ABSTRACT

BRIGHTWELL, ROBERT JOHN. Interactions between the Invasive *Linepithema humile* and Honeydew-Producing Hemiptera. (Under the direction of Jules Silverman).

Honeydew producing Hemiptera are important to the survival of the Argentine ant in its introduced range. We investigated the role honeydew-producing Hemiptera had on Argentine ant survival in an infestation in Raleigh, North Carolina. Within this infestation there was a facultative mutualism between the Argentine ant and the endemic terrapin scale on red maples prevalent during the warmer months. We investigated the effects of excluding Argentine ant foragers from the host tree canopy on each participant. We also investigated whether denying access to honeydew-producing Hemiptera would result in an increase in toxicant consumption. During the winter months, we investigated the apparent role loblolly pine plays in providing a winter food source for the Argentine ant.

The Argentine ant entered into a mutualism with the native terrapin scale on the host red maple. This mutualism imposed an indirect cost on the host tree with smaller seeds and leaves on Argentine ant infested red maple. When Argentine ants were excluded from trees, terrapin scale populations collapsed and local Argentine ant nests relocated away from excluded trees. Bait consumption by Argentine ants was lower where access to honeydew-producing Hemiptera was denied. We attribute this to longer distances reducing foraging effort of Argentine ant colonies.

The Argentine ant cannot survive extended periods when temperatures remain below minimum foraging limits but above freezing. Argentine ants aggregated around loblolly pines and we suspect that this aggregation was not to escape cold temperatures but to concentrate around a reliable winter food source. We found the bark temperature on sun-exposed loblolly pines were elevated during the winter months to temperatures suitable for successful Argentine ant foraging. We found that workers trailing down the trunk were heavier than those trailing up the trunk indicating that the Argentine ants are feeding on a liquid food source, probably honeydew.

Interactions between the Invasive *Linepithema humile* and Honeydew-
Producing Hemiptera

by
Robert John Brightwell

A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Entomology

Raleigh, North Carolina

2008

APPROVED BY:

_____   _____
R.R. Dunn   F. Gould

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J. Cardoza   Jules Silverman
                                                          Chair of Advisory Committee

DEDICATION

This thesis is dedicated to the memory of my father the late

Robert William Brightwell

BIOGRAPHY

Robert John Brightwell was born January 9th 1964 and is the son of Norma Agnes and the late Robert William Brightwell. He was raised in Wellington New Zealand, a picturesque harbour city with a stunning café culture and nightlife. John enjoys rugby, a game he has, sadly, grown too old to play and cricket, a game he had, sadly, no aptitude or skill to play. Growing up he developed a taste for outdoor adventures such as tramping and mountain biking and is an enthusiastic, but uncoordinated skier. John enjoys traveling, both in New Zealand and abroad. He has traveled extensively around the world including South East Asia, Europe, Africa, North America and Mexico. He still has hankering to go wandering and his greatest wish is to see a bear in the wild.

John's tertiary academic career began later in life at the age of 35 when he enrolled at Victoria University of Wellington. In 2002 he graduated with a B.Sc. with 1st class Honours majoring in Ecology and Biodiversity under the supervision of Dr Phil Lester. Whilst attending Victoria University he met his future wife Karen Louise Martin, an American citizen who was visiting New Zealand on a one year working visa. In 2002 Karen's visa ran out and she returned to the U.S.A. It seemed best to both John and Karen that he look for graduate opportunities in the U.S. and this is how he found himself in Dr. Jules Silverman's laboratory at North Carolina State University in August 2004.

In 2007 Karen, now a high school teacher, and John were married on Virginia Beach and currently live in a small two-bedroom apartment with a willful orange tabby named The

Sultan of Brunei. John harbours dreams of becoming an academic and is grateful to all within the Department of Entomology, North Carolina State University for the opportunities and friendships made during his time there. He has an opportunity to take up a Post Doctoral Research position within the department and he is looking forward to beginning a new phase of his career.

ACKNOWLEGDEMENTS

Finally, I would like to express my deep gratitude to my wife Karen. Throughout my time at NC State she has been supportive of my efforts, ready to kick my backside when I needed motivation and willing to listen when things were not going according to plan. She would fill in as a field technician with little complaint even though the tasks were

tedious and demanding. Without Karen, this thesis would never have been completed.

Thank you.

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Chapter one

The Argentine ant – a worldwide pest in North Carolina

The Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), has become an important invasive ant species worldwide (Holway et al. 2002a). Originating in Argentina's lower Paraná River drainage (Wild 2004), *L. humile* is now found on six continents and numerous oceanic islands (Suarez et al. 2001b). *Linepithema humile* prefers a Mediterranean climate but its distribution may extend into sub-tropical and temperate climates (Hartley et al. 2006b, Roura-Pascual et al. 2004b). The first records of *L. humile* in the United States are from 1891 in New Orleans (Foster 1908) and was thought to have arrived on coffee ships from Brazil (Newell and Barber 1913a). In the continental United States of America the distribution of *L. humile* is currently concentrated in California and the southeastern states (Suarez et al. 2001b). Its distribution is apparently decreasing in the Gulf States where it is being displaced by *Solenopsis invicta* (Wilson 1951). In the southeastern states the northward expansion of *L. humile* has currently stalled in North Carolina (Suarez et al. 2001b). This was thought to be due to cold winter temperatures limiting survival of *L. humile* away from human structures (Buczkowski et al. 2004).

Linepithema humile is polgynous with up to 16.3 queens per 1000 workers (Keller et al. 1989) and as much as 3.5% of a colony's biomass (Markin 1970d), depending on the season. Queens can produce up to 60 eggs per day (Newell 1908) and with multiple queens, egg production can lead to very high worker numbers (Newell and Barber 1913a). Queens mate intranidally in spring, shed their wings in the nest and immediately start laying eggs (Newell and Barber 1913a). Colonies expand by budding, where one or

more queens and a sizeable number of workers will walk away from their home nest and establish a new nest in a suitable location (Newell and Barber 1913a). Satellite and home nests remain connected with up to 50% of workers moving between connected colonies (Markin 1968). The polygynous and polydomous structure of a colony can lead to truly enormous colonies known as supercolonies, the best known examples being located in California (Suarez et al. 1999, Tsutsui and Case 2001, Tsutsui et al. 2000) and southern Europe (Giraud et al. 2002). This has been explained as a consequence of *L. humile* propagules passing through a series of genetic bottlenecks (Tsutsui et al. 2000). Alternatively Giraud et al (2002) has suggested the selective loss of rare recognition cues resulted in the dominance of the most common recognition cues amongst populations and, therefore, a loss of intraspecific aggression. In North Carolina, however, supercolonies are far smaller than those found elsewhere (Buczkowski et al. 2004). One explanation for the small size of these supercolonies has been the greater genetic diversity found in the Southeast keeping intraspecific aggression high enough to prevent supercolony fusion (Buczkowski et al. 2004).

Linepithema humile has become a major urban pest in its introduced range (Silverman and Brightwell 2008). *Linepithema humile* has also proved itself a pest in agricultural systems where it forms facultative mutualisms with numerous damaging honeydew-producing Hemiptera (listed in Holway et al. 2002a). It may not, perhaps, be as serious a pest as it once was in areas of the Gulf States now overrun by *S. invicta* (Newell and Barber 1913a). *Linepithema humile* has also proven capable of penetrating natural

communities in California (Human and Gordon 1996, Ward 1987), South Africa (Bond and Slingsby 1984) and Europe (Gomez and Oliveras 2003, Way et al. 1997). This does not appear to happen in North Carolina's Piedmont and it maybe that penetration during the warmer months is hampered from a lack of suitable honeydew-producing mutualist partner (Rowles and Silverman in review). The evidence for arthropod displacement by *L. humile* is mixed in California (Holway 1998, Human and Gordon 1997) although in Hawaii, where there are no endemic ant species, *L. humile* presence was associated with reduced arthropod diversity (Cole et al. 1992, Holway 1998, Krushelnycky and Gillespie 2008). Similarly, in South Africa's Cape floristic region the presence of *L. humile* appeared to displace floral arthropods (Lach 2008). *Linepithema humile* is capable of disrupting the local ant community in natural communities in virtually all of its introduced areas (reviewed in Holway et al. 2002a). This ant does not appear to effect vertebrates as commonly as other invasive ant species (Holway et al. 2002a). However, *L. humile* has been shown to promote nest failure in the California gnatcatcher, *Poliptila melanura*, (Sockman 1997), negatively affect the abundance and fitness of the coastal horned lizard, *Phrynosoma coronatum*, (Fisher et al. 2002, Suarez and Case 2002), and depress capture rates of the gray shrew, *Notiosorex crawfordi*, (Laakkonen et al. 2001).

Linepithema humile has long been noted for its affinity for honeydew (Hölldobler and Wilson 1990a, Holway et al. 2002a, Ness and Bronstein 2004). Ant tending is primarily thought to provide protection from the natural enemies of honeydew-producing Hemiptera (Way 1963). Another benefit claimed for the Hemiptera by *L. humile* tending

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is sooty mold removal through consumption of the excreted honeydew (Bach 1991). Honeydew consists mainly of sugars (Fischer et al. 2002) and is the main food resource for *L. humile* workers (Markin 1970a). This resource is thought to be the primary fuel that allows some ant species, many in the Dolichoderine subfamily, to maintain and fuel the overwhelming numbers of workers required to dominate an ant community (Davidson 1997a, 1998, Davidson et al. 2003). Indeed, *L. humile* have an enlarged proventriculus allowing workers to carry more honeydew (Davidson 1998). As noted above, *L. humile* is often associated with outbreaks of honeydew-producing Hemiptera and it seems likely that increased honeydew production from increased Hemipteran numbers may help *L. humile* achieve the truly astonishing colony sizes noted around the world (Giraud et al. 2002, Suarez et al. 1999, Tsutsui and Case 2001, Tsutsui et al. 2000).

The apparent importance of honeydew to the success of *L. humile* in its introduced range led us to investigate three aspects of honeydew consumption by *L. humile* in North Carolina. Chapter two described our efforts in understanding the effects on the local abundance of *L. humile* of honeydew produced by the terrapin scale, *Mesolecanium nigrofasciatum* on red maple, *Acer rubrum*. Specifically, we investigated the effect of denying access to *M. nigrofasciatum* on local *L. humile* abundance. It is known that *L. humile* will shift nests to and from a food resource as the resource is discovered and then depleted (Holway and Case 2000). This suggests that denying access to honeydew will encourage *L. humile* to move resulting in a decrease in local abundance, and thereby affect indirect control (Rust et al. 2003). We also tested whether honeydew denial results

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in increased sugar-based toxic bait consumption. If access to honeydew resources is curtailed *L. humile* should switch to an alternate food source, such as a sugar-based bait, increasing bait efficacy.

As mentioned previously, honeydew-producing hemipteran abundances often explode when tended by *L. humile*. These elevated abundances frequently crash when the tending ant is excluded from the host plant (reviewed in Strysky and Eubanks 2007a). In Chapter three we investigated the effect on the abundance of *M. nigrofasciatum* on *A. rubrum* of *L. humile* exclusion from the tree canopy. Increased hemipteran loads from ant tending may negatively affect the fitness of the host plant (Buckley 1987, Way 1963). However, many studies have concluded an indirect host plant fitness benefit from hosting an ant-hemipteran mutualism through the deterrence of even more damaging herbivores, often foliar herbivores (reviewed in Strysky and Eubanks 2007a). We investigated the effect on the fitness of the host *A. rubrum* from the *L. humile-M. nigrofasciatum* mutualism. *Acer rubrum* rarely suffers from extensive foliar herbivory (Johnson and Lyon 1988) and we predicted that this mutualism would result in a fitness cost to the host plant.

Winter conditions in the North Carolinian Piedmont are generally considered too harsh to support nests unless they take refuge in human structures (Buczkowski et al. 2004). *Linepithema humile* does not survive freezing temperatures for more than a few hours (Jumbam et al. 2008) yet the Piedmont can suffer subfreezing temperatures for extended periods. *Linepithema humile* is known to aggregate into large colonies and relocate nests

down into the soil to escape cold weather (Newell and Barber 1913a). However, escaping subfreezing temperatures may not be the biologically important temperature threshold. *Linepithema humile* suspends foraging when temperatures fall below $5^{o}C$ (Markin 1970c). It is possible that restricted worker foraging may prove equally lethal to an *L. humile* nest from starvation given time (Jumbam et al. 2008). At one *L. humile* infestation in the Piedmont we observed *L. humile* trailing up and down loblolly pine, *Pinus taeda*, apparently foraging for honeydew even though ambient temperatures were below $5^{o}C$. In Chapter four we investigated the role of honeydew in the survival of *L. humile* away from human structures in the Piedmont. Specifically we tested the ability of *L. humile* to survive extended periods at temperatures below foraging limits but above freezing. We also mapped the aggregation of *L. humile* during the winter months and where the nests were coalescing. During the winter months we investigated just how *L. humile* can forage on *P. taeda* even though temperatures should preclude foraging and whether it is, indeed, feeding on a liquid food source.

The following three chapters are written for journal publication and conform to the following layout: abstract, methods and materials, results, discussion then literature cited sections. Tables can be found at the end of the literature cited section followed by the graphs for the chapter. The legend for each graph can be found immediately following the corresponding graph. For ease of reading the title page has been modified and Keywords have been eliminated. Chapter two has the results and discussion sections combined to conform to the layout for the Journal of Economic Entomology. The

appendix contains an article published in Insectes Sociaux in 2007 titled "Argentine ant foraging activity and interspecific competition in complete vs. queenless and broodless colonies". This article does not fit the theme of this thesis, however, it was completed during my time as a graduate student at North Carolina State University.

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Chapter two

Effects of honeydew-producing hemipteran denial on local Argentine ant distribution and boric acid bait performance

Abstract

The Argentine ant is well known for its attraction to honeydew and is often associated with hemipteran outbreaks in agricultural and urban environments. It has been suggested that Argentine ants may be controlled by restricting access to honeydew and forcing the ants to move or by encouraging increased liquid toxicant intake. We tested this possible control strategy by restricting Argentine ant access to the honeydew-producing terrapin scale within the canopy of red maple trees and monitoring ant numbers with pitfall traps and nest counts in the mulch around the tree base. Terrapin scale numbers were reduced when Argentine ants were excluded from the host tree canopy, consistent with the assumptions of Argentine ant protection from natural enemies. Argentine ant nest numbers fell dramatically in the mulch around ant-excluded trees; however there was no reduction in Argentine ant numbers caught in pitfalls around trees with or without canopy access. We added 0.5% boric acid bait stations at the base of the red maples and monitored bait consumption. Bait consumption was lower around ant-excluded trees, even though pitfall and nest counts remained unaffected, suggesting that restricting access to honeydew-producing Hemiptera did not enhance bait performance. We attribute this result to the increased distance Argentine ant workers had to trail from nest to bait station when not tending nearby terrapin scale. We suggest an alternative management strategy concentrating direct control of Argentine ants around a few host plants infested with honeydew-producing Hemiptera by controlling Hemiptera in nearby host plants.

Introduction

The Argentine ant, *Linepithema humile*, is a significant worldwide urban and agricultural pest (Silverman and Brightwell 2008). One feature of Argentine ant biology, in common with many invasive ant species, is their predilection for the honeydew produced by phloem-feeding Hemiptera (Holway et al. 2002a). It is thought that honeydew is the fuel that allows some ant species to achieve and maintain the extraordinary densities recorded (Davidson et al. 2003) and honeydew is an important source of carbohydrate for the Argentine ant (Flanders 1951, Holway et al. 2002a, Ness and Bronstein 2004). It has been suggested that controlling honeydew-producing Hemiptera may indirectly reduce Argentine ant infestations (Rust et al. 2003).

Although Rust et al. (2003) saw some reductions in Argentine ant foragers after treating hemipterans with systemic insecticides, this treatment provided poor Argentine ant control. One problem the authors considered was that the Argentine ant foragers counted at their monitoring stations had traveled from nests located at a distance. Argentine ant workers may forage up to 60m (Vega and Rust 2003) and therefore any effects on local Argentine ant numbers may have been masked by individuals from distant nests. Another possible problem involved measuring Argentine ant foraging activity through the removal of sugar water from monitoring stations (described in Reierson et al. 1998). Argentine ants are polydomous and may shift nests towards food sources (Holway and Case 2000, Newell and Barber 1913a, Silverman and Nsimba 2000). The addition of monitoring stations containing sugar water may have had the effect of substituting one

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carbohydrate resource for another. Argentine ant foragers and nests that may have moved away following collapse of the honeydew producing Hemiptera may have been attracted back into the treatment area by the monitoring stations. The Argentine ant prefers sugary liquids over other food types (Baker et al. 1985b, Newell and Barber 1913a) and a bait that mimics honeydew appears to be the most acceptable to the Argentine ant worker (Silverman and Roulston 2001). This raises the possibility that pest ants, such as the Argentine ant, may be encouraged to consume more aqueous carbohydrate based toxicant if the foraging workers are excluded from alternate or competing sources of carbohydrate, such as honeydew (Silverman and Brightwell 2008).

A commercial park in Raleigh, North Carolina has a large infestation of Argentine ants with workers commonly seen foraging up into the canopy of many trees and bushes planted within the park. Red maple trees (*Acer rubrum*) heavily infested with the native terrapin scale (*Mesolecanium nigrofasciatum*) are common within the Argentine ant infested areas of the commercial park (Brightwell and Silverman in prep). The terrapin scale has a univoltine life history and is known to produce copious amounts of honeydew (Simanton 1916a). Herein we report on the effect that limiting access to terrapin scale populations has on the local abundance of Argentine ant populations. We also report on the consumption and efficacy of aqueous boric acid bait when Argentine ant workers were denied access to the red maple canopy.

Materials and Methods

Effect of excluding ants from scales on ant numbers

Mature red maple trees are used extensively in ornamental plantings within the park and are grouped in rows of four or more red maple trees separated by *Magnolia grandiflora* bushes or driveway entrances on each side of the road. Individual red maple trees within each row were spaced ca. 7m apart; with the rows of red maple separated by at least 39m. In 2006 eight rows of red maple trees were selected for the Argentine ant exclusion experiments. Six of these rows of red maple contained five trees and two rows contained four red maple trees. Four rows were assigned to be Tanglefoot® banded (The Tanglefoot Company, Grand Rapids, Michigan, USA) with treatment and control allocated 19 tree each. Tanglefoot® treatment consisted of a 80mm wide band applied directly to each treatment trunk and was inspected and reapplied weekly to prevent failure.

Argentine ant worker numbers were assessed with pitfall trapping. Four PVC collars were dug into the mulch around each tree; orientated north, south, east and west and were 31mm in internal diameter and 120mm in length. The top of the collar was situated ca. 40mm under the surface mulch and ca. 300mm from the trunk of the tree. These collars allowed the placement and removal of the pitfall traps with minimum disturbance to the pine straw mulch. The pitfalls were a 50ml centrifuge tube (Evergreen Scientific, Los Angeles, California USA) containing 30mls of a 99:1 Ethanol : glycol mixture and left *in situ* for 24hrs. Pitfall samples were started August 3rd, 13 days before the Tanglefoot®

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banding was applied, and which were repeated 30, 57 and 87 days after the first pitfall samples.

Insecticidal bait efficacy

In 2007 we modified the preceding experiment by increasing the Tanglefoot® banded treatment rows and control rows to five each, and by adding 0.5% boric acid liquid bait to all treatments thirty six days after Tanglefoot® treatment. Tanglefoot® treatment rows were allocated by using the treatment rows from the previous year and randomly assigning one of the new rows of trees. All trees within a treatment row were banded with Tanglefoot® as described previously. Four pitfalls were arranged as described previously but only around those trees at the end of each row. This arrangement ensured that the distance between these end trees were at least 40m for every row. Pitfall samples were started August 2nd, five days before the Tanglefoot® banding was applied, and was repeated fortnightly. Nest counts were also undertaken weekly in the mulch around the experimental trees. The mulch layer was carefully peeled back by hand and nests counted. As with Silverman et al. (2006), we found that ants would remain in the inspected area with this procedure.

Increased bait efficacy through Tanglefoot® banding was considered effective if we observed a decrease in pitfall counts of Argentine ants and numbers of Argentine ant nests in the mulch surrounding the tree. Boric acid bait stations were added to one of the end trees in each row thirty-six days after Tanglefoot® treatment. The bait stations were

placed into the mulch of the tree approximately 300mm from the trunk and offset 45° from being in line with other trees in its row. Two PVC collars, as described above, were sunk into the mulch with the top 10mm left protruding above the mulch of the tree at both ends of a row. One end of each row was randomly assigned to receive the boric acid bait station with the other left as the bait control. The bait stations were 50ml centrifuge tubes filled with 40ml of 0.5% boric acid solution, the anhydrous boric acid being dissolved in 25% sugar water (Klotz et al. 1998). The lid had a 3mm hole through its center and a scintillation vial lid floating on the boric acid solution to prevent the mass drowning of Argentine ants in the bait solution. A control bait station with virus mesh glued over the hole in the cap was paired with the bait station to control for evaporation. Bait stations were changed every one to two days until the trial was completed. Bait consumption was determined by the difference in bait volume after placement in the field, adjusted for any evaporation.

Effect of ant exclusion on scale numbers

In May 2008 we undertook a survey of terrapin scale on all red maples used in experiments conducted in 2006 and 2007. This survey assessed the direct effect of Argentine ant exclusion on the numbers of terrapin scale infesting these red maples. Terrapin scales are found at the distal ends of the branches, concentrated on the new growth. For each tree, branches reachable from the ground were surveyed and any terrapin scale females were counted. As we couldn't survey every branch of each tree a relative abundance of terrapin scale was assessed. To reduce any bias, we started

surveying on the south side of each tree and progressed clockwise until four terrapin scale infested. We then summed the terrapin scale count and divided by four to calculate the relative abundance of terrapin scale per branch for the differing treatments. Not every red maple had four reachable terrapin scale infested branches. In such cases, the total count for that tree was divided by four to reflect the relative abundance of terrapin scale per branch between trees.

Data analysis

Statistical analyses were performed using SAS v.9.1 (SAS Institute, Cary, North Carolina). The 2006 pitfall data and the boric acid consumption data were analyzed with PROC MIXED repeated measures ANOVA with barrier treatment as the between-subjects variable with time as the within-subjects variable. The 2007 pitfall data and nest count data were tested using PROC MIXED repeated measures ANOVA with barrier treatment and bait station presence as the between-subjects variables with time as the within-subjects variable. The terrapin scale survey was analyzed using PROC GLM with banding treatment as the independent variable and terrapin scale numbers as the dependant variable along with pairwise comparisons between the year(s) of Tanglefoot banding and no banding. Data for 2006 pitfall counts and bait volume consumption were log transformed to stabilize variances whilst data for 2006 pitfall counts and nest count data were square root transformed for the same purpose.

Results and Discussion

In this study we investigated the effect of honeydew denial to the foraging of nearby Argentine ants. We found no difference in pitfall capture rates of Argentine ants around Tanglefoot[®] and non-Tanglefoot[®] banded trees in 2006 ($F_{1,10.8} = 3.30$, $p = 0.0970$; Fig 1), or in 2007 ($F_{1,8.16} = 2.41$, $p = 0.1582$; Fig 2). There was no difference in the number of Argentine ants found in pitfalls around those trees Tanglefoot[®] banded for two years compared to those trees Tanglefoot[®] banded for one year only ($F_{1,3.33} = 0.02$, $p = 0.8985$). Alder and Silverman (2004) found that pitfall sampling produces large daily sampling variation compared to other methods such as worker counts at baits and this large variation may have masked any reduction in foraging Argentine ant worker numbers. We did find evidence that denying access to honeydew-producing hemipterans can alter local Argentine ant densities. Argentine ants shifted virtually all of their nests away from those Tanglefoot[®] banded experimental trees, presumably to nearby ornamental hedges and trees (Table 1; Fig 3). The Argentine ant workers collected in the pitfall traps around the Tanglefoot[®] banded experimental trees were then most likely foragers from distant nests. The Argentine ant is noted for its propensity to move nests close to food resources and away when that resource is depleted (Holway and Case 2000, Newell and Barber 1913a, Silverman and Nsimba 2000). Honeydew is one carbohydrate resource that allows a dominant ant species, including invasive ant species, to maintain the large worker numbers required for community dominance (Davidson 1997a, Davidson et al. 2003, Holway et al. 2002a).

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Argentine ants rely on a mass recruitment foraging strategy, keeping a proportion of workers at the nest ready to be recruited to forage newly discovered resources, thereby limiting the number of workers that may be committed to resource exploration (Roulston and Silverman 2002). Those Argentine ant nests situated at the base of the control trees appear to dedicate most of their workers to honeydew retrieval from the red maple canopy. These nests may not, therefore, commit many more foragers to searching the mulch at the base of these trees than Argentine ant nests situated further away. A large number of ornamental plantings were well within 60m of the experimental trees, the distance Argentine ants have been documented to trail (Vega and Rust 2003). Previous studies investigating the effect on Argentine ant foraging numbers after insecticidal treatment of honeydew-producing Hemiptera returned equivocal results (Rust et al. 2003). Given the proximity of surrounding nests it is reasonable to expect that any decrease in foraging activity around Tanglefoot[®] banded trees would be modest at best.

The addition of 0.5% boric acid bait in 2007 had no effect on the numbers of Argentine ant workers caught in the pitfalls, regardless of whether the tree was Tanglefoot[®] banded or not ($F_{1,8.15} = 0.72$, $p = 0.4193$; Fig 2). Nor did the addition of the 0.5% boric acid bait have any effect on Argentine ant nest numbers (Table 1; Fig 3). We did find that boric acid bait consumption was lower around Tanglefoot[®] banded trees than around control trees ($F_{1,10.7} = 5.41$; $p = 0.0409$; Fig 4). One assumes this was due to fewer foragers to the bait station. Klotz et al (1998) who tested the efficacy of 0.5% boric acid in 25% sucrose water in the field, also saw a reduction of Argentine ants recruiting to the boric

acid bait stations compared to control stations filled with 25% sucrose water only. Low concentrations of boric acid are not repellent to a variety of ant species including *Campanotus abdominalis floranus* (Klotz and Moss 1996), *Monomorium pharaonis, Tapinoma melanocephalum* as well as the Argentine ant (Klotz et al. 1996) The Argentine ant nests at the base of the control trees were probably contributing the bulk of the foragers visiting the boric acid bait stations at these trees. In contrast, the Argentine ant workers visiting bait stations at the base of Tanglefoot® banded trees were traveling from nearby ornamental plantings.

Ant foraging effort has been shown to lessen over distance in *Formica* species, an ant which employs a central-place foraging strategy (Karhu 1998, Wimp and Whitham 2001). The Argentine ant with its dispersed central-place foraging strategy and polydomous colony structure will rapidly establish nests near a food source (Holway and Case 2000, Newell and Barber 1913a). It is, therefore, surprising that Argentine ant nests did not relocate close to our boric acid bait stations. It is unclear why our bait stations did not attract Argentine ants to locate close by. Our bait stations held a maximum of 40ml of toxicant with access limiting the number of Argentine ant workers that can feed simultaneously. It may be that limiting the number of simultaneously feeding workers may not have allowed foraging trails to grow sufficiently large to trigger nest movement. An alternative explanation is that there were alternative carbohydrate resources within the environment plentiful enough to preclude nest movement to our bait stations. The extended distances from nests to bait stations at the base of Tanglefoot® banded trees

probably limited foraging effort to the bait stations as distance increased from nests to bait.

Terrapin scale populations were lower when the host tree had Argentine ants excluded from the canopy than trees with canopy foraging Argentine ants, regardless of year or length of time ($F = 21.89$, d.f. 3, $p < 0.0001$; Fig 5). We found no difference, however, in terrapin scale numbers per branch between trees Tanglefoot[®] banded for 2006, 2007 or banded for both years (Fig 5). While excluding Argentine ants from tree canopies did not result in increased boric acid bait consumption it did reduce the abundance of terrapin scale in the study trees. Sap-sucking Hemiptera can inflict considerable fitness costs to the host plant including increased phloem removal, fouling from sooty mould and increased risk of hemipteran-vectored pathogens (Buckley 1987, Way 1963). Host red maples with terrapin scale numbers reduced by denying Argentine ant access to the canopy showed an increase in plant fitness with increased dry seed mass and larger leaves (Brightwell and Silverman in prep).

The Argentine ant is known to be a facultative mutualist to numerous honeydew-producing Hemiptera and has been associated with elevated numbers of the partner Hemiptera (Holway et al. 2002a, Ness and Bronstein 2004). This appears to be the case for the terrapin scale. It is thought that protection from parasitoids and predators are the main benefit to the mutualist Hemiptera of ant attendance (Buckley 1987, Way 1963). The terrapin scale is known to normally suffer from high rates of parasitism in North

Carolina (Devorshak 1994, Meyer et al. 2001). This may explain the decrease in scale abundance when Argentine ants were prevented from foraging in the red maple canopy. We have previously reported that Tanglefoot® banding individual infested branches resulted in almost total collapse of terrapin scale numbers on those branches (Brightwell and Silverman in prep). The terrapin scale populations seen on trees Tanglefoot® banded in 2006 or 2007 reflects the ability of the crawlers to readily disperse via ballooning, birds etc (Simanton 1916a).

Argentine ants will move nests to food resources and away when those resources have been exhausted (Holway and Case 2000, Newell and Barber 1913a). We did not see a reduction in Argentine ant numbers or nests around canopy accessible red maples after 0.5% boric acid bait was introduced. Denying access to terrapin scale, which resulted in Argentine ant nests moving away, suggests an alternate management strategy derived from trap-mulching (Silverman et al. 2006). Treating the majority of honeydew producing Hemiptera but leaving some plants infested should encourage the Argentine ant nests to move closer to those infested plants. This should concentrate Argentine ants nests into a known concentrated area allowing for targeted, cost effective, treatment of the infestation. Daane et al. (2006) suggested that initial deployment of liquid baits in spring should encourage bait consumption when honeydew is still limited and when winter aggregations are breaking up (Newell and Barber 1913a). Our proposed strategy may be most effective under these conditions but we have shown nests can be encouraged

to move in the height of summer and, therefore, may prove a useful strategy from early

spring through to late summer.

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Table 1: Repeated measures ANOVA table for mean Argentine ant nest numbers in 2008 Tanglefoot[®] banding and 0.5% boric acid bait trials. Period has three variables 1) pre Tanglefoot[®] banding; 2) post Tanglefoot[®] banding but pre boric acid bait introduction and 3) post Tanglefoot[®] banding and boric acid bait consumption

Effect	d.f.	F value	p value
Banding	1,31.7	122.66	<0.0001
Bait	1,31.7	0.32	0.5728
Week	10,122	7.62	<0.0001
Banding x Bait	1,31.7	2.44	0.1279
Banded x Week	10,122	7.64	<0.0001
Bait x Week	10,122	0.57	0.8343
Banded x Bait x Period	10,122	1.04	0.4125
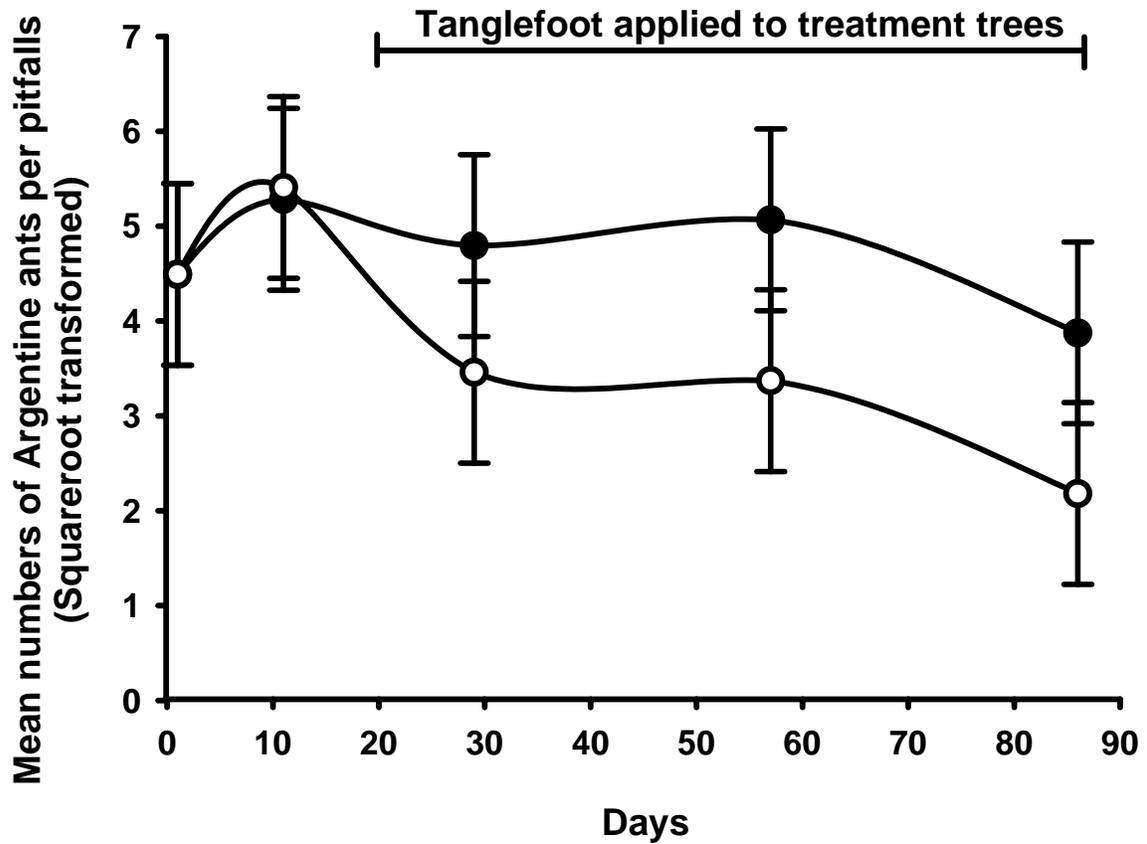
Figure 1: Mean (± S.E.) numbers of Argentine ants caught in pitfalls placed in the pine needle mulch around red maple trees in 2006. (●): indicates Argentine ants caught in pitfalls around trees where canopy foraging was permitted. (○): indicates Argentine ants caught in pitfalls around Tanglefoot[®] banded trees. The reduction in Argentine ants from Tanglefoot[®] banding was not significant at α = 0.05.
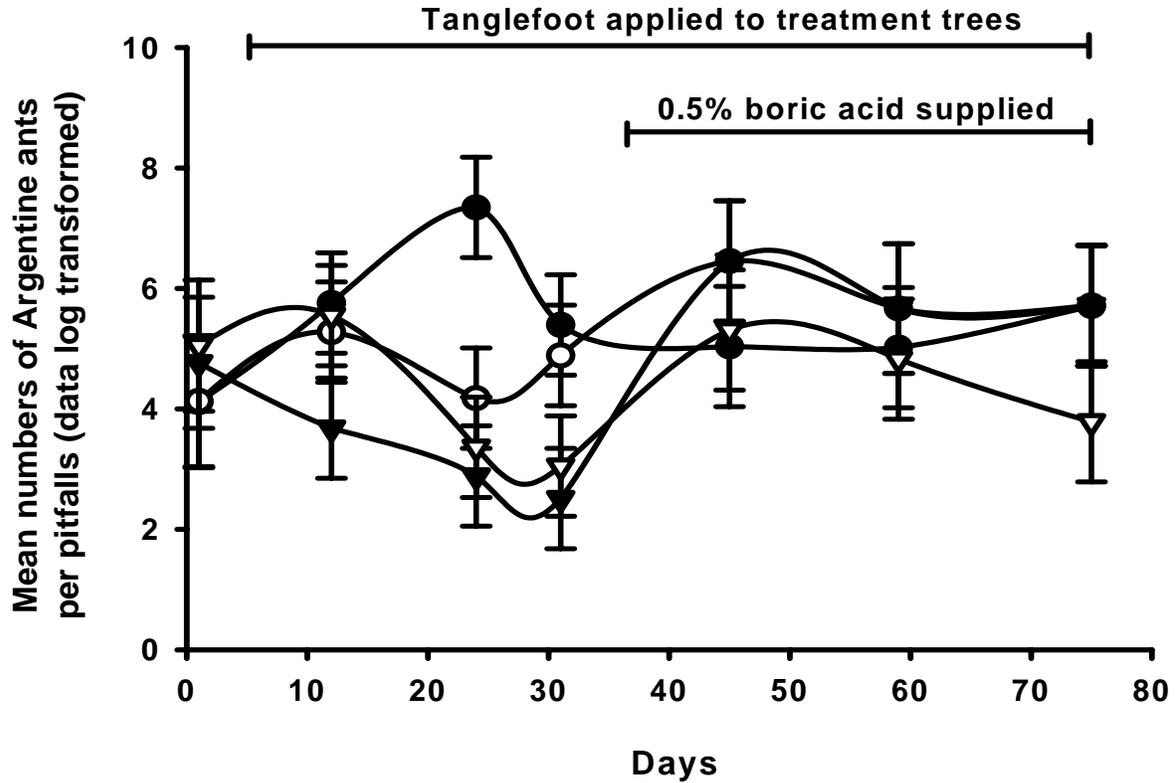
Figure 2: Mean (± S.E.) numbers of Argentine ants caught in pitfalls placed in 2007 in the pine needle mulch around red maple trees separated into pre and post 0.5% boric acid treatment. (●): indicates Argentine ants caught in pitfalls around all trees where canopy foraging was permitted before without boric acid treatment. (○): indicates Argentine ant caught in pitfalls around Tanglefoot® banded trees without boric acid treatment (▼): indicates Argentine ants caught in pitfalls around unbanded trees with boric acid bait treatment. (▽): indicates Argentine ant caught in pitfalls around Tanglefoot® banded trees with boric acid treatment, bait treatment begun 36 days after Tanglefoot® banding applied. The reduction in Argentine ants from Tanglefoot banding was not significant, nor was adding 0.5% boric acid at α = 0.05.
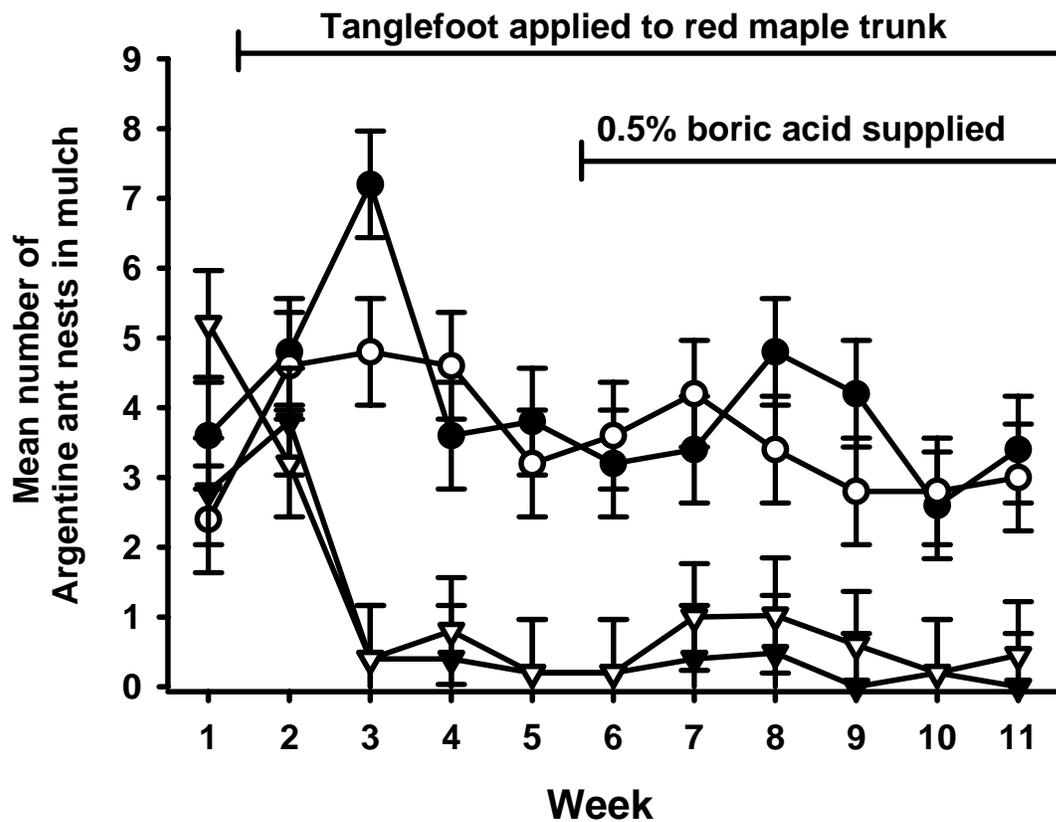
Figure 3: Mean (± S.E.) numbers of Argentine ant nests located in the pine needle mulch around red maple trees in 2007. (●): indicates Argentine ant nests located around trees where canopy foraging was permitted without 0.5% boric acid bait station added to surrounding mulch. (○): indicates Argentine ant nests located around trees where canopy foraging was permitted with 0.5% boric acid station added to surrounding mulch. (▼): indicates Argentine ant nests around Tanglefoot® banded trees without 0.5% boric acid bait station added to surrounding mulch. (▽): indicates Argentine ant nests around Tanglefoot® banded trees with 0.5% boric acid bait station added to surrounding mulch.
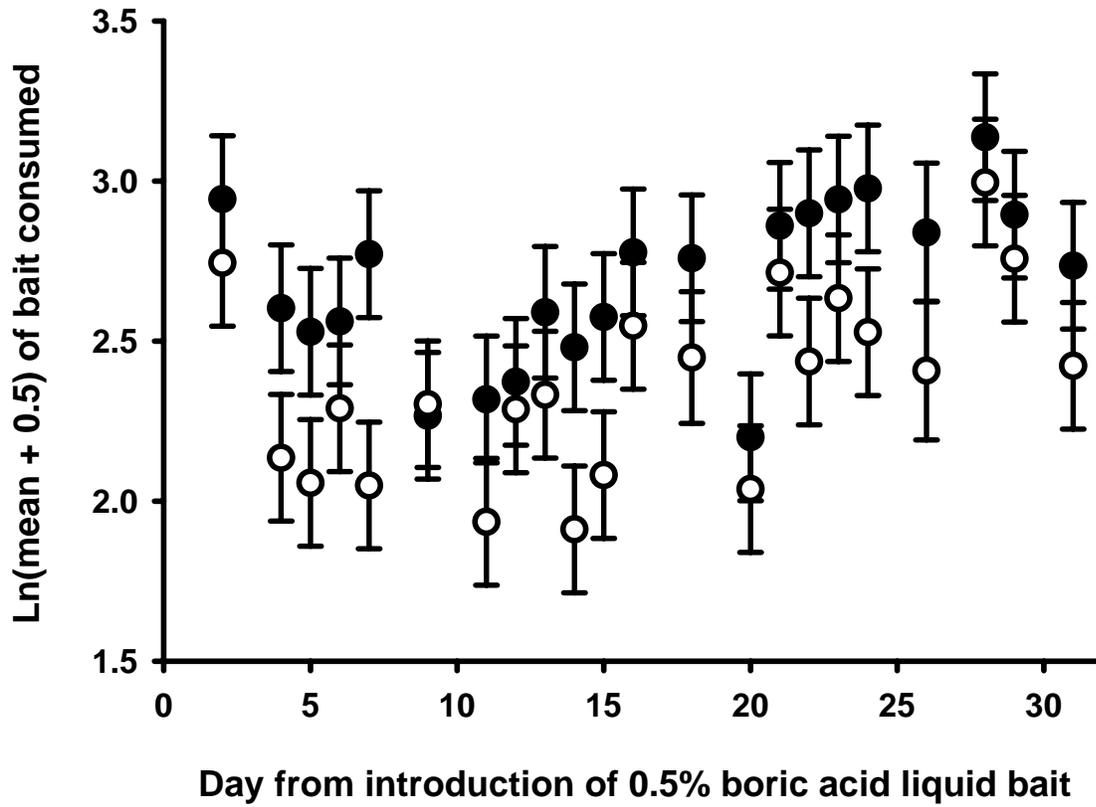
Figure 4: Mean (± S.E.) milliliters of 0.5% boric acid solution removed from bait stations in 2007. Volume data log (+ 0.5) transformed. (●): indicates volume of boric acid removed from bait stations around trees where canopy foraging by Argentine ants was permitted. (○): indicates volume of boric acid removed from bait stations around Tanglefoot® banded trees.
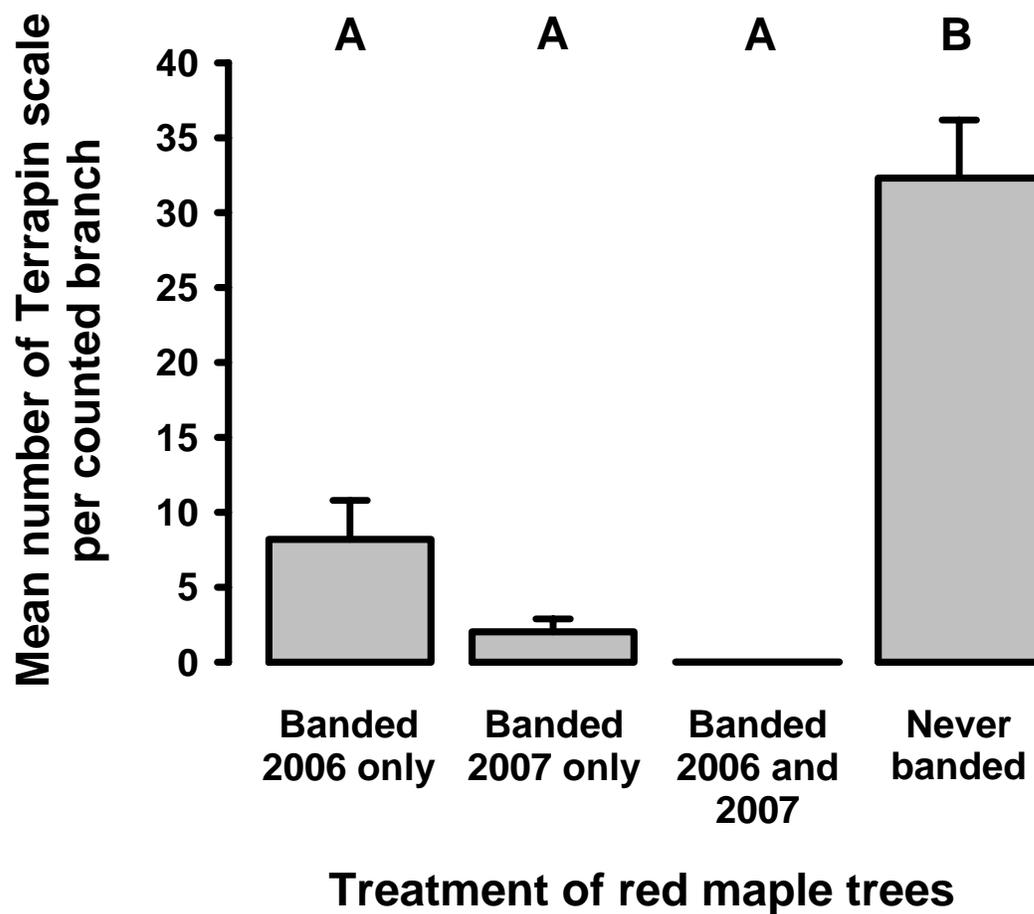
Figure 5: Mean (± S.E.) numbers of terrapin scale found per branch on Tanglefoot[®] banded and unbanded red maples in 2008. Branches on each tree were searched for terrapin scale until four infested branches counted or all branches surveyed. Those trees with less than four infested branches had zero terrapin scale recorded until four branches were recorded.

Chapter three

Title: Invasive Argentine ants reduce fitness of red maple via a

mutualism with an endemic coccid

Abstract

Many invasive ant species form mutualisms with honeydew-producing Hemiptera, offering protection in exchange for food. Invasive ants, such as the Argentine ant, have often been associated with hemipteran outbreaks in natural and agricultural ecosystems. A number of studies have demonstrated a net benefit to host plant fitness from ant-hemipteran mutualisms, however, few studies have quantified the effect an invasive ant-hemipteran mutualism would have on host plant fitness. We investigated the effects of a mutualism between the invasive Argentine ant and the endemic terrapin scale on scale density and the fitness of their host red maple. The terrapin scale has numerous natural enemies and we predicted that scale numbers would collapse once Argentine ants were excluded from the host tree canopy. Red maples rarely suffer high levels of non-hemipteran herbivory and we predicted that the elevated numbers of terrapin scale associated with this mutualism would impose a net fitness cost on the host red maple. We found that excluding Argentine ants from the host tree canopy led to a collapse of terrapin scale numbers. Red maples hosting this mutualism had lower seed mass and smaller leaves indicating that this Argentine ant-terrapin scale mutualism imposed a net fitness cost to the host tree. We conclude that for host plants that do not suffer from a high non-hemipteran herbivore load, invasive ant-hemipteran mutualisms are likely to impose a net cost to the fitness of the host plant.

Introduction

Biotic community processes are traditionally described in terms of competition and predation; however, the role of mutualisms in structuring ecosystems has received increasing recognition in recent decades (Bronstein 1994, Bruno et al. 2003). Many ant species form mutualisms with phloem-feeding Hemiptera and these mutualisms have been described as keystone interactions due to their extraordinary influence over the structure of a community (Eubanks and Strysky 2006). The tending ant species offers protection from predators and parasitoids to the honeydew-producing Hemiptera in exchange for honeydew, a ready source of carbohydrate (Hölldobler and Wilson 1990b). Access to honeydew can lead to incredible local abundances of ants, both in forest canopies (Davidson 1997b, Davidson et al. 2003) and on the ground (Hölldobler and Wilson 1990b) and this increased abundance in ants may have a cascading effect on food webs (Mooney 2007, Wimp and Whitham 2007).

Human activity has greatly increased the risk of biotic invasions around the world leading to a biotic homogenization of the worlds regions (Vitousek et al. 1997). Amongst exotic species, a small number of invasive ant species have proved to be particularly problematic, with the ability to penetrate and alter natural, agricultural and urban environments (Holway et al. 2002a). This small suite of ants has affected the abundances and species diversity of vertebrates, invertebrates and plants and is capable of disrupting whole ecosystems (reviewed in Holway et al. 2002a). The Argentine ant, *Linepithema humile*, among the most wide spread of invasive ants, is now found on six continents and

numerous oceanic islands (Suarez et al. 2001a). As with other invasive ant species, this ant appears set to extend its introduced range as opportunities for human mediated translocation increase with increased local, regional and global trade. This suggests that *L. humile*, along with other invasive ants, will remain a threat to as yet uninfested environments, as well as continue to alter biotic communities within the existing introduced range.

Tending of honeydew-producing Hemiptera by invasive ant species usually result in an increase in hemipteran densities (Abbott and Green 2007, Altfield and Stiling 2006, Coppler et al. 2007, Daane et al. 2007, Ness and Bronstein 2004) with *L. humile* consistently linked to outbreaks in honeydew-producing Hemiptera (Bartlett 1961, Buckley 1987, Daane et al. 2007, Lach 2003, Ness and Bronstein 2004, Way 1963) However, the direction of the indirect effect of a mutualist ant on host plant fitness may range from negative to positive, and is usually conditional on the level of non-hemipteran herbivory suffered by the plant. The fitness benefits to the host plant of any ant-mediated reduction in non-hemipteran herbivory has to outweigh the costs of increased hemipteran herbivory associated with ant-hemipteran mutualisms (reviewed in Strysky and Eubanks 2007b). Most studies investigating host fitness effects of ant-hemipteran mutualisms have involved native ant species (Strysky and Eubanks 2007b). There have been both net costs and benefits reported by those studies that have investigated the host fitness effects of an invasive ant-hemipteran mutualism (Altfield and Stiling in press, Blancafort and

Gomez 2005, Hill et al. 2003, Kaplan and Eubanks 2005, Lach 2007, O'Dowd et al. 2003).

We examined the effects on the hemipteran partner, and host tree, of an invasive ant-coccid mutualism involving *L. humile*. *Linepithema humile* tends infestations of the endemic terrapin scale, *Mesolecanium nigrofasciatum*, on their native red maple host, *Acer rubrum*. As noted above, for an ant-hemipteran mutualism to confer a net fitness benefit to the host plant, its non-hemipteran herbivore load must be high. *Acer rubrum* does host a number of herbivores although none are considered damaging (Johnson and Lyon 1988), and suffers relatively low foliar herbivory, averaging 4% of leaf area (Seastedt et al. 1983). We hypothesized, therefore, that the presence of *L. humile* in the canopy of *A. rubrum* contributed to the high numbers of *M. nigrofasciatum* seen in infested trees and that this interaction negatively affects host tree fitness. Our two predictions are that 1) *M. nigrofasciatum* numbers would decline in the absence of foraging *L. humile* and 2) the fitness of *M. nigrofasciatum* infested *A. rubrum* would be lower for those trees where *L. humile* workers are permitted to forage in the canopy than those trees where canopy foraging by *L. humile* was restricted.

Methods and Materials

Study species and site

The distribution of *A. rubrum* stretches from Manitoba to Newfoundland in the north and east Texas to south Florida in the south of North America (Walters and Yawney 1990). It is commonly found in forests in North Carolina and elsewhere and is extensively used as an ornamental tree. *Mesolecanium nigrofasciatum* is an endemic coccid found from Ontario to Florida, mainly to the east of the Mississippi River (Simanton 1916b, Williams and Kosztarab 1972b). It has been found on over 30 host plant species (Williams and Kosztarab 1972b). This coccid has occasionally proved damaging to blueberries and fruit trees although this damage was not linked to ant tending (Milholland and Meyer 1984, Simanton 1916b). *Linepithema humile* is an invasive species with a world-wide distribution including North Carolina (Suarez et al. 2001a) It is noted for its aggressiveness, and its propensity to enter into mutualisms with a wide variety of honeydew-producing Hemiptera (Holway et al. 2002a).

The site of this study is a commercial business park located outside Raleigh, North Carolina, USA. Within this site is a contiguous infestation of *L. humile* covering approximately 42Ha. The site has extensive ornamental plantings utilizing *Quercus, Pinus, Ilex* and *Lagerstroemia* species as well as *A. rubrum*. Many of the roads running through the site have lines of *A. rubrum* planted along the curbs. All trees selected for our experiments were of a similar size and were planted at the same time. Pine needle mulch is used around all ornamental plantings and is where the majority of *L. humile* nests are

found. There are remnants of secondary growth forest along stretches of park boundary. These remnants mainly consist of *Pinus, Quercus* and *Acer* species. Extensive surveying for *M. nigrofasciatum*, both within and outside the *L. humile* infestation, was undertaken in mid-July 2005 when 3rd instar females moved from the leaf and settled on twigs (Simanton 1916b). During these surveys *M. nigrofasciatum* was never observed on *A. rubrum* outside of the *L. humile* infestation even though native ant species such as *Tapinoma sessile, Crematogaster* spp., and *Formica* spp. were often seen foraging on these trees. *Mesolecanium nigrofasciatum* was only observed on those *A. rubrum* with foraging *L. humile*. The *A. rubrum* trees selected for our experiments were watered daily in 2006 through the irrigation system run by the commercial park management. In 2007, the watering regime was reduced to once weekly due to a severe drought that gripped North Carolina. In both years, new mulch and commercial, pelletized, fertilizer was added to all trees in early spring, as a standard component of tree management within the commercial park.

Effect of Argentine ant presence on terrapin scale numbers

A line of ten mature, *M. nigrofasciatum*-infested, *A. rubrum* trees was selected for this experiment. An initial count of *M. nigrofasciatum* and foraging *L. humile* was undertaken 20th July on four selected branches per tree before *L. humile* was excluded from the canopy of treatment trees. This count was recorded as week one. One or more stems on each of the four branches were chosen for the scale count, these branches were three to four meters in length with the scale settling approximately 500mm from the stem

tip. Initial coccid counts per branch ranged from 35 to 444 individuals. For each tree, a Tanglefoot® pest barrier (Tanglefoot Company, Grand Rapids, Michigan, USA) was applied to two of the four branches; the treatment branches being chosen at random. The barrier was positioned as close to the trunk of the tree as practicable and consisted of a wide band of duct tape covered with Tanglefoot®. Weekly counts of *M. nigrofasciatum* and *L. humile* were conducted on the 10 experimental trees after the initial count until week 10 and then fortnightly to week 18. Counts of *L. humile* were taken just trunk side of the *M. nigrofasciatum* infestation with workers travelling both to and from the infestation included. Tanglefoot® banded trees were checked weekly for barrier failure and Tanglefoot® reapplied as necessary. Data was tested using PROC MIXED repeated measures ANOVA, with barrier treatment as the between-subjects variable and time as the within-subjects variable, and was performed using SAS v.9.1.3 (SAS Institute Inc.).

Effect of Argentine ant canopy foraging on red maple fitness

Along one road of the commercial park are rows of *A. rubrum*, each consisting of four to five mature trees. Six of these rows of *A. rubrum* were chosen for this experiment. Three of these rows were randomly selected for Tanglefoot® treatment, consisting of tanglefooting all trees in the row at breast height in early August, excluding foraging *L. humile* from the canopy until leaf drop in November. Tanglefoot® was reapplied twice weekly to prevent *L. humile* from gaining access to the treatment tree canopy. Leaf and seed samples were collected in April the following year. The winged fruit (samaras) were collected in mid-April shortly before leaf development (Walters and Yawney 1990).

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Seed dispersal lasts one to two weeks and collection was delayed as long as possible to collect mature seeds (Walters and Yawney 1990). Samaras were collected from all 18 trees in this experiment. The samaras were collected by running a closed fist down samara-bearing twigs. All samaras capable of being reached from the ground were collected from each tree, samaras being collected from all points around the tree bearing samaras. We chose to compare dried seed mass of treatment and control trees; dried seed mass minus dispersal structures having been suggested as the best and easiest of many methods of seed measurement (Westoby 1998). The samaras from each tree were placed in a paper bag and dried in an oven (60°C) for 7 days. We then randomly selected 3 bags from each row for weighing. From each bag, 40 samaras were randomly selected and the dried seed excised from the samara. Each seed was weighed on a Cahn C27 Electrobalance (Cahn Instruments Inc. Cerritos California, U.S.A.).

Leaf growth in *A. rubrum* is indeterminate with two to three early, or preformed, pairs of leaves emerging from the budscale before subsequent late leaves develop (Critchfield 1971). Early leaf size was chosen as a measure of plant fitness as these early leaves were formed the previous season from resources accumulated when the Tanglefoot[®] treatment was in place on treatment trees (Kikuzawa 1983). Therefore, faster developing early leaves should indicate more stored resources allocated to leaf growth and, therefore, indicate higher fitness of the tree. Leaves were collected in late April from all trees in each of the six rows before the development of late leaves. Leaf collection consisted of plucking the two or three pairs of early leaves (Critchfield 1971) from individual twigs

from those branches with leaves on them; ensuring leaves were collected from all points of the tree with branches bearing leaves. In all, 1494 leaves from the 18 selected *A. rubrum* trees were then measured. Larger mean leaf size would indicate earlier leaf bud and/or faster growth. Leaf size was determined by measuring the distance between the tips of the outer lobes of the leaf. Some of the smallest leaves were not sufficiently developed to distinguish the outer lobes and in this case all leaves from the twig were discarded. Gottschalk (1994) demonstrated that shading could significantly decrease average *A. rubrum* leaf size. The trees selected for this experiment were all situated so that they received full sun. Leaf width and seed weight data were tested using Proc GLM ANOVA, and was performed using SAS v 9.1.3 (SAS).

Results

Mesolecanium nigrofasciatum abundance on *A. rubrum* was dependent on the presence of canopy foraging *L. humile*. The Tanglefoot® banding was effective in reducing *L. humile* foraging with *L. humile* presence on Tanglefoot® banded branches reduced to near zero once the banding was applied ($F_{1,18} = 32.42$, $p < 0.0001$; fig 1). There was a reduction in *M. nigrofasciatum* abundance on the treatment branches compared to the control branches once the Tanglefoot® band was applied with *M. nigrofasciatum* numbers being reduced to virtually zero on the treated branches ($F_{1,38} = 29.25$, $p < 0.0001$; fig 1).

The mean dried seed mass from the Tanglefoot® banded trees was heavier than the mean dried seed mass from the unbanded trees ($F_{5,12} = 6.03$, $p = 0.0051$; fig 2) as would be expected where more resources allocated to each seed produced on those *A. rubrum* where *L. humile* was excluded from the canopy than in the control trees. Likewise, leaves from the Tanglefoot® banded trees were wider than those from the unbanded control trees ($F_{5,12} = 5.48$, $p = 0.0075$; fig2). This indicates that *A. rubrum* leaf bud was earlier and/or leaf growth was stronger in those trees where *L. humi*le was denied access to the canopy.

Discussion

The presence of *L. humile* supported the high abundances of *M. nigrofasciatum* on *A. rubrum*, with *M. nigrofasciatum* abundance decreasing when *L. humile* attendance was reduced. The fitness of *A. rubrum* increased when *L. humile* was denied access to their canopies, with heavier dried seed mass seen in the following spring along with larger leaves indicating earlier leaf bud and/or faster leaf growth.

An extensive survey in 2005 for *M. nigrofasciatum* outside of the *L. humile* infestation found no individuals even though there are numerous suitable host plants within crawler ballooning distance. This, unfortunately, prevented any direct comparison of the benefits offered by native ants and *L. humile* to *M. nigrofasciatum*. It does strongly suggest, however, that native ant-*M. nigrofasciatum* mutualisms offer little protection to the coccid from natural enemies. Ant-coccid mutualisms are usually facultative and highly conditional, dependent on honeydew quality (Henneberry et al. 2000) and protein requirements of the ant colony (Cushman 1991, Ness and Bronstein 2004). In their introduced range, *L. humile* is well known as a facultative mutualist partner with a wide variety of honeydew-producing Hemiptera (Holway et al. 2002a). In previous studies, the presence of *L. humile* has consistently been associated with increased numbers of its mutualist partner (Altfield and Stiling 2006, Daane et al. 2007, Ness and Bronstein 2004).

Mesolecanium nigrofasciatum is subject to predation from a host of predators and is known to be parasitized by numerous parasitoids (Simanton 1916b, Williams and

Kosztarab 1972b) with very high rates of parasitism reported in North Carolina (Devorshak 1994, Meyer et al. 2001). Invasive ant species, such as *L. humile*, readily enter into mutualist interactions with honeydew-producing Hemiptera, the main benefits thought to be protection from parasitoids and predators (Buckley 1987, Way 1963). On our study trees, *L. humile* offered an effective though not complete protection for *M nigrofasciatum*. The larvae of the Coccinellid, *Hyperaspis binotata*, and the predaceous Lepidoptera, *Laetilia coccidivora*, are known predators of *M. nigrofasciatum* (Simanton 1916b), and were found in *L. humile* tended *M. nigrofasciatum* infestations.

The increased numbers of *M. nigrofasciatum* could be expected to exert some direct fitness costs upon the host *A. rubrum* through increased phloem removal. In a number of studies this direct cost has been offset for the host plant through the indirect fitness benefits accrued from the hemipteran-tending ant species deterring non-hemipteran herbivores (Eubanks and Strysky 2006, Strysky and Eubanks 2007b). The indirect effects of the presence of an ant-hemipteran interaction on a plants' fitness can range from negative through neutral to positive and is usually conditional on the level of non-hemipteran herbivory suffered by the plant (Ness and Bronstein 2004, Strysky and Eubanks 2007b). Sap-sucking Hemiptera can place considerable stress on a plant and non-hemipteran herbivory levels need to be high before the ant-hemipteran interaction becomes the preferred stress (Strysky and Eubanks 2007b).

Strysky and Eubanks (2007b) found most studies on ant-hemipteran mediated effects demonstrated net benefits to host plant fitness. An invasive ant-hemipteran mutualism may prove beneficial to the fitness of those host plants with severe herbivore loads. For those plants rarely threatened with extensive insect defoliation, the cost of elevated hemipteran densities promoted by invasive ants, such as *L. humile*, may overwhelm any fitness benefit from reduced defoliation. Studies investigating the effect on plant fitness of an invasive ant-hemipteran mutualism have found both positive and negative effects. Kaplan and Eubanks (2005) found a net positive benefit to the host cotton plant from a mutualism between the invasive ant *Solenopsis invicta* and the cotton aphid, *Aphis gossypii*. However, most studies reported negative effects. In the Seychelles the presence of the yellow crazy ant, *Anoplolepis gracilipes*, was shown to reduce the leaf size of two trees, *Pisonia grandis* and *Scaevola sericea*, and reduce the foliage cover of *P. grandis* (Hill et al. 2003). On Christmas Island, an *A. gracilipes* promoted coccid outbreak resulted in canopy die back from increased sooty mould cover (O'Dowd et al. 2003).

Invasive ant-hemipteran mutualisms are known to disrupt insect pollination of some entomophilous plants (Blancafort and Gomez 2005, Lach 2007). *Acer rubrum* is considered amphiphilous, being pollinated by both wind and insects. (Wodehouse 1945). There is the possibility that canopy foraging by *L. humile* may have exerted a fitness cost through deterrence of potential pollinators from infested *A. rubrum*. *Acer rubrum* flowers are highly attractive to bees, however, the plant is considered to be mainly wind

pollinated and any such effect likely has minimal effect on tree fitness (Wodehouse 1945).

We found that the presence of *L. humile* in the host canopy was associated with a reduction in two indicators of *A. rubrum* fitness. This is presumably due to elevated phloem removal associated with the positive relationship between *L. humile* foragers in the canopy and *M. nigrofasciatum* abundances. In contrast, Altfield and Stiling (in press) found that the coastal shrub *Baccharis halimifolia* suffered lower mortality through decreased numbers of the stemborer *Oidaematophorus balanotes* on those shrubs where *L. humile* tended an aphid. These opposite conclusions highlight that a net positive effect on the host plant of such mutualisms require other, more damaging, herbivores to be deterred by the ant.

Herbivory can induce a compensatory response from plants (reviewed in Trumble et al. 1993). It is possible that host *A. rubrum* allocated more resources to seed production and leaf growth in response to a possible increase in foliage herbivory after the exclusion of *L. humile* from their canopies. We did not quantify abundances of defoliating herbivores and, therefore, cannot rule out this possibility. However, long lived woody plants, such as *A. rubrum*, have a low intrinsic growth rate and, therefore, any capacity for compensatory response to herbivory is limited (Stamp 2003). A number of studies have reported that native ant-hemipteran mutualisms deterred damaging defoliating herbivores in woody plant hosts (e.g Floate and Whitham 1994, Karhu 1998, Warrington and

Whittaker 1985). Woody plants would also be expected to invest heavily in other herbivore defences, including chemical defensive measures, and relinquish the option for compensatory growth (Coley et al. 1985). *Acer rubrum* invests heavily in direct, chemical, antiherbivore defences. (Abou-Zaid et al. 2001, Loughrin et al. 1997, Müller-Schwarze et al. 1994), As previously mentioned, *A. rubrum* suffers relatively low levels of herbivory. *Acer rubrum* is, however, susceptible to attack from a variety of phloem and xylem feeders (Johnson and Lyon 1988). The apparent reliance of *A. rubrum* on predators and parasitoids to control sap-sucking Hemiptera breaks down in the presence of an invasive ant species, such as *L. humile*, with a proclivity for entering into mutualisms with sap-sucking Hemiptera. It appears that those *A. rubrum* with *L. humile* foraging in their canopy simply suffered an increased net fitness cost from increased phloem loss induced by high numbers of ant-protected Hemiptera.

Our results contrast with those of Kaplan and Eubanks (2005) in an invasive ant-aphid mutualism on cotton, where the evolution of extrafloral nectaries on the host cotton plant indicates a reliance on ant-hemipteran mutualisms as part of its antiherbivore defence. They also contrast with the indirect benefits host trees accrued from native ant-native hemipteran mutualisms (Floate and Whitham 1994, Karhu 1998, Whittaker and Warrington 1985) where the host tree may have evolved to take advantage of such mutualisms and the host plants face damaging defoliating herbivores. In our system the host tree *A. rubrum,* like many trees, invests heavily in chemical antiherbivore defences such that any fitness advantage from naturally occurring ant-hemipteran mutualism are

likely to be modest at best. We conclude that for host plants that do not suffer from a high non-hemipteran herbivore load, invasive ant-hemipteran mutualisms are likely to impose a net cost to the fitness of the host plant.

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a) Foraging *L. humile* per branch

b) *M. nigrofasciatum* per branch

WEEK
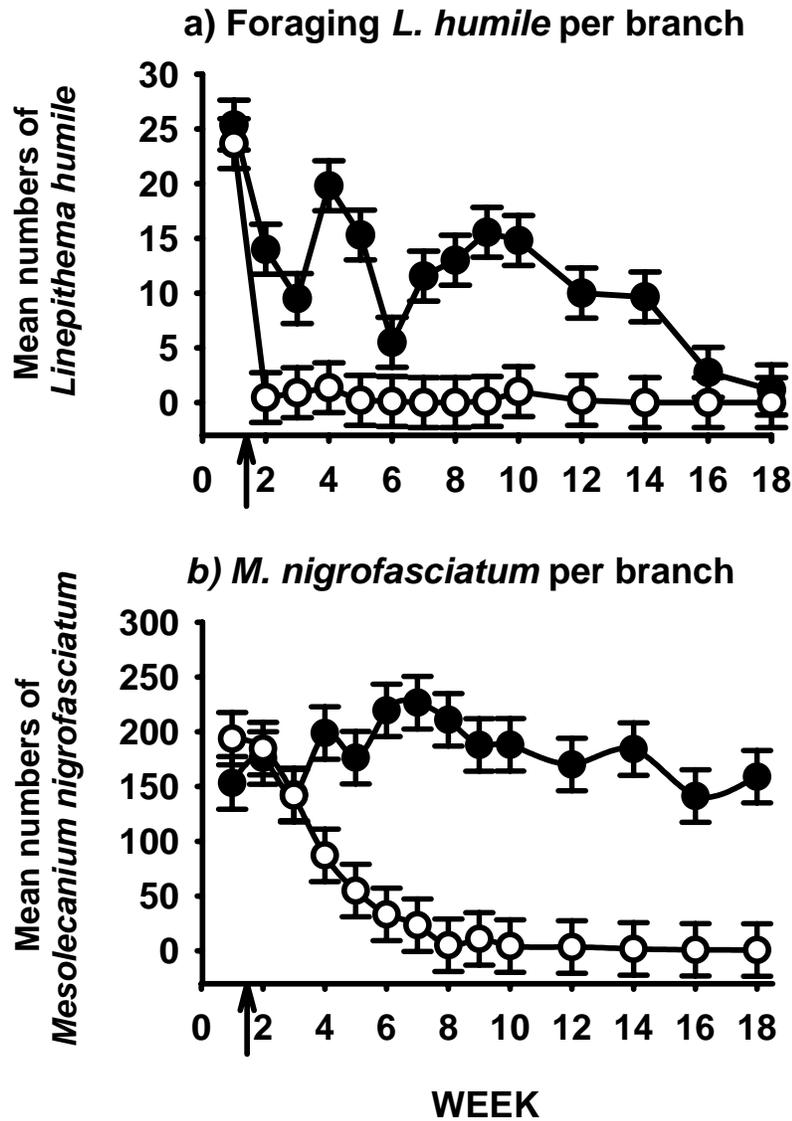
Figure 1: Mean (± S.E.) numbers of a) *L. humile* foraging per branch and b) *M. nigrofasciatum* per branch of *A. rubrum* trees with branch access permitted or denied to foraging *L. humile*. (●): indicates *L. humile* permitted to forage on branches. (○): indicates *L. humile* excluded from branches. Arrow indicates day when Tanglefoot® banding was applied to treatment branches.
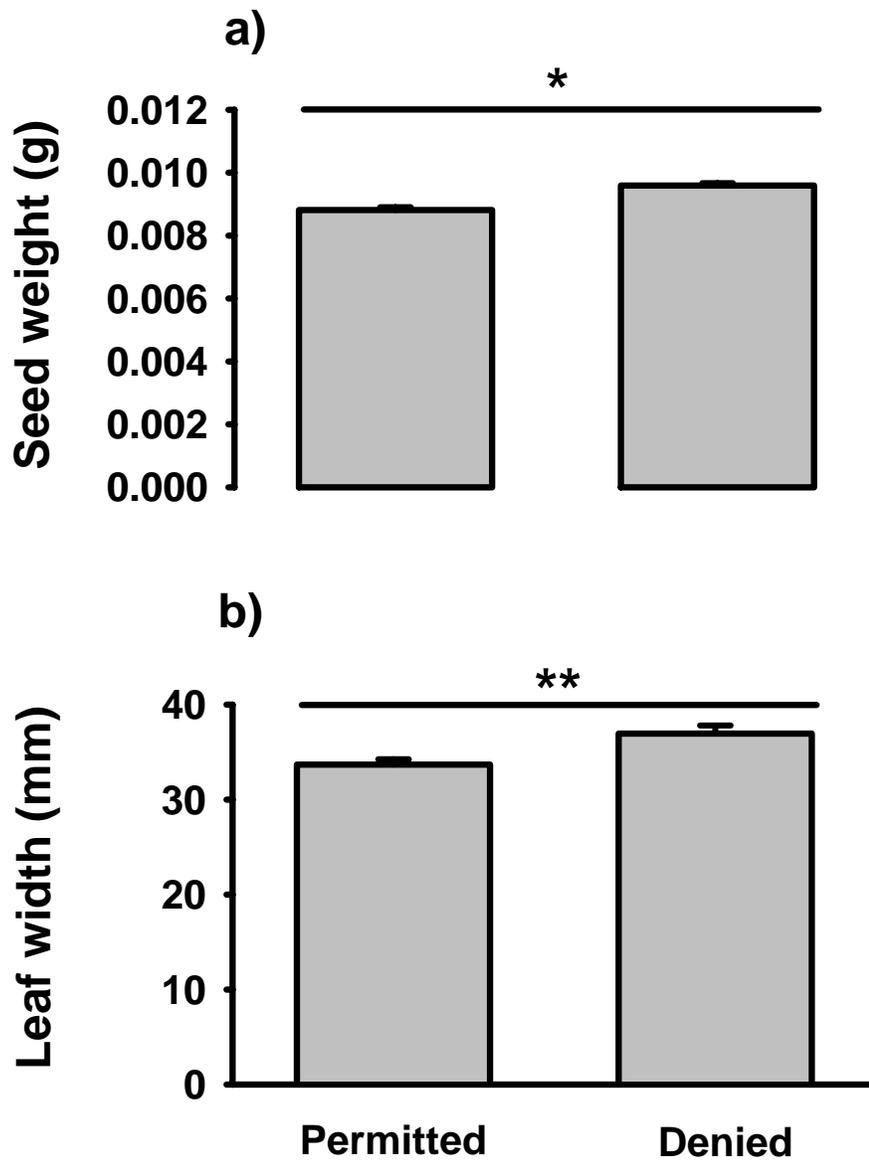
Figure 2: Effects on *A. rubrum* fitness the following spring when *L. humile* canopy foraging was permitted or denied. (**a**) Mean (± S.E.) dried seed weight, with 40 seeds weighed per tree. (**b**) Mean (± S.E.) leaf width. Wider leaf width infers earlier leaf bud and/or faster leaf growth. Asterisk indicates significant difference between treatments by general linear model: $* < 0.05$; $** < 0.005$.

Chapter Four

Pine trees facilitate the survival of an invasive ant at the edge of its

distribution

Abstract

All organisms have distribution boundaries set by abiotic and biotic constraints. Facilitative interactions, where one participant exerts a positive effect on another, may allow an organism to overcome abiotic constraints at the edge of their range. We report on an interaction whereby a tree made an otherwise inaccessible food resource available to an ant. The invasive Argentine ant is distributed worldwide with many areas at the edge of its introduced range considered too cold for survival away from human structures. At the northern edge of its range in the eastern United States we observed Argentine ants foraging on loblolly pines during the winter months even when ambient temperatures should have prevented all foraging. In the laboratory we investigated if Argentine ants could survive temperatures above freezing but below their minimum foraging temperature of 5°C. In the field we recorded the distribution of Argentine ant colonies to determine where their winter aggregation sites were located. Temperatures of the trunks of loblolly pine were recorded and Argentine ant workers trailing up and down were collected and weighed. We found that the Argentine ant starves to death when temperatures remain below 5°C. The Argentine ants aggregated within loblolly pine stands where they nested in the soil. Even when ambient temperatures were below the Argentine ants foraging temperature envelope the southern (sun-facing) side of the trunk absorbed enough radiant energy to raise bark temperatures high enough to allow Argentine ant foraging. Argentine ant foragers trailing down the trunk were consistently heavier than foragers trailing up the tree indicating the Argentine ant is feeding on a food source we suspect is honeydew from hemipterans dwelling in the canopy. Loblolly pine

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provided a suitable microclimate that allowed the Argentine ants access to an otherwise inaccessible liquid food source that is required to survive cold winters away from human structures. We believe that this abiotic/biotic interaction may be more common than realized and may explain the persistence of some organisms at the edge of their range.

Introduction

All organisms have distribution boundaries set by abiotic and biotic constraints. Release from a biotic constraint, such as a new food source, can extend the distribution of an organism into previously unsuitable environments (Pope et al. 2008, Zimmerman 1973). Similarly, an altered microclimate provided by one organism may allow another to overcome abiotic constraints (Armas and Pugnaire 2005, Drezner 2006, 2007). These facilitative interactions may also extend the distribution patterns of an organism even though this facilitation is unintentional. (Bertness and Callaway 1994). Facilitative interactions that modify a microclimate to allow access to previously inaccessible food sources may be a common, but little reported, mechanism for the survival of an organism along the edge of its ranges. Here we report on one such occurrence.

Invasive species represent an interesting opportunity to understand the extent to which mutualisms, and their absence might influence the fundamental and realized niches of species. Invasive species have been shown to facilitate the establishment of each other in new environments (reviewed in Simberloff and Von Holle 1999). Exotic species have even been considered facilitators of native species (Rodriguez 2006). The small suite of invasive ants may prove useful for examining the role of mutualisms in range expansion. Invasive ants are noted for readily entering into mutualisms with many native and exotic honeydew-producing Hemiptera and extra floral nectaried plants (Ness and Bronstein 2004) and these mutualisms may be important in the successful establishment of the ant (Helms and Vinson 2008, Holway et al. 2002).

One invasive ant species, the Argentine ant *Linepithema humile* may be particularly suitable for understanding the effect of facilitation in extending an organisms range as it readily engages in mutualisms with many honeydew-producing Hemiptera. *Linepithema humile* is a highly invasive ant originating from the lower Paranà River drainage system of Argentina (Wild 2004). Considered a tramp species (Hölldobler and Wilson 1990, Passera 1994), it has now spread to all continents except Antarctica as well as many oceanic islands (Suarez et al. 2001). This ant species prefers Mediterranean climates but can be found in some subtropical and warm temperate regions of the world (Roura-Pascual et al. 2006). *Linepithema humile* are thought to be severely restricted by cool weather (Buczkowski et al. 2004, Hartley and Lester 2003, Krushelnycky et al. 2005) with subfreezing temperatures being lethal to *L. humile* (Herbert 1932). Foraging is restricted to ambient temperatures above 5°C which has direct impacts on survival (Markin 1970b). While it has long been known that *L. humile* takes advantage of mutualist partners, it has never been clear whether mutualists allow this ant to extend their fundamental niche or simply to just perform better within it. However, our preliminary observations suggest that at the edges of its distribution, *L. humile* may rely on honeydew-producing Hemiptera where they would otherwise be confined to human structures during the cold winter months.

Linepithema humile are known to coalesce into fewer, but larger, nests during the winter months and will often relocate nests down into the soil to escape cold temperatures

(Heller and Gordon 2006, Newell and Barber 1913). It is unknown if *L. humile* can survive long periods of cold that would prevent foraging for food. We had previously observed *L. humile* nests amongst loblolly pine, *Pinus taeda*, stands during winter months. We also observed *L. humile* trailing up and down *P. taeda* even though the ambient temperature was well below the minimum foraging temperature of 5°C. *Linepithema humile* were observed apparently collecting honeydew from *Toumeyella virginiana* on *P. taeda* during the coldest months of the year (A.D. Rowles pers. comm.).

Observing this foraging when conditions suggest that *L. humile* foraging should be curtailed, we asked three questions: 1) could *L. humile* survive extended periods at temperatures that should curtail foraging and if nearby food could forestall death? 2) did *L. humile*, indeed, forage in the field at temperatures below their reported foraging minimum temperature and was this disproportionately on *P. taeda*? 3) were *L. humile* collecting food when foraging on *P. taeda* during apparently unsuitable foraging conditions?

Methods and Materials

Our study site was a commercial park near Raleigh, North Carolina with a large infestation of *L. humile*. The site has extensive ornamental plantings with a variety of large trees including *Acer*, *Quercus*, and *Lagerstroemia* species as well as extensive plantings of *P. taeda*. These *P. taeda* plantings are located in a number of windbreaks within the commercial park.

Feeding at low temperatures

Linepithema humile nests were created from laboratory colonies collected from the field and consisted of one queen and 100 workers and a few brood. The experimental nests were housed in a 591ml plastic containers (S.C. Johnson and Son Inc., Racine WI 53403) with anti-viral insect screen panels glued into the lid and bottom of the container to allow airflow. Each nest contained a moistened plaster nest measuring 80mm in diameter. The experimental nests were subjected to differing temperature and food regimes. Nests were allocated to a constant temperature of either $4^{o}C$ or $26^{o}C$ with relative humidity held at 50%. For each temperature five nests were assigned to one of three food regimes consisting of water, water plus 25% sucrose-water, or water, 25% sucrose-water and canned tuna. Water and sucrose-water were presented in 6 x 50mm disposable culture tubes (FisherScientific, 2000 Park Lane Drive, Pittsburgh, PA 15275 USA) with a cotton wool wick. Approximately 1g of tuna was presented in a 40 x 40 x 8mm weighing dish (FisherScientific, 2000 Park Lane Drive, Pittsburgh, PA 15275 USA). Weekly counts of surviving queens and workers were undertaken for 3 weeks. Each week after surviving

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queens and workers were counted, dead ants were removed and all water tubes, sucrose-water tubes, and tuna were replaced, ensuring that nests did not run out of any allocated food resource. Treatment effects on ant mortality were analyzed using GLM repeated measures ANOVA (Systat).

Aggregation of L. humile *during winter months*

Four *P. taeda* windbreaks were selected to follow the distribution of *L. humile* through the surveyed months. These windbreaks were located between parking lots that had landscaped traffic islands scattered through them planted with *Acer*, *Quercus* and/or *Lagerstroemia* species. The ornamental trees within these traffic islands provided convenient points to survey for *L. humile*. All traffic islands had at least one ornamental tree with pine straw mulch at the base. *Linepithema humile* nest searching was modified from Heller and Gordon (2006) to avoid destroying the nests. We first carefully peeled back the mulch around the ornamental tree to be surveyed. *Linepithema humile* do not relocate after disturbance by temporary pine straw mulch removal (Silverman et al. 2006). The ground exposed around the tree was intensively probed with a thin length of steel wire. Workers would rapidly appear on the soil surface to defend the disturbed nest. Monthly surveys for *L. humile* nests were conducted from November 2007 to May 2008. Our method could not quantify the size of the disturbed nests and, consequently, could not determine how many nests were around each tree base. Therefore, we recorded the presence or absence of nests surrounding each surveyed trees. Monthly differences in *L. humile* distribution within the survey plots was examined by calculating similarity indices

for tree species with *L. humile* nests at the base by month using Bray-Curtis similarity coefficients. The relative similarity of months and of species was plotted using a non-metric multidimensional scaling algorithm.

Foraging of L. humile *on* P. taeda *trunks*

One tree from three of the *P. taeda* windbreaks was chosen for temperature and *L. humile* foraging surveys. These three trees were chosen because they received sunlight from sunrise to sunset, the shortest illumination time being approximately 10 hours per day in early January 2008. All temperature and foraging surveys were taken at 1300 hrs on days with full sun with ambient temperatures ranging from 1°C to 17.2°C. The trunks of these three *P. taeda* had received approximately 5.5 hrs of direct sun before surveying, ensuring that the trunks had time to warm sufficiently for *L. humile* foraging (U.S.N.O. 2008). Temperature was recorded at breast height on the trunk with a mini infrared thermometer (FischerScientific, 2000 Park Lane Drive, Pittsburgh, PA 15275 USA) at the mid point of the sun-illuminated section and shaded sections of the trunk. Ambient temperature was obtained from the National Weather Service Automated Surface Observing System weather station at Raleigh-Durham Airport, North Carolina (SCO 2008). This weather station is located ~5km from the study site. This weather station records ambient temperature hourly and the 1250 hrs reading were used for our ambient temperature reading.

After temperatures were recorded from each tree, 10 *L. humile* workers trailing up the

trunk of each tree and 10 workers trailing down from the tree were collected separately

by aspiration. We chose workers moving in a steady trail and aspirated the next ant

approximately 20 seconds after the previously worker was collected to reduce any

sampling bias. We did not observe *L. humile* foraging on the shaded side of the *P. taeda*

trunks across the ambient temperatures surveyed. The collected workers were sealed in a

50ml centrifuge container (Evergreen Scientific, Los Angeles, California USA) and

stored in a polystyrene container to keep the ants cool until they could be frozen (-20°C).

Care was taken to ensure that the *L. humile* workers aspirated were from a trail and not

displaying searching behavior (Deneubourg et al. 1990). The frozen ants were weighed

on a Cahn C27 Electrobalance (Cahn Instruments Inc. Cerritos California, U.S.A.). Both

trunk temperatures and ant weights were analyzed using GLM ANOVA (Systat).

Results

Linepithema humile nests subjected to 26°C and provided with sucrose water or sucrose water and tuna had high worker survival through three weeks (Fig 1; Table 1). Those *L. humile* nests subjected to 4°C had poor survival regardless of the food regime offered and were extirpated by the third week (Fig 1; Table 1). Interestingly, those nests subjected to water only but were kept at 26°C showed similar mortality to those nests kept at 4°C, indicating that mortality was through starvation rather than cold.

There were monthly distributional differences in *L. humile* nests within our survey plots as shown by the non-metric scaling algorithm (Fig 2). The coldest months; January, February and March, contrasted strongly with the four warmest months. Individual tree species showed a difference in *L. humile* nest association through the surveyed months with most of the *L. humile* nests located around *P. taeda*, the only evergreen tree that was surveyed (Fig 3). *Quercus* and *Pinus taeda* fell into the same quadrant indicating they shared some similarities in *L. humile* nests associated with the base of the trees, although the distance between them is large. Interestingly, both species have bark that is darker and rougher than either *Lagerstroema* or *Acer rubrum*. Both *Lagerstroema* and *Acer rubrum* contrast strongly with each other and with *Quercus* and *Pinus taeda*.

The temperature on the sun-exposed side of the *P. taeda* trunk was higher than the temperature on the shaded side across the range of ambient temperatures until ambient temperature reached 20°C (F = 34.167, df 1, p < 0.0001; Fig 4). The weights of *L. humile* workers trailing down the trunk of *P. taeda* were heavier than those workers trailing up

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the trunk indicating that *L. humile* workers were feeding in the *P. taeda* canopy (F = 119.235, df 1, p < 0.0001; Fig 5). There was a remarkable increase in ant weight for those workers trailing down trunks when ambient temperatures were over 12°C than when ambient temperatures were under 12°C indicating that workers may be able to consume more food above this foraging temperature threshold (F = 45.401, df 1, p < 0.0001; Fig 5).

Discussion

Linepithema humile can not survive long periods without a food source. When temperatures were consistently below the minimum foraging temperature of $5^{\circ}C$, *L. humile* starved even with food available. In areas where winter temperatures remain below suitable foraging temperatures for extended periods, *L. humile* should not be able to establish away from human structures. In our study site, winter aggregations of *L. humile* tended to concentrate around *P. taeda* stands that provided a suitable temperature permitting worker foraging. We suspect that *T. virginiana* adults and nymphs infesting these *P. taeda* provided a consistent winter honeydew resource to sustain *L. humile* nests made accessible by the elevated bark temperature.

An important question in understanding the limits to the distribution of species is how mutualisms may sustain a population at the edge of its range. Finding a suitable partner may be critical for the range expansion of a number of species reliant on mutualisms for survival or reproduction. For example, invasive plants require a new suite of suitable pollinators and/or seed dispersers to replace those found in the invaders home range (Richardson et al. 2000). Invasive ants often provide protection to exotic, and native, honeydew-producing Hemiptera (Ness and Bronstein 2004). Honeydew is thought to allow some ant species to dominate an ant community through maintaining large numbers of workers (Davidson 1997, 1998). *Solenopsis invicta* colonies grew larger on a diet of honeydew and arthropod protein than just protein alone (Helms and Vinson 2008) and *L. humile* are noted for their affinity for carbohydrate-based resources such as hemipteran

honeydew (Holway et al. 2002, Newell and Barber 1913). Indeed, liquid sugar-based food, such as honeydew, is the largest component of retrieved food for a colony throughout the entire year (Abril et al. 2007, Markin 1970a), the main consumer of sugar-based liquids being workers (Markin 1970c). Hee et al. (2000) demonstrated that queen survival is dependent on the presence of workers. Our experiment investigating feeding at low temperatures showed that workers succumbed quickly when access to sucrose-water was curtailed, demonstrating the importance of a reliable year-round carbohydrate source for the success of *L. humile* colonies.

We previously noted the presence of *T. virginiana* on *P. taeda*, where it settles mainly under the bark, along with two other *Toumeyella* species, *T. pini* and *T. parvicornis,* both of which prefer to settle at the branch tips (Williams and Kosztarab 1972). *Toumeyella virginiana* is known to readily associate with other ant species (Williams and Kosztarab 1972). A number of *Cinara* species are also known to infest *P. taeda* in North Carolina (D. Stephan, pers. comm.). It is unknown if they feed through the winter months, however, it is possible *Cinara* may provide an alternate winter honeydew source for *L. humile*. We noted a marked increase in *L. humile* worker weights traveling down the trunks of *P. taeda* when ambient temperatures reached 12°C or more. Ambient temperatures above 12°C may allow *L. humile* to forage along the length of the branches and gain access to *T. pini* and *T. parvicornis* and possibly some *Cinara* species for potential honeydew collection. Alternatively, the infesting honeydew-producing Hemiptera may require the substrate to be 12°C or higher to feed and ambient

temperatures above 12°C would allow those Hemiptera on shaded bark and at branch tips to feed and produce honeydew.

It is well known that invasive species can alter their new environment to favor themselves and other native or invasive organisms (Mondor and Addicott 2007, Simberloff and Von Holle 1999). Introduced vertebrates, invertebrates and plants can modify their new environments to allow other invasive plants and animals to thrive (reviewed in Simberloff and Von Holle 1999). However, these examples are habitat modification on a large scale. As mentioned above, *L. humile* is noted for causing outbreaks of honeydew-producing Hemiptera and it is likely that the *T. virginiana* infestation observed at our study site persists only through the presence of *L. humile*.

There are few known examples of a new food source extending other species ranges. The Pacific coast aquatic garter snake, *Thamnophis sirtalis*, preys on both fish and amphibians (Lind and Welsh 1994). The introduction of exotic trout into lake and water systems on the Pacific coast of the United States of America allowed *T sirtalis* to expand its range into waterways previously inhabited by amphibians only (Pope et al. 2008). On the Pacific North coast, Anna's hummingbird, *Calypte anna*, has extended north of its historical range of California and is now found as far north as northern British Columbia, Canada throughout the year (Taylor and Kamp 1985). This range extension is thought to be due to urban plantings of exotic plants providing nectar throughout the year (Zimmerman 1973). The presence of *T. virginiana* on *P. taeda* provided a potential year-

round supply of honeydew for *L. humile*. However, it was previously thought that winter nests of *L. humile* were restricted to human structures as the winter conditions in the southeastern U.S. would prevent foraging away from human structures (Buczkowski et al. 2004).

We found a foraging refuge for *L. humile* on the southern side of *P. taeda* where the bark absorbs radiant energy from the sun. By 1:00pm the temperature of sun-exposed bark of *P. taeda* had reached 15° to 30^oC, the optimal foraging temperature range for *L. humile* (Markin 1970b), across the recorded range of ambient temperatures for this experiment. In California and Southern Europe *L. humile* has invaded natural habitats where it is known to consume honeydew (Abril et al. 2007, Heller and Gordon 2006, Holway 1995, Krushelnycky et al. 2004, Way et al. 1997). Buczkowski et al (2004) noted that the winter temperatures endured by supercolonies in the U.S. Southeast averages $\sim 5^oC$ lower than for the California and southern Europe supercolonies. It appears that *L. humile* requires a temperature refuge provided by *P. taeda* to allow active foraging at these lower winter temperatures.

We are unaware of any report of similar abiotic/biotic interactions where a microclimate is provided that allows access to a previously inaccessible food resource extending the distribution of an organism. Microclimate alteration has been shown to extend the distribution of other organisms, notably plants. A number of plant species rely on the modified microclimate under nurse plants to increase their chances of establishment and

survival in harsh climates (Armas and Pugnaire 2005, Drezner 2006, Gass and Barnes 1998, Jurena and Van Auken 1998, Reay and Norton 1999, Valientebanuet and Ezcurra 1991). *Linepithema humile* infestations in northern California's Jasper Ridge Biological Reserve aggregate their nests around the south side of bushes during the colder winter months (Heller and Gordon 2006). This aggregation concentrates around the coyote bush, *Baccharis pillularis*, and *L. humile* have been observed tending aphids on this shrub (D. Gordon pers. comm.). *Linepithema humile* are known to move nests close to food sources as they become available (Holway and Case 2000). Winter temperatures may not preclude foraging by *L. humile* workers in California. However, it does appear that *L. humile* nests at Jasper Ridge Biological Reserve aggregated to a warm winter nest site with a readily available winter food source in aphid honeydew.

Our system demonstrated an interesting abiotic/biotic interaction that benefited *L. humile* by allowing workers to forage during otherwise unsuitably cold weather. A honeydew resource was made accessible only by the sun-warmed bark providing a suitable foraging microclimate. We believe that this abiotic/biotic interaction may not be exclusive to *L. humile*, but similar interactions may ensure the viability of a number of other species, along the edges of their range.

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Table 1: Repeated measures analysis of Argentine ant nest survival at cold temperature (4°C) compared to mid-optimal foraging temperature (26°C) when subjected to three food regimes; water only, water and sucrose-water and water, sucrose-water and tuna.

Variable	F value	df	p value
Food regimes	62.257	2	<0.0001
Temp regimes	172.359	1	<0.0001
Temp x Food interaction	42.741	2	<0.0001
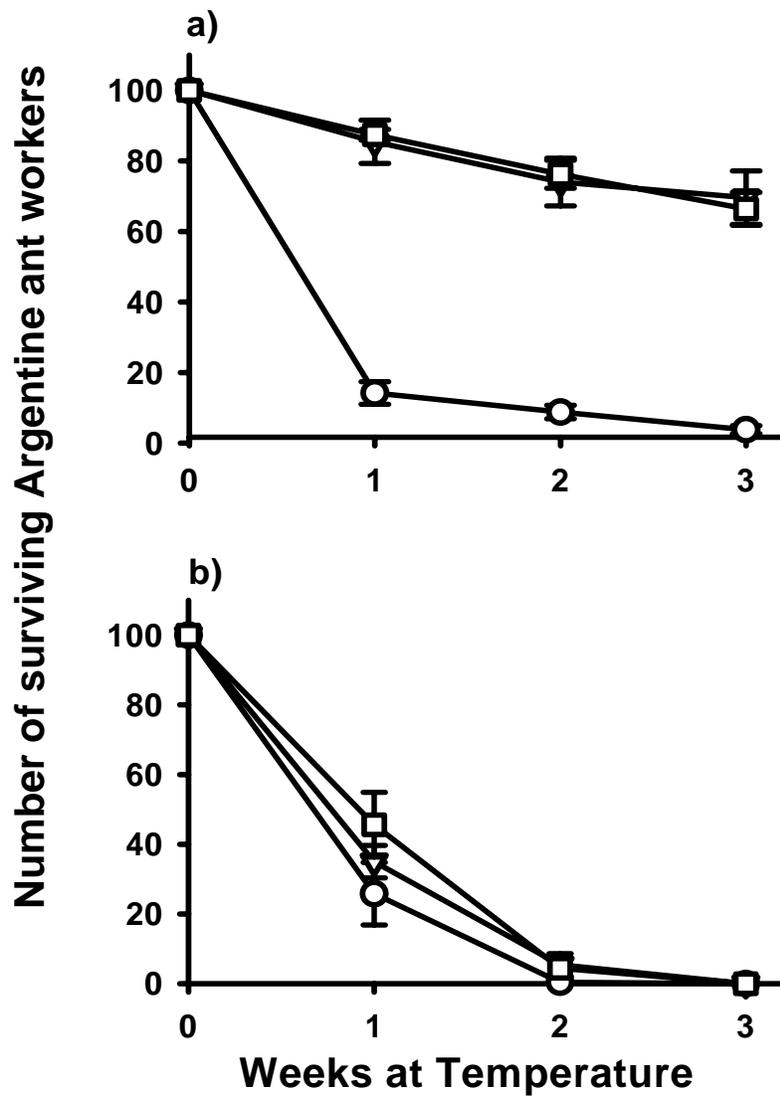
Figure 1: Mean (± S.E.) numbers of surviving Argentine ant workers through three weeks under differing food regimes at a constant temperature of a) 26°C and b) 4°C. All nests consisted of 1 queen, 100 workers and a few brood. (○): nests were subjected to a food regime of water only. (▽): nests were supplied with water and sucrose-water. (□): nests were supplied with water, sucrose-water and ~1g of tuna.
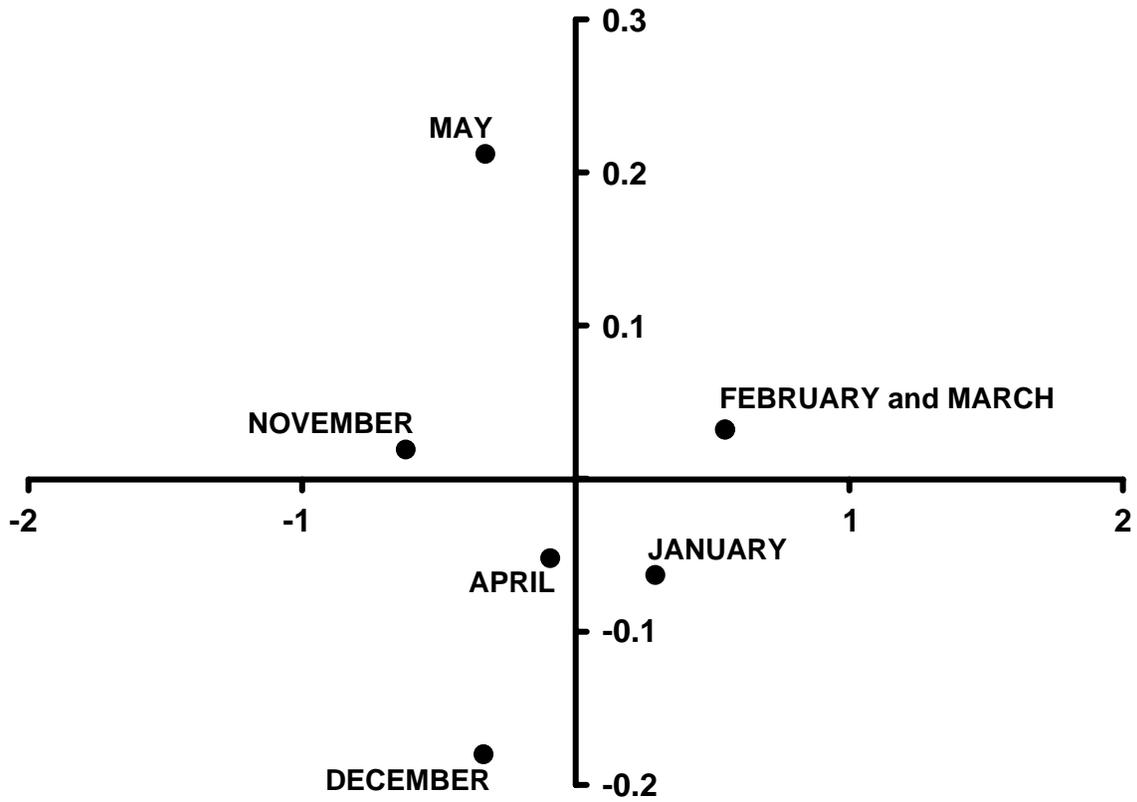
Figure 2: Bray-Curtis Ordination of the monthly difference in *L. humile* nests at the base of surveyed trees. The ordination falls into two main groups with the coldest months; January, February and March strongly contrasting with warmer months.
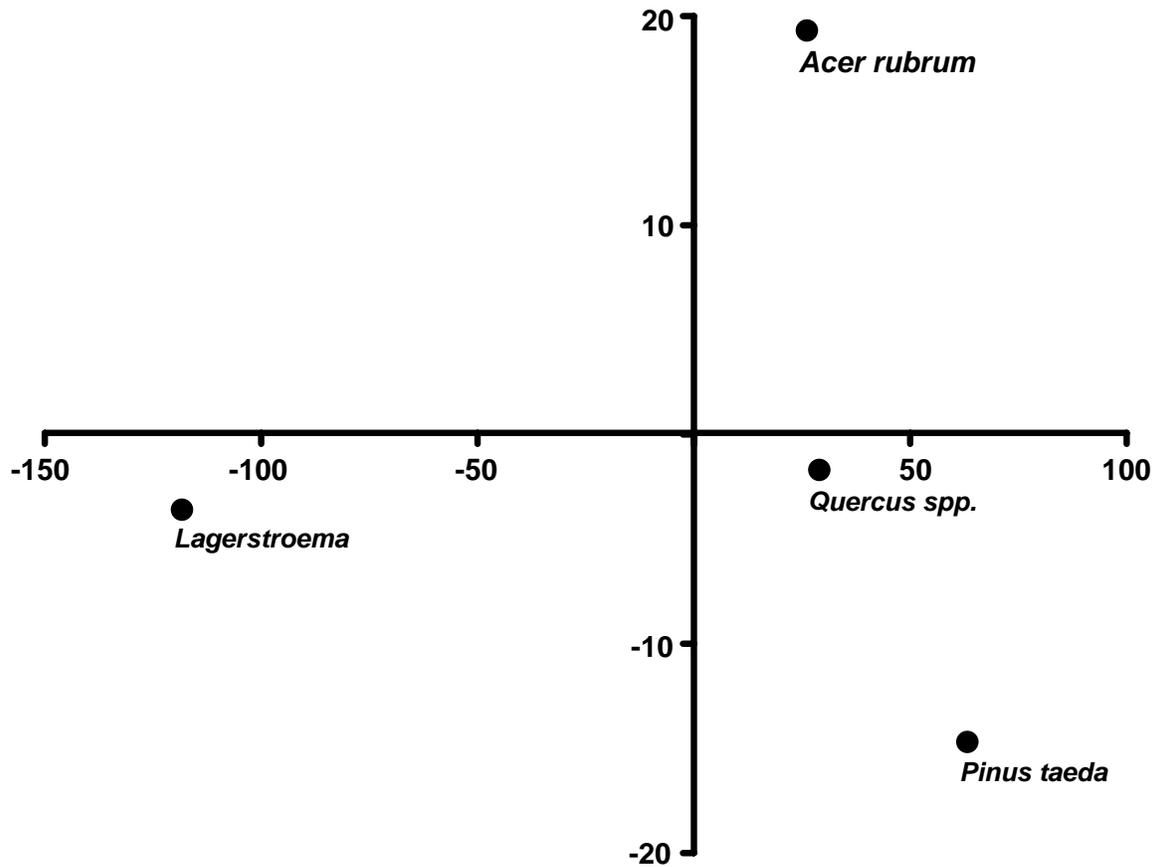
Figure 3: Bray-Curtis Ordination of the tree species difference in *L. humile* nests at the base of surveyed trees. The ordination shows that *Lagerstroema* spp. and *Acer rubrum* strongly contrast with *Quercus* spp. and *Pinus taeda*. *Quercus* spp. and *Pinus taeda* share some similarities in terms of *L. humile* nest abundance.
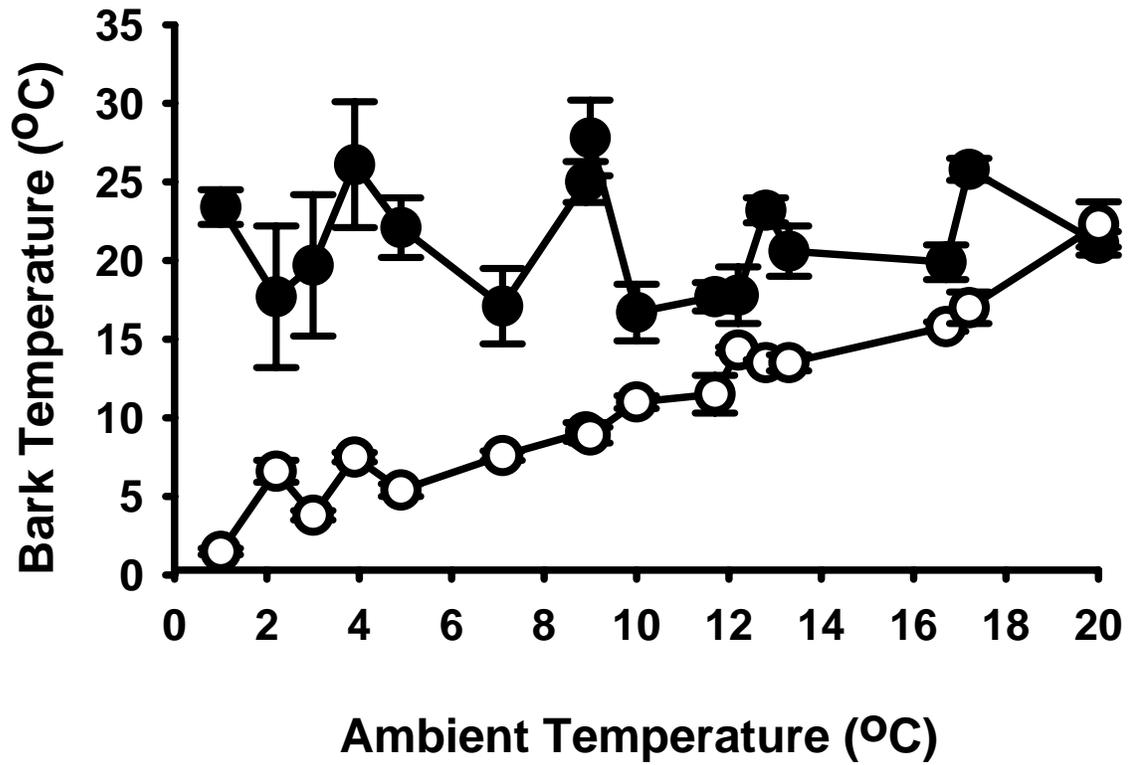
Figure 4: Mean (± S.E.) bark temperature of the southern (sun-facing) and northern (shaded) trunk face of loblolly pine compared to ambient temperatures. All recordings were taken on sunny days from December to February. (●): are bark temperatures recorded on the southern face of loblolly pine trunks. (○): are bark temperatures recorded on the northern face of loblolly pine trunks.
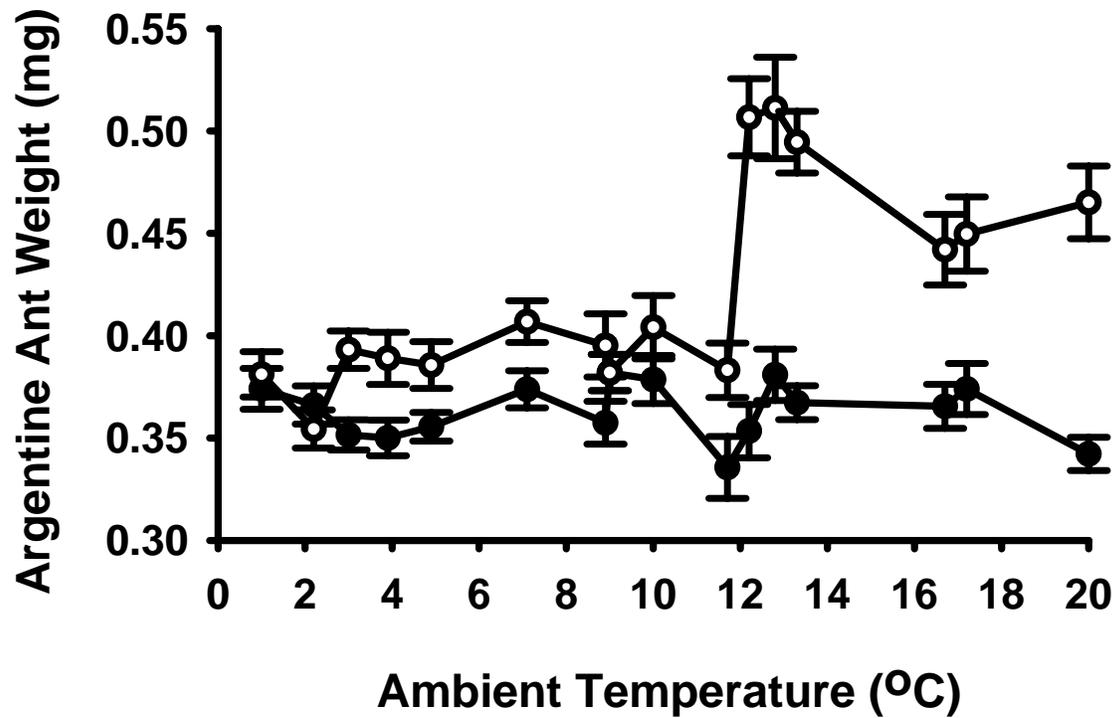
Figure 5: Mean (± S.E.) weights in milligrams of Argentine ant foragers found on the south face of the loblolly pine trunks compared to ambient temperatures. All Argentine ants were collected on sunny days from December to February. (●): are weights of Argentine ant workers trailing up the trunk of loblolly pines. (○): are weights of Argentine ant workers trailing down the trunk of loblolly pines.

Chapter five

Conclusion

In previous chapters we have explored the interactions between the Argentine ant, *Linepithema humile*, and it's facultative mutualism with the terrapin scale, *Mesolecanium nigrofasciatum*, found on the red maple host, *Acer rubrum,* within our study site. We investigated the effects on local abundances of both *L. humile* and *M. nigrofasciatum* when *L. humile* is denied access to the host trees' canopy. *Mesolecanium nigrofasciatum* numbers deceased precipitously when *L. humile* was excluded, however, foraging *L. humile* worker numbers did not decrease when access to *M. nigrofasciatum* was curtailed. *Linepithema humile* nests did move away from those trees where access to the canopy, and honeydew-producing Hemiptera, was curtailed indicating that *L. humile* will shift nests when a food resource is denied, or depleted. However, the high abundances of workers seen in *L. humile* infestation suggest that some foraging activity will be seen even in those areas where honeydew access is denied. The indirect effect of *L. humile* foraging in the canopy of the host *A. rubrum* was detrimental to the fitness of the host tree with smaller seeds and smaller leaves seen on Tanglefoot® banded trees. This is presumably through elevated levels of sap-sucking Hemiptera.

Interestingly, denying access to carbohydrates in the form of honeydew did not result in an increase in 0.5% boric acid consumption when dissolved in 25% sucrose water. Indeed, consumption was lower from the bait stations situated near those trees where canopy access was denied to *L. humile*. This appeared to be related to the proximity of *L. humile* nests to the boric acid bait stations. Where access to the host canopy was allowed, bait consumption was higher, apparently because there was strong trailing from nearby *L.*

humile nests. Where canopy access was denied, trails necessarily originated from far

nests and resulted in lower consumption. The addition of boric acid had no effect on

local *L. humile* abundances nor did the addition of boric acid on local *L. humile* nest

numbers. Any affect on *L. humile* numbers and nests had been seen shortly after canopy

access was denied.

We had previously observed *L. humile* trailing up and down *Pinus taeda* during the cold

winter months; even when ambient temperatures were below minimum foraging

temperatures of $5^{o}C$ (Markin 1970). We had also observed an apparent aggregation of *L.*

humile around *P. taeda* stands during the winter months. *Linepithema humile* is known

to aggregate during the winter months (Heller and Gordon 2006, Newell and Barber

1913), apparently to conserve, or even generate, colony heat (Seeley and Heinrich 1981).

The Piedmont region of North Carolina periodically suffers from extended bouts of cold

temperatures that should restrict *L. humile* colonies to human structures during the winter

months, yet colonies have managed to persist for some years away from human

structures. We know that *L. humile* nests can escape from cold temperatures by nesting

in the soil (Heller and Gordon 2006, Newell and Barber 1913) and this has been seen at

our study site (Silverman unpubl. data). Soil temperatures in the North Carolinian

Piedmont rarely, if at all, drop to subfreezing temperatures (SCAN 2008). However,

subfreezing temperatures may not be the biologically relevant temperature limits for *L.*

humile colonies. Temperatures that restrict colony foraging but does not directly kill the

colony could still prove lethal to the colony over time due to foraging restriction leading to starvation (Jumbam et al. 2008).

We investigated whether *L. humile* could, indeed, aggregate around *P. taeda* and if *L. humile* can survive above freezing, but below the minimum foraging temperature of 5°C. In a laboratory experiment *L. humile* cannot forage successfully at a constant temperature of 4°C and the nests died after a few weeks, even when protein and carbohydrate resources are readily available. It, therefore, appears that *L. humile* has little ability to survive off fat reserves or enter into some sort of suspended animation to help colonies survive extended cold temperatures.

Linepithema humile colonies appeared to aggregate around *P. taeda* during the cold winter months. Our monthly survey of *L. humile* nests at the base of a number of tree species showed a decrease in *L. humile* nests around *Quercus* , *Acer rubrum* and *Lagerstroema* species as we moved from November 2007 to March 2008. *Pinus taeda* was the only surveyed tree species that maintained *L. humile* nests at their base for all the months surveyed. Given that the soil temperature at our study site remains above freezing temperatures and *L. humile* had been observed trailing on the trunks in cold ambient temperatures, it seemed that *L. humile* might be aggregating to secure a steady food resource.

We took the bark temperature of *P. taeda* on the sunny and shaded side of the trunk at 1:00pm on winter days. Bark temperatures on the sunny side of the trunk were well into the optimal foraging temperature range of *L. humile* even when ambient temperature were below minimum foraging temperatures (Markin 1970). This microclimate did not extend to the shaded side of the trunk. *Linepithema humile* workers that were trailing down the trunk were heavier than *L. humile* workers trailing up the trunks of *P. taeda* indicating that the workers were feeding on a liquid food resource in the tree.

A reliable source of carbohydrates, in the form of honeydew is critical to ecologically dominant ant species (Davidson 1997, Davidson et al. 2003). *Linepithema humile*, along with other invasive ant species have a high affinity for honeydew and readily enter into facultative mutualisms with honeydew producers (Holway et al. 2002, Ness and Bronstein 2004). The facultative nature of these mutualisms for *L. humile*, and other invasive ants, may mean that there are multiple honeydew resources available, and tended by the colony. Although denying tending by an invasive ant may be catastrophic to the hemipteran partner, the facultative nature of these mutualisms means it is unlikely to have ameasurable effect on the invasive ant species. Unlike other studies (Altfield and Stiling in press, Kaplan and Eubanks 2005), we have shown that such mutualisms can have deleterious consequences on the fitness of the host plant. These differing results appear to hinge on the severity of the non-hemipteran herbivore load. For host plants with a high non-hemipteran herbivore load an invasive ant-hemipteran mutualism may confer an indirect fitness benefit. However, for those hosts that do not suffer such herbivory, an

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invasive ant-hemipteran mutualism is likely to impose a net cost to the fitness of the host plant.

The cold winters suffered in North Carolina's Piedmont may severely restrict the number of mutualist partners available to *L. humile*. The inability to survive long periods without foraging requires *L. humile* nests to move close to potential winter honeydew resources and these appear to be located on *P. taeda*. Bark microclimate on the host tree allows foraging *L. humile* workers access to this winter food resource and, thereby, reduces the chances of colony starvation. It would appear that the ability of *L. humile* to survive away from human structures in North Carolina's Piedmont is due to the combination of a suitable foraging microclimate permitting access to a winter food resource. Honeydew is important to ant species, such as *L. humile*, to maintain ecological dominance during the warmer months of the year. Along the edges of its worldwide distribution, a reliable honeydew resource, combined with a microclimate that allows access, may be critical for the continued establishment of ant species such as the invasive *L. humile*.

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Appendix

Argentine ant foraging activity and interspecific competition in

complete vs. queenless and broodless colonies.

Argentine ant foraging activity and interspecific competition in complete vs. queenless and broodless colonies.

R.J. Brightwell[1]

J. Silverman[1,2]

[1] Department of Entomology, North Carolina State University, Raleigh NC 27695

[2] Corresponding Author: jules_silverman@ncsu.edu

Running Title: Worker-only colonies of Argentine ants

Abstract

Introduction experiments may prove useful in understanding the mechanisms underlying the successful establishment of invasive ant species into new areas. These manipulative introductions could be particularly helpful in exploring the interactions between invasive species and the local fauna and flora. However, the inherent risk of accidental establishment in such experiments poses unacceptable ethical concerns. Some of the worst invasive species are tramp ant species, which can adversely affect biodiversity and community structure after establishment. We conducted laboratory and field experiments investigating a safe methodology for carrying out introduction experiments using the sterile workers of the invasive Argentine ant, *Linepithema humile*, as a model. We found no difference in foraging rate between worker-only colonies of *L. humile* and complete colonies, containing queens, workers and brood. Worker-only *L. humile* colonies showed the same exploitative and interference ability as complete colonies in bait dominance trials with the odorous house ant, *Tapinoma sessile*, in both laboratory and field trials. We suggest that for those invasive ant species with sterile workers, worker-only colonies may be substituted for complete colonies in short-term field experiments in new areas.

Introduction

A number of studies have sought to predict the success of introductions through analysis of the abiotic and biotic parameters thought to be significant in the spread and establishment of invasive ant species (Hartley et al. 2006a, Hartley and Lester 2003b, Korzukhin et al. 2001, Lester 2005, Roura-Pascual et al. 2004a, Roura-Pascual et al. 2006b, Suarez et al. 2005). However, there is still a need for more manipulative field experiments to help advance understanding of the mechanisms involved in successful colonization of invasive ant species (Holway et al. 2002b, Moller 1996). To date, most manipulative field experiments with invasive ant species have been constrained to within the ants' native or introduced range or along the invasion boundary to mitigate any accidental introduction. However, with the ability of invasive ant species to drastically alter community structure, any interspecific interactions within or close to their introduced range may not accurately reflect what would happen in a new area. Understanding these interactions may prove important in comprehending the mechanisms involved in the successful establishment of an invasive ant species. Introduction experiments into new areas holds promise in understanding some of the mechanisms of a successful establishment, however, concerns over accidental introductions have limited the use of deliberate invasive ant species introduction in manipulative experiments (Holway et al. 2002b, Moller 1996) .

The Argentine ant, *Linepithema humile* (Mayr), is an invasive ant species that is now successfully established on six continents and numerous oceanic islands (Suarez et al.

2001c). Although already widespread, models indicate that this ant species has the capacity to expand it's introduced range further (Hartley et al. 2006a, Hartley and Lester 2003b, Roura-Pascual et al. 2004a). It shares many life history traits in common with other invasive ant species including polygyny, unicoloniality, a high degree of interspecific aggression and small monomorphic workers (Hölldobler and Wilson 1990b, McGlynn 1999b, Passera 1994). As with other invasive ant species, *L. humile* has the ability to significantly alter the biodiversity and community structure within its introduced range (Holway et al. 2002b, Sanders et al. 2003) as well as being a significant urban and agricultural pest (Vega and Rust 2001).

By their very nature, invasive ant species pose a high risk of successful establishment and growth in a new area. In the case of *L. humile* for example, although workers are sterile, sexuals can still be reared from eggs and brood in the absence of queens (Vargo and Passera 1991) and Aron (2001) suggests that the successful establishment of a propagule is possible in the absence of queens. To mitigate this possibility experiments have been conducted in uninfested areas utilizing boxes that allowed the sterile *L. humile* workers egress and ingress while restricting queen movement (Holway 1999, Human and Gordon 1996, 1999).

Linepithema humile is noted for its readiness to shift nest sites (Markin 1970e, Newell and Barber 1913b, Passera 1994). Considering this propensity to abandon nests, utilizing these restrictive boxes significantly reduces the risk of accidental introduction of an

invasive ant species. These methods, however, require close and constant attention, Holway (1999) noted that he had occasion to halt his experiment upon the observation of queens or workers carrying brood exiting the nest box. Although caged colonies would appear to pose a very low risk of accidental establishment, the severe consequences to a habitat inherent with the introduction of a major pest ant species such as *L. humile* necessitates a methodology with zero risk of accidental introduction if introduction experiments are to be undertaken in an uninvaded area.

Ingram (2002) noted that the *L. humile* nests on the edge of her study site in Haleakala, Hawaii often contained workers and brood but no queens. This suggests that colonies of *L. humile* lacking queens, and possibly brood, behave similarly to nests containing queens, brood and workers. In this study, we present an alternative methodology, utilizing *L. humile* as a model, in which colonies consisting of workers only are substituted for colonies containing queens, brood and workers, defined hereon as a complete colony. We compared the foraging behavior of worker-only and complete colonies of *L. humile* to a carbohydrate and protein source. We also compared *L. humile*'s interference competitive ability with the odorous house ant, *Tapinoma sessile*, an ant species native to the United States with similar life history traits to *L. humile* and, also, capable of being a significant pest. We propose that worker-only colonies of *L. humile* would be a safe alternative to complete colonies, even in the event of escape, and may prove an ethically acceptable way of conducting field introduction experiments in new areas.

Methods

Colony Foraging

We conducted laboratory foraging trials to determine if there was any difference in foraging rate between complete and worker-only *Linepithema humile* colonies to a standard sucrose source. Experimental colonies of *L. humile* were formed from laboratory colonies collected from Research Triangle Park, Wake County, North Carolina and maintained at 26°C and 50% relative humidity with a 16:8 light-dark cycle. The experimental colonies consisted of, either, complete colonies of 5 queens and brood plus 1000 workers, or worker-only colonies consisting of 1000 workers. Our collection method for worker-only colonies utilized *L. humile* workers propensity to swarm up structures in defense of their disturbed their nests. A small linoleum tile was placed on edge into the laboratory colonies and the swarming workers aspirated along the top edge.

These colonies were kept in plastic nest containers lined with Fluon® to prevent escape. Each container contained a plaster nest chamber (100x15mm) moistened once per week. All colonies had a test-tube filled with water and plugged with cotton wool to provide moisture and were supplied with 25% sucrose-water *ad libitum* and fed freshly killed cockroaches (*Blattella germanica*) once per week. Worker-only colonies were kept isolated in a queen and brood free room for more than one week before conducting the study to prevent any possible pheromone influence from queens. The last worker-only replicates were tested 72 days after the colony was assembled.

We compared foraging behavior between *L. humile* worker-only and complete colonies by measuring trailing activity across a bridge between the colony and a food source. Worker-only colonies were tested first to prevent any possible queen pheromone influence. All colonies were starved of sucrose-water for 24 hours before a cotton twine bridge, 1mm in diameter, was added to link the colony to a feeding tube. Half of the colonies were given access to 25% sucrose-water in the feeding tube while the rest were given access to an empty feeding tube as a control. Five minute counts of *L. humile* workers crossing the bridge, in both directions, were recorded at 10 minute intervals for the first hour, then at 90 minutes, 120 minutes and then hourly to 420 minutes (7 hours). Each of the four treatments was repeated five times.

Bait dominance

Interference competition trials were undertaken to determine if there was any competitive difference between complete and worker-only *L. humile* colonies. Bait dominance trials against *Tapinoma sessile* were conducted both in the laboratory and the field. *Linepithema humile* colonies were formed from the same laboratory colonies as above. For the laboratory bait dominance trials, *T. sessile* colonies were formed from a laboratory colony originally obtained from Rocky Mount, Nash County, North Carolina. All laboratory *T. sessile* colonies were complete colonies, as were one-half of the *L. humile* colonies, the remainder consisted of workers only.

All colonies for the laboratory bait dominance trials contained approximately 1500 workers with the complete colonies including at least five queens plus brood. The colonies were kept in fluon-lined containers with one colony of *T. sessile* and one colony of *L. humile* attached to opposite ends of a foraging container, measuring 170 by 250 mm, with 8mm internal diameter vinyl tubing, plugged to keep the colonies separate. Nest chambers constructed of a water-filled, aluminum foil wrapped, 50ml centrifuge tube plugged with cotton wool were placed within each colony container to keep the nest moist. Complete *L. humile* colonies were confined within this centrifuge tube by a cap with $0.5mm^2$ mesh capable of allowing workers ingress and egress but impassable by the queens or brood. This was necessary as complete *L. humile* colonies were prone to depositing their eggs and brood in the vinyl tubing (R.J.B. pers. obs.). Their feeding regime was the same as described above.

Both colonies were deprived of sucrose-water for 24 hours. *Tapinoma sessile* colonies were then given access to the foraging container containing bait of approximately 10 grams of strawberry jam for two hours before the *L. humile* colonies were permitted access. The times to bait discovery and bait domination by the *L. humile* colonies were recorded. Bait discovery by *L. humile* was defined as the first physical contact of the bait by a worker and bait domination was defined as only *L. humile* workers left in the foraging arena. These laboratory trials were discontinued after *L. humile* colonies dominated the foraging container.

For the field dominance trials, a site with a large *T. sessile* population was identified bordering the *L. humile* infestation at Research Triangle Park. Worker-only colonies consisting of approximately 5000 *L. humile* workers and complete colonies consisting of same the number of workers with more than five queens and approximately 0.1g of brood were prepared and housed in sealed 3.07 liter containers. A relatively large colony fragment was chosen as Walters and Mackay (2005) demonstrated that high worker numbers were required to compete successfully against a large competitor colony. These containers had a moistened bed of pine needle mulch, a vent in the lid covered with a screen of fine mesh and an 8mm internal diameter vinyl exit tube with a removable plug. Both colony types had a water-filled 50ml centrifuge tube plugged with cotton wool to keep the nest moist and some freshly killed cockroaches. The complete colony queens and brood were contained within this centrifuge tube by a cap with a 0.5 mm^2 mesh capable of allowing workers ingress and egress but impassable to queens and brood.

Bait cards, measuring 74 x 120 mm with approximately 10 grams of strawberry jam, or approximately 10gm of canned tuna in water, were placed along the *T. sessile* infestation boundary one hour before the *L. humile* colonies were introduced. This ensured that *T sessile* was recruiting to the bait cards before the placement of the *L. humile* colonies. The sealed containers of complete or worker-only *L. humile* colonies were placed by the bait stations with the container exit tube approximately 100mm from the bait station. The colonies were allowed to acclimatize for two hours before the exit plug was removed and the times to bait discovery and domination were recorded. Bait discovery was defined as

above and bait domination was defined as only *L. humile* workers on the bait card. All nests were replugged and retrieved after two hours. Five replicates were performed for each treatment.

Data Analysis

Statistical analysis and significance tests ($P < 0.05$) were performed using SAS v.9.1 (SAS Institute, Cary, North Carolina). Data for the colony foraging experiment were log transformed to comply with assumptions of constant variance and tested using repeated measures analysis of variance with complete and worker-only colonies as the between-subjects variable and time as the within-subjects variable. Two way ANOVA and pooled t-tests were performed to compare complete vs. worker-only colony performance in the laboratory and field bait dominance trials.

Results

In the colony foraging experiment, both worker-only and complete *L. humile* colonies demonstrated a pattern of an early spike in bridge crossing activity followed by a low constant foraging level after 90 minutes of the bridges' introduction (fig. 1). There was no difference in foraging rate between worker-only and complete *L. humile* colonies, nor was there a difference in foraging rates to the sucrose-water filled and empty feeding tubes (Table 1).

There was no difference in time to bait discovery or bait dominance between complete and worker-only colonies in the laboratory trials (fig 2, Table 2), nor was there a difference in the time taken from bait discovery to bait dominance between the colony types ($t = -1.43$, df 8, $p = 0.1919$). Likewise, in the field trials there were no differences in the discovery and dominance times, to either the strawberry jam or tuna bait, between the colony types (fig 3, Table 2). Both complete and worker only colony types showed a preference for the strawberry jam baits with faster discovery ($F_1 = 5.47$, $p = 0.0326$) and dominance times ($F_1 = 7.81$, $p = 0.0130$) to strawberry jam baits than to the tuna baits.

Discussion

In this study, we found no difference in the exploitative competitive ability between complete and worker-only colonies of the invasive *L. humile*. Nor did we find any difference in their exploitative and interference competitive abilities in the laboratory, or in the field, with *T. sessile*. *Linepithema humile* showed a preference for a carbohydrate resource by finding and acquiring the offered carbohydrate resource quicker than the protein resource, regardless of colony type. However, in all trials *L. humile* eventually discovered and dominated the offered food resource, whether it is carbohydrate or protein based.

Our investigation into the interactions between *L. humile* and *T. sessile* suggests that the use of worker-only colonies in investigating interspecific interactions reflect interactions of complete colonies. Invasive or tramp ant species are noted for their unusually aggressive nature (Passera 1994). Intraspecific aggression for invasive ant species are often measured by assays pitting individual, or a few, isolated workers against similar numbers of conspecific workers, as described and tested by Roulston et al. (2003). Interspecific aggression between *L. humile* and other invasive or native ant species have also been measured using worker vs. worker assays (Holway 1999, Morrison 2000). However, aggression assays between individual ant workers are limiting in that they do not reflect colony-level interference competitive ability. Complete colonies have been used in colony level interference competition assays as previously described. Vander Meer and Alonso (2002) demonstrated that the interspecific aggression of queenless and

broodless *Solenopsis invicta* was not significantly different from queenright colonies. We have demonstrated that this holds true for *L. humile* also.

Both worker-only and complete *L. humile* colonies preferred strawberry jam to canned tuna in the field bait dominance trials. While high protein levels promote greater production of *L. humile* sexuals and brood (Aron et al. 2001), sugar is preferred by workers over proteins even with queens and brood present (Baker et al. 1985a, Markin 1970b). We found no difference between worker-only and complete colonies in foraging effort to a protein source. Our protein source, however, was not live and caution should be exercised when using worker-only colonies in assessing the predation effects of invasive ants on non-myrmicine arthropods in a new area.

The methodology we present here is intended to remove the small but inherent risk posed by the current short-term colony introduction methodologies (Holway 1999, Human and Gordon 1996, 1999). The noted characteristic of *L. humile* colonies to readily move nest sites in adverse conditions may complicate longer term field experiments with worker-only colonies (Heller and Gordon 2006b, Markin 1970e, Newell and Barber 1913b, Passera 1994, Vega and Rust 2001). The disturbance inherent in transporting the colony coupled with a possible sub-optimal artificial nest structure may not encourage the worker-only colony to remain faithful to the nest for an extended period of time. Different environments are likely to require variations in the construction of the introduction nest to promote longer-term residency. However the possibility of longer-

term introduction experiments, through testing and refining artificial nests in a new area is both possible and safe with worker-only colonies.

Many invasive ant species share similar life history traits (Hölldobler and Wilson 1990b, Moller 1996, Passera 1994). They are aggressive exploitative and interference competitors (McGlynn 1999a) and often capable of altering arthropod community structure (reviewed in Holway et al. 2002b). In this study we present a new methodology for safely conducting manipulative field experiments using invasive ant species in uninfested areas. To date, testing hypotheses explaining how invasive ant colonies establish in new areas have been difficult to conduct safely in new areas. Using worker-only colonies of invasive ant species with sterile workers may facilitate testing some of these hypotheses.

Acknowledgements

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Table 1: Summary of F statistics for tests of the fixed effects of treatments and interactions from repeated measures ANOVA on foraging activity of complete and worker-only colonies to a feeding tube with 25% sucrose-water or an empty feeding tube.

Effect	df	F	p-value
Time	4, 21.5	2.53	< 0.0001
Colony type	1, 19.9	0.24	0.6330
Sucrose	1, 19.9	3.33	0.0829
Colony type x time	13, 100	0.27	0.9943
Colony type x food	1, 19.9	3.21	0.0884
Sucrose x time	13, 100	0.79	0.6663
Colony type x sucrose x time	13, 100	0.67	0.7835

Table 2: Student t-test results for times to discovery and dominance of offered bait between worker-only and complete Argentine ant colonies. Carbohydrate bait was strawberry jam while protein bait was canned tuna. Laboratory trials were performed with carbohydrate bait only.

| Variable	df	t-value	p-value
Laboratory trials			
Bait discovery	8	-0.89	0.3989
Bait dominance	8	-1.79	0.1114
Field trials			
Carbohydrate bait discovery	8	-0.61	0.5573
Carbohydrate bait dominance	8	-0.33	0.7541
Protein bait discovery	8	0.47	0.6491
Protein bait dominance	8	-0.24	0.8159
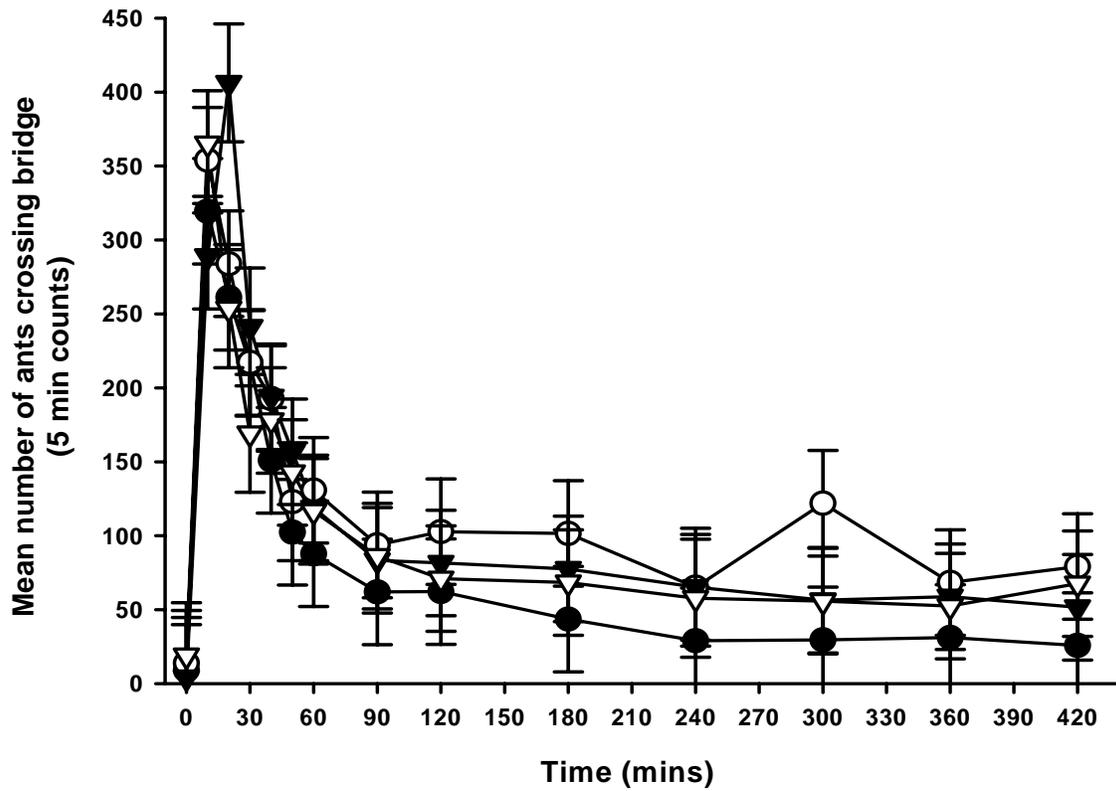
Figure 1: Mean (± SE) foraging activity of worker-only and complete colonies of *L. humile* to 25% sucrose solution and a control of an empty feeding tube. (●): complete colony foraging to 25% sucrose solution. (○): complete colony foraging to empty feeding tube. (▼): worker-only colony foraging to 25% sucrose solution. (▽): worker-only colony foraging to empty feeding tube.
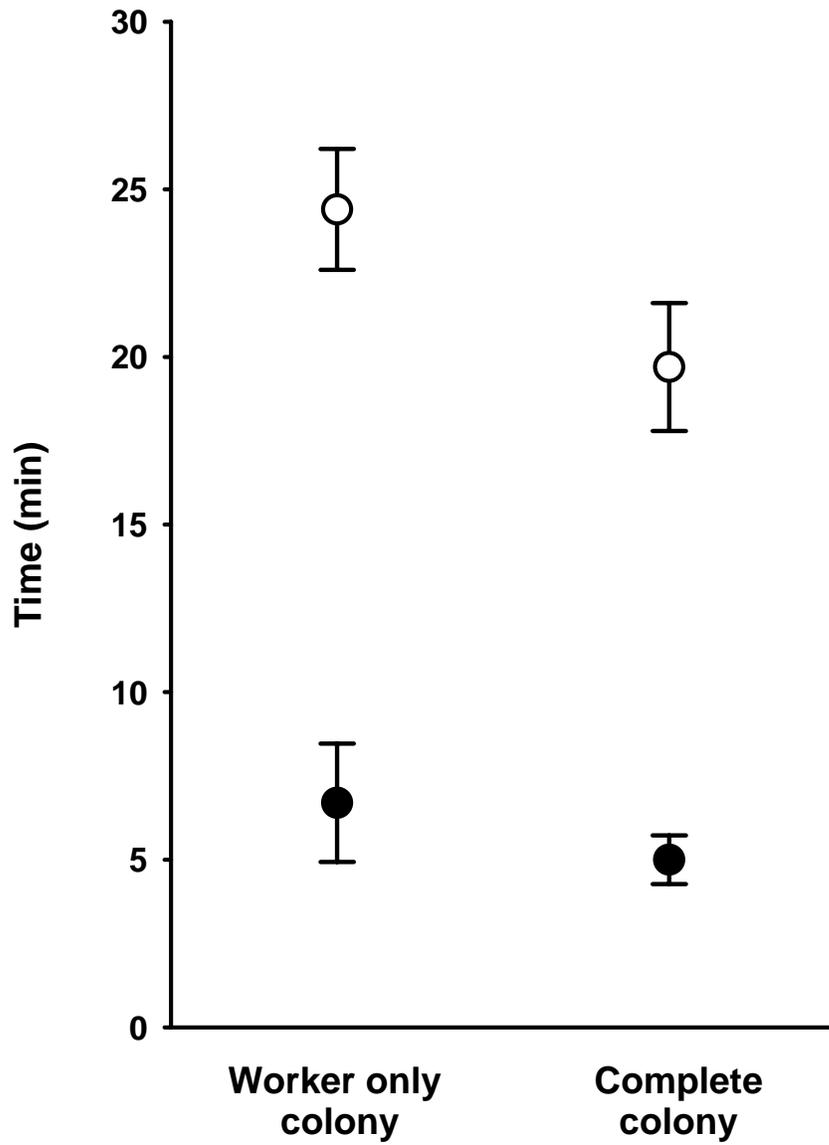
Figure 2: Mean (± SE) time to discovery and dominance of *T. sessile* dominated bait by worker-only and complete colonies of *L. humile* in laboratory trials. (●): time to bait discovery. (○): time to bait dominance. Time to bait discovery equals first physical touch of the bait by a *L. humile* worker. Time to bait dominance equals exclusion of *T. sessile* from the foraging container.
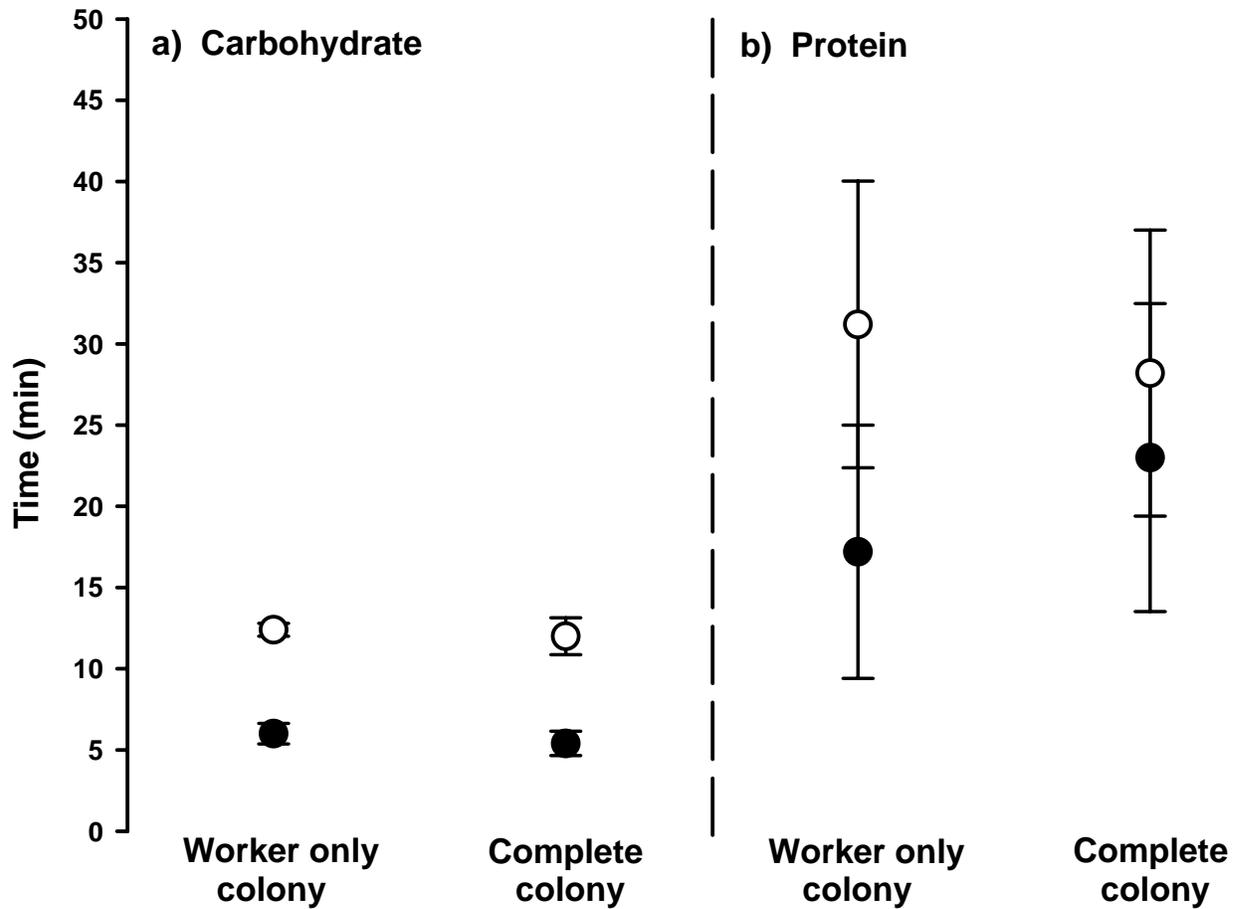
Figure 3: Mean (± SE) time to discovery and dominance by worker-only and complete colonies of *L. humile* of *T. sessile* dominated bait in field trials. Baits consisted of a) Strawberry jam as a carbohydrate source and b) canned tuna as a protein source. (●): time to bait discovery. (○): time to bait dominance. Time to bait discovery equals first physical touch of the bait by a *L. humile* worker. Time to bait dominance equals exclusion of *T. sessile* from the bait card.