignano 1990). Workers in the genus *Dorymyrmex* usually forage alone, discovering and exploiting a food resource before other ant species arrive (Andersen 1997). When other ant species arrive in high numbers *Dorymyrmex* workers abandon the food resource (Andersen 1997). Despite the contrasting foraging behavior, both ant species share the same trophic level. This suggests foraging behavior is not an indicator of trophic level. At the MOTT, two other species, *Prenolepis imparis* and *Formica pallidefulva* also occupied a nearly identical trophic level with fire ants. Because only two colonies of

each species were collected, I did not statistically test differences between them and fire ants. Foraging behavior of *P. imparis* and fire ants is similar. *Prenolepis imparis* recruits as many as 200 workers to a food source and subsequently defends it from most other ant species (Lynch et al 1980).

Three native ant species, *Aphaenogaster rudis, Monomorium minimum*, and *Forelius mccooki*, at the MOTT occupied a trophic level above fire ants. The δ^{15}N of these three ant species did not overlap with the green lynx spider, suggesting all three ant species feed on some plant-based resources. Previous foraging studies indicate a strong reliance on prey resources by these three species. For example, *A. rudis* is known to kill and remove Eastern termite colonies, *Reticulitermes flavipes* (Buczkowski and Bennet 2007). Adams and Traniello (1981) report *M. minimum* feeds almost exclusively on small arthropods. No previous dietary analysis of *F. mccooki* is reported. However, *F. mccooki* does occupy a similar trophic level in Alabama as it does in California, suggesting a prey rich diet that is consistent across spatial scales (Tillberg et al 2007).

The estimated trophic level of fire ants between sites was similar. However, basal ^{15}N was higher at TNF. Differences in the basal ^{15}N could be attributed fertilizers. At TNF, the fields are fertilized once a year with 13-13-13 fertilizers and the application of fertilizers is known to increase basal ^{15}N.

My study provides evidence for a decline in the trophic level of fire ants in their introduced ranges relative to their native range (LeBrun et al 2007). Fire ants occupied an intermediate trophic level between leaf-chewing herbivores and predacious arthropods at my field sites in Alabama. The shift by fire ants to a more herbivorous diet could help explain how fire ants maintain high worker abundances in their introduced ranges. Future

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studies should address changes in the trophic level of native ants in response to the

removal of fire ants. This could provide valuable insight into the response of an ant

community to the invasion of a dominant ant species. Future studies should also quantify

differences in the trophic level of monogyne fire ants and polygyne fire ants. Polygyne

fire ant colonies have multiple queens and workers reach higher spatial densities than

monogyne colonies due to a lack of spatial territories (Porter 1992). Plant-based

resources could support these higher densities, causing further δ^{15}N depletion of fire ants.

Fig. 1 The trophic position of ants, predators, and herbivores at the Mary Olive Thomas Tract. Numbers in graph are sample identification graph.

Key to sample identification number followed by taxa name and (sample size): 1 *Aphaenogaster fulva* (1), 2 *Forelius mccooki* (3), 3 *Monomorium minimum* (2), 4 *Prenolepis imparis* (2), 5 *Formica pallidufulva* (2), 6 *Temnothorax pergandei* (1), 7 *Paratrechina faisonensis* (2), 8 *Brachymyrmex patagonicus* (5), 9 *Crematogaster ashmeadi* (3), 10 *Camponotus americanus* (3), 11 *Camponotus pennsylvanicus* (3), 12 *Cyphomyrmex rimosus* (3), 13 *Camponotus nearcticus* (6), 14 Hemiptera: Psillidae (1), 15 Coleoptera: Curculionidae (1), 16 Chrysomelidae: Alticinae (1), 17 Hemiptera: Cicadellidae (1), 18 Hemiptera: Aphididae (1), 19 Araneae: Oxyopidae (3)
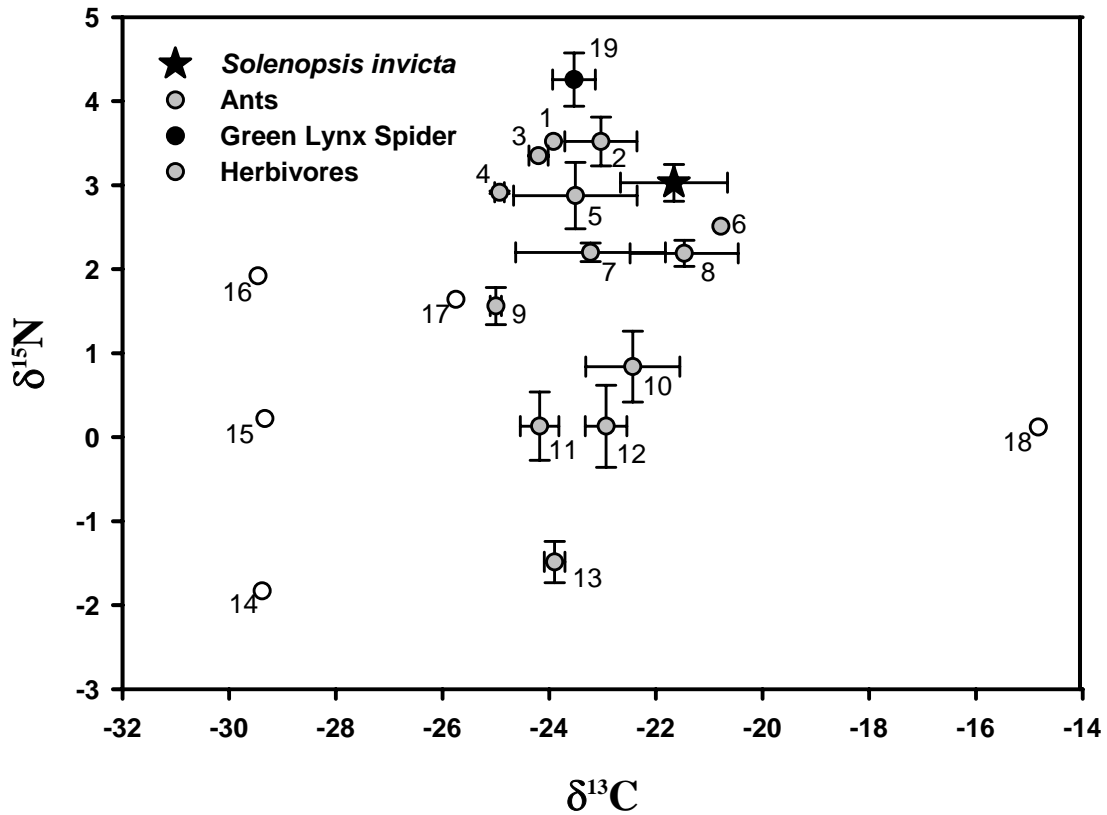
**Fig. 2** The trophic position (± SE) of ants, predators and herbivores at Tuskegee National Forest. Numbers in graph are sample identification number.

Key to sample identification number followed by taxa name and (sample size): 1 *Pheidole bicarinata* (2) 2 *Dorymyrmex bureni* (5) 3 Chrysomelidae: Alticinae (1) 4 Hemiptera: Cicadellidae (1) 5 Coleoptera: Curculionidae (1) 6 Araneae: Oxyopidae (1)
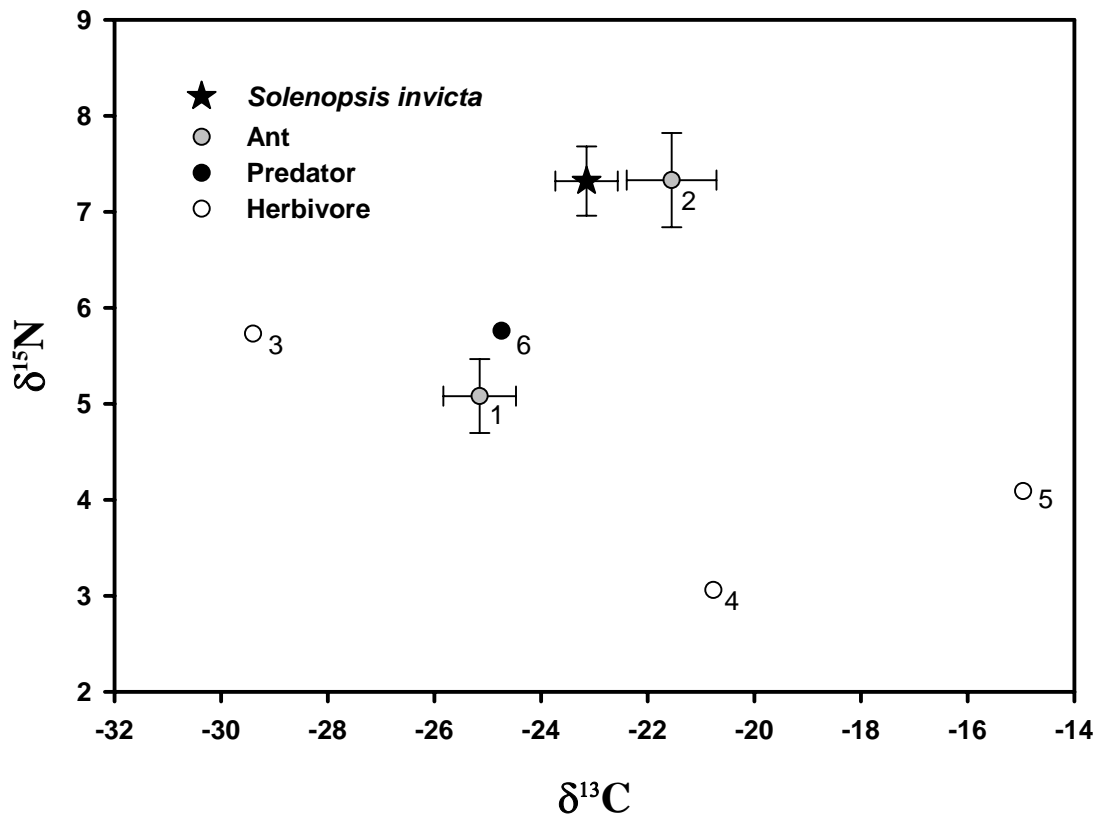
Fig. 3 The estimated $\delta^{15}N$ of fire ants at the Mary Olive Thomas Tract and Tuskegee National Forest.
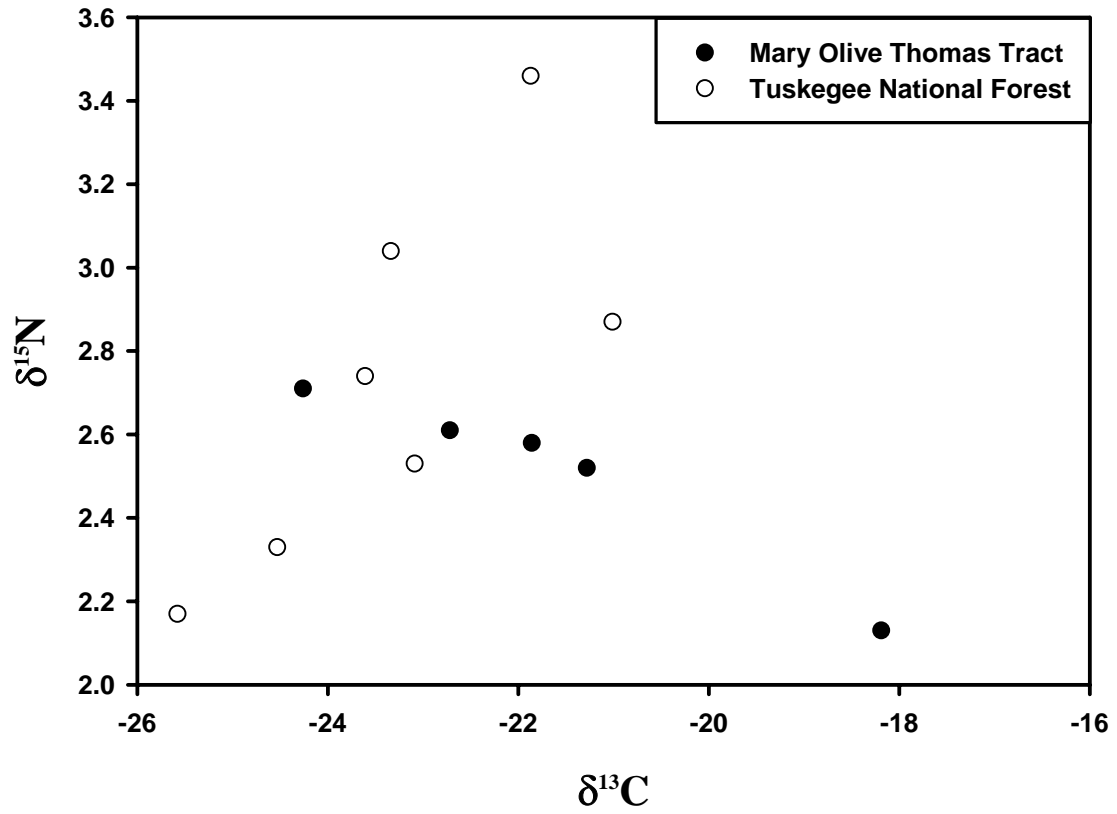
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